17TH ASEAN FOOD CONFERENCE 2023
24-27 OCT

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MINISTRY OF SCIENCE, TECHNOLOGY AND INNOVATION

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A joint activity of ASEAN Committee on Science, Technology and Innovation
&
Federation of Institutes of Food Science and Technology in ASEAN

17th ASEAN FOOD CONFERENCE 2023

FUTURE OF FOOD IN ASEAN: WHAT'S NEXT?

24 - 27 October 2023

Borneo Convention Centre, Kuching, Sarawak, Malaysia

e-Proceeding

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Perpustakaan Negara Malaysia Cataloguing-in-Publication-Data

Siti Noorbaiyah Abdul Malek, Nor Khaizura Mahmud Ab Rashid, Lim Seng Joe and Chang Lee Sin. 2023. e-Proceeding of the 17th ASEAN Food Conference. Malaysian Institute of Food Technology. pp. 410

e ISBN 978-967-9986-04-4

WELCOME MESSAGE

MINISTER OF SCIENCE, TECHNOLOGY AND INNOVATION, MALAYSIA

MINISTRY OF SCIENCE, TECHNOLOGY AND INNOVATION

It is an honour for me to welcome all speakers and participants to the 17th ASEAN Food Conference (AFC2023) with the theme: "FUTURE OF FOOD IN ASEAN: WHAT'S NEXT?".

AFC2023, a signature event of the Sub-Committee on Food Science and Technology (SCFST) under the ASEAN Committee on Science, Technology and Innovation (COSTI), is an important platform to discuss directions on topics of common interest within the realm of the Future of Food in ASEAN. This conference is also essential for the experts involved and delegates to be able to interact and share ideas for the advancement of food science and technology in the ASEAN region. It also indirectly reflects our hard work in fostering innovative solutions and enhancing global competitiveness of regional education, research, and innovation.

With the current global uncertainty of food security and health crisis, we have faced significant geo-political, economic, social, and environmental challenges. With an emphasis on increasing food production, enhancing producers' lives, promoting agri-based investments, and creating favourable markets and fair trade, integration and cooperation in food, agriculture, and forestry within ASEAN have become even more engaged. With continued coordination among ASEAN Member States (AMS) and stakeholders, we will be able to build a secure, sustainable and resilient ASEAN food industry.

Therefore, the AFC2023 comes at an opportune moment and is very pertinent because it highlights developments and new findings in food science and technology. AFC2023 aims to provide the underpinning science in food safety and regulatory science, food sustainability, Internet of Things (IoT) and digitalisation, food industry trends and consumer perspectives, halal auditing and certification, food processing and engineering, food chemistry and microbiology, technology transfer and the commercialisation of traditional food.

On that note, I would like to congratulate Malaysian Institute of Food Technology (MIFT) and the collaborators of AFC2023, namely the International Union of Food Science and Technology (IUFoST), Ministry of Science, Technology And Innovation, Malaysia and the Malaysian Agricultural Research and Development Institute (MARDI) as well as the AFC2023 institutional partners, for their efforts in making this event a reality today.

I encourage everyone to take this unique opportunity to strengthen partnerships, working collectively towards a more sustainable future in food. It is my fervent hope that this AFC2023 will succeed in realising its goals.

YB Chang Lih Kang
Minister of Science, Technology and Innovation

WELCOME MESSAGE

Organising Committee Chairman, 17th ASEAN Food Conference 2023

On behalf of the Organising Committee, we are delighted to extend our warmest greetings to all participants of the 17th ASEAN Food Conference, where we will explore the intriguing theme of "The Future of Food in ASEAN: What's Next?". We are proud to announce that the ASEAN Food Conference (AFC) is a collaborative effort between the ASEAN Committee on Science, Technology, and Innovation (ASEAN COSTI) and the Federation of Institutes of Food Science and Technology in ASEAN (FIFSTA). This biennial event rotates among ASEAN member countries that host their own Institute of Food Science and Technology and are FIFSTA members.

This year, the Malaysian Institute of Food Technology (MIFT), serves as the host for the 17th AFC. Notably, our conference draws participants not only from ASEAN nations but also from countries beyond, including Japan, South Korea, Taiwan, India, Bangladesh, Algeria, Poland, Canada, Australia, and the United Kingdom.

Over the span of three days, this conference promises a dynamic exchange of ideas through plenary sessions, scientific presentations, poster exhibitions, and informal gatherings. Eminent scientists and experts from academia, government, industry, trade associations, and consumer groups in both ASEAN and the global community will engage in thought-provoking discussions. The primary focus will be on addressing current and emerging challenges while exploring the pivotal role of food science and technology in advancing sustainable food supply chains that are accessible to all. Additionally, the conference provides an invaluable platform for knowledge sharing, fostering research networks, and forging new collaborative opportunities among experts in the field.

Lastly, thank you to all our collaborators and supporters for making the AFC 2023 possible. We sincerely hope that this conference will prove beneficial to all participants, enriching their respective interests, businesses, and areas of expertise. Moreover, we trust that you can enjoy the exciting adventure in Sarawak, a place which is also known as "Bumi Kenyalang" - a diverse and culturally rich destination. It boasts 27 ethnic groups speaking 45 languages, each with its own unique traditions, making it a vibrant and exciting place to visit. Welcome to the 17th ASEAN Food Conference 2023!

Thank you.

Sharidah Yusoff
Organising Chairman
17th ASEAN Food Conference 2023

WELCOME MESSAGE

President, MIFT & FIFSTA

A warm welcome to the ASEAN Food Conference 2023! It is an honour and a privilege for MIFT to host this 17th edition in Malaysia again as we gather to discuss, collaborate, and innovate in the realm of food security, sustainability, and culinary diversity within the ASEAN region.

I would like to extend my heartfelt gratitude to each and every one of you for joining us at this significant event. The ASEAN Food Conference provides a platform for us to come together, share our knowledge, and work towards addressing the complex challenges that lie ahead in the world of food, agriculture, and nutrition.

As we stand at the crossroads of an ever-changing world, the importance of food cannot be overstated. It is not only a basic human need but also a pivotal element in the cultural, economic, and social fabric of our nations. The ASEAN region, with its rich diversity in cuisines, agricultural practices, and culinary traditions, is a testament to the incredible variety and depth of our food heritage.

During this conference, we will delve into a wide range of topics, from sustainable agricultural practices and food security to innovations in food technology and the preservation of our cultural culinary heritage. Our discussions will revolve around finding solutions that not only meet our present needs but also secure a better future for generations to come.

We have a stellar line-up of speakers and experts from across the ASEAN member countries and beyond who will share their invaluable insights and experiences. This conference is a unique opportunity for networking, collaboration, and the exchange of ideas, and I encourage you to take full advantage of it.

In addition to the formal sessions and presentations, we have organised various cultural events and food exhibitions that will allow you to explore the diverse flavours and traditions that define our region. I encourage you to engage in these activities to gain a deeper appreciation of our rich food heritage.

As we embark on this journey over the next few days, let us keep in mind the importance of our collective efforts in shaping the future of food in ASEAN. Let our discussions be productive, our interactions be enriching, and our shared commitment to sustainable, accessible, and nutritious food for all be unwavering.

Once again, I extend my warmest welcome to all of you, and I am confident that together, we will make the 17th ASEAN Food Conference 2023 a resounding success. Thank you for being here and let us now officially commence this conference.

Enjoy the conference!

Dr. Koh Yew Ming
President
MIFT & FIFSTA

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Abstract: Propolis is a resinous substance collected by stingless bees containing bioactive compounds which exert various biological properties. The present study focused on the evaluation of chemical and biological profiles produced by three Indo-Malayan stingless bee propolis, namely *Tetrigona apicalis*, *Tetrigona binghami*, and *Heterotrigona fimbriata*, extracted using ethanol and water. The bioactive compounds of propolis extracts were analyzed using gas chromatography–mass spectrometry (GC-MS), while the antibacterial activity of the extracts was investigated using disc diffusion method against Gram-positive and negative bacteria. The angiotensin converting enzyme (ACE) activity of the extracts was determined spectrophotometrically. *H. fimbriata* ethanolic extract displayed the best biological activities compared to the other propolis extracts, and thus was chosen for the anti-obesity study on high-fat diet-induced mice. Significant differences were detected in total weight gained and food efficiency ratio (FER) between groups since $p<0.05$, but concentrations of serum profile parameters between all groups were found to be insignificant. In conclusion, this study shows that Malaysian stingless bee propolis contain bioactive components that have great potential to be used for their therapeutic and medicinal benefits. *H. fimbriata* extract may also potentially be utilized as a diet supplement since it was able to suppress excessive weight gain effectively.
***Keywords*:** Anti-obesity, biological activity, chemical composition, propolis, stingless bee

INTRODUCTION

According to Centre for Disease Control and Prevention (CDC), obesity is the major leading cause of death worldwide and has been shown to have severe impacts on human health and reduces the quality of life. Therefore, solutions are much needed nowadays to address the issue at hand. Stingless bees such as *Homotrigona fimbriata* and *Trigona* sp. are native to Malaysia and are noted to be resistant to parasites and diseases brought by the honey bees, *Apis mellifera* (Azmi et al. 2018). However, studies on these stingless bees and their products, especially those derived from the *Tetrigona* sp., are still scarce (Sulaeman et al. 2019). The use of extracts derived from the propolis of the stingless bees may provide an alternative approach to suppress weight gain or even promote weight loss due to the polyphenolic compounds present. Propolis is high in bioactive compounds that can be utilized for its medicinal properties. Yet, there is still limited studies or research done, especially on the Malaysian stingless bee propolis. A study conducted by Cai et al. (2020) has proven that Chinese propolis extract to be effective in preventing weight gain as well as fat accumulation in the liver of mice induced with high fat diet. Besides, a study carried out by Sakai et al. (2017) using Brazilian propolis has also managed to significantly reduce the fat weight in mice fed with the propolis extract. Therefore, the use of Malaysian stingless bee propolis such as *H. fimbriata*, *T. binghami* and *T. apicalis* in anti-obesity studies should be conducted to provide an alternative in addressing the obesity issue at hand. Moreover, this research also aimed to determine the antibacterial and Angiotensin I-converting enzyme (ACE) activities of the propolis extracts studied.

MATERIALS AND METHODS

Stingless Bee Propolis Sample Extraction

The extraction process was done according to Suriyatem et al. (2018) with slight modifications by crushing 20g of each crude propolis sample into smaller pieces and placed in the extracting solvents using the ratio 2:5 *m/v*. Distilled water and 70% ethanol were used as the extracting solvents. Then, the samples were heated and stirred constantly on a hot plate at 70°C for 5 min. Next, the samples were let cool at room temperature and placed in darkness for 24 hours before they were being filtered using Whatman No. 1 filter papers.

GC-MS Analysis of Propolis Extracts for Bioactive Components

The chemical composition the propolis extracts were determined using gas chromatography-mass spectrometry (GS-MS) analysis method on the PerkinElmer Clarus 600 Gas Chromatograph system with TurboMatrix Headspace Sampler 40. The equipment was also supported by the National Institute Standard and Technology (NIST) library data. The experimental conditions for the Elite 5MS capillary column were as followed: length=30m, ID=250mm, film thickness=0.25μm. the column initial temperature, 80°C held for 4 min, was increased to 250°C at 3°C/min, then to 300°C, which was held for 15 min, at 20°C/min. The acquisition specifications were as detailed: the temperature of the injector was set at 250°C and the source temperature was at 280°C; split ratio used was 100:1; helium was used as the carrier gas; flow rate was at 1 mL/min; scan range was 35-500 Da; solvent delay was 2 min; and the injected volume was 0.4 mL in each vial (Asgharpour et al. 2020). The compounds showing 80% similarity with chemical compounds from the NIST library database were chosen for this study.

Antibacterial Activity

The antibacterial study was carried out using disc diffusion method with Mueller Hinton Agar (MHA), as employed by Rechenchoski et al. (2017) using common Gram positive and negative bacteria in testing the antimicrobial activity of the propolis extracts. Gram positive bacteria chosen for this study were *Staphylococcus aureu*s and *Bacillus subtilis*, while Gram negative strains were *Escherichia coli* and *Salmonella typhimurium*. The bacterial strains were spread on the MHA plates evenly using sterilized cotton swab. Next, 6 mm discs soaked with propolis extracts, cefotaxime (positive control) and sterile distilled water (negative control) were placed on labelled plates and incubated at 37°C for 24 hours. All of the steps were done in triplicate. The inhibition zones formed around the disc were measured.

Angiotensin I-Converting Enzyme (ACE) Inhibitory Activity

The ACE inhibitory action of the propolis samples were determined using the method of hippuric acid (HA) liberation from hippuryl-L- histidyl-L-leucine (Hip-His-Leu, HHL) catalyzed by the ACE from rabbit lung tissue (Sigma Aldrich, USA), as described by Cai et al. (2020) with slight modifications. 50 μL of the propolis sample with concentrations ranging from 20 to 100 μL were mixed with 50 μL ACE solution (25mU/mL) in potassium phosphate buffer solution pre-incubated for 10 min at 37°C. Then, 150 μL of HHL solution (5mM HHL, 0.1M potassium phosphate with 0.3M sodium chloride at pH 8.3) was added into the samples and incubated at 37°C for 1 hour. 8μL of 5M HCL was then used to stop the reaction. Captopril was used as the positive control for this assay and different concentrations were prepared to obtain the standard curve, while solution without enzyme and sample was used as blank. The absorbance was measured using a UV-VIS Spectrophotometer (DU 730 Beckman Coulter) at λ=228 nm.

Anti-obesity Study

The procedures described below were approved by the Animal Ethics Committee of the Universiti Putra Malaysia (Ref: UPM/IACUC/AUP-R008/2021). A total of thirty male Balb/c (20.58±3.03 g) mice (4 weeks old) were acclimated for a week under control laboratory conditions (24±2 °C, 60-70% relative humidity and 12 h light-dark cycle) during the experimental period, as done by Lim et al. (2016). The mice were then randomly grouped into five different cages (n=6) after acclimatization based on assigned diets, which were: i) Normal diet (ND) (standard mice pellet Gold Coin, Malaysia); ii) Normal diet with 500 mg/kg *H. fimbriata* ethanolic extract (ND+P); iii) High-fat diet (HFD) (Mazuri Rat & Mouse Diet); iv) High-fat diet with 500 mg/kg propolis supplemented (HFD+P) and v) High-fat diet with orlistat (HFD+Or) (control). Animals were allowed to feed their respective diets and water was provided *ad libitum* throughout the experiment. The mice were allowed a sedentary lifestyle, without specific physical activity or exercise regime throughout the course of the study. After 4 weeks, the animals were denied food and fasted for 16 hours in preparation for blood collection through cardiac puncture under anesthesia. Body weight of each mouse was weighed weekly using an electronic balance. The total diet intake consumed by the mice in each group was measured weekly by subtracting the balance quantity with the initial quantity supplied.

RESULTS AND DISCUSSION

Generally, the present study conducted showed that ethanol-extracted propolis was able to extract more chemical compounds and exhibited better biological properties compared to the water-extracted propolis samples. Polar solvents are highly favoured in the extraction of herbs and plant-based products including propolis since they are better in solubilizing and attracting compounds such as terpenoids and polyphenols (Mello et al. 2010). The GC-MS analyses carried out revealed that the propolis extracts from the three different stingless bee species consisted of major groups such as sugar, carboxylic acid, terpenoid, sugar alcohol, hydrocarbon, ketone, aldehyde and amino acid. According to Hikmawant et al. (2021), ethanol is able to extract components such as flavanols, polyphenols, sterols, alkaloids and terpenoids better than water-extracted samples. On the other hand, analysis of the water extracts was able to identify considerable amounts of sugars and their derivatives in all of the propolis samples tested. This is because carbohydrates such as sucrose, fructose, glycose including sugar alcohols are water-soluble compounds (Zhong et al. 2021). Therefore, the presented GC-MS analyses of the samples were able to shade some light on the chemical constituents that made up the Malaysian stingless bee propolis studied by evaluating both types of extract.

Table 1 showed that both extracts exhibit antibacterial properties. Gram positive bacteria, *S. aureus* and *B. subtilis*, are more susceptible to the propolis samples compared to Gram positive bacteria tested. These results are also agreeable with those reported by Abdullah et al. (2019), in which *E. coli* and *Salmonella* strains tested showed weaker antibacterial activities, compared to *S. aureus* and *B. subtilis* that yielded better inhibition zones when tested against stingless bee propolis.

Table 1. Zone of inhibition diameters (mm) of propolis ethanolic extracts against Gram positive and negative bacteria tested

Bacteria / **Propolis**	***S. aureus***		***B. subtilis***		***E. coli***		***S. typhimurium***	
	Ethanolic	Water	Ethanolic	Water	Ethanolic	Water	Ethanolic	Water
H. fimbriata	14.0±2.0*	14.0±2.0*	13.0±1.0*	13.5±1.0	0±0.0	9.5±1.0*	0±0.0	0±0.0
T. apicalis	16.5±1.0*	16.5±1.0*	17.0±1.0*	13.5±1.0	0±0.0	8.0±2.0*	0±0.0	0±0.0
T. binghami	17.5±1.0*	12.0±1.0*	15.0±1.0*	12.0±1.0*	0±0.0	7.0±1.0*	0±0.0	0±0.0
Cefotaxime	18.0	15.0	18.0	15.0	18.0	15.0	18.0	15.0
Distilled water	0±0.0		0±0.0		0±0.0		0±0.0	

*Significant differences detected between all groups since $p<0.05$ using ANOVA.

Table 2. Total phenolics, flavonoids, antioxidant and ACE inhibitory activities of propolis ethanolic and water extracts

Propolis	Total Phenolic Content (mg/mL)		Total Flavonoid Content (mg/mL)		ACE Inhibiting Activity (IC_{50})	
	Ethanolic	Water	Ethanolic	Water	Ethanolic	Water
H. fimbriata	42.15 ± 0.03	13.21 ± 0.26	57.43 ± 0.36	34.53 ± 0.11	3.10	3.45
T. apicalis	55.48 ± 0.05	7.60 ± 0.13	53.88 ± 0.04	34.50 ± 0.24	4.19	3.64
T. binghami	31.25 ± 0.03	10.11 ± 0.19	54.75 ± 0.07	34.17 ± 0.25	3.40	4.06

Table 2 displayed results tabulated for the ACE inhibitory actions of the propolis tested. According to a study done by Guler et al. (2021), flavonoids found in propolis extracts had the potential to inhibit the activity of angiotensin-converting enzyme (ACE) due to their immunomodulatory and inhibition properties. Ethanolic extracts also showed better ACE inhibition action due to their polyphenolic compounds that contribute to the higher inhibitory activity shown by them, compared to the water extracts.

Table 3. Average daily weight gain, food intake and percentage of FER calculated for each diet group studied

Group	ND	ND+P	HFD	HFD+P	HFD+Or
Weight gain (g/day)	0.35±0.04*	0.26±0.04*	0.97±0.05*	0.37±0.06*	0.18±0.05*
Food intake (g/day)	20.93±0.35	20.68±0.39	24.82±0.48	24.46±0.34	22.40±0.33
FER (%)	1.7±0.16*	1.3±0.18	3.9±0.13*	1.5±0.2*	0.8±0.17*

*Correlation is significant at $p<0.05$.

Table 3 revealed that mice fed with HFD gained the highest amount of weight, followed by the ND (control) group. ND+P and HFD+P groups showed that the propolis supplemented were able to significantly prevent excessive weight gain effectively. Koya-Miyata et al. (2009) claimed that propolis was a pharmacological inhibitor of weight

gain. This is because the food intake by the mice from the control and propolis administrated groups for 10 days did not differ significantly during the experimentational period, suggesting that propolis extracts did not influence the total diet intake of the mice. The authors postulated that propolis were important in the regulation of carbohydrate and lipid metabolism by boosting energy expenditure and inhibiting adipogenesis to discourage extreme weight gain in the mice studied.

CONCLUSIONS

This research was carried out to identify and profile the chemical compounds and biological activities of propolis collected from three different native Malaysian stingless bee species, which allows association between the bioactive compounds identified in the propolis extracts with the pharmacological properties exhibited by them that can further contribute to their characterization and commercialization values. Propolis used in the current study shows promising prospect in the discovery of potent bioactive compounds that can contribute to a wide array of biological activities such as antioxidant, antibacterial, ACE inhibition and anti-obesity. Some future prospects that can be explored are by employing different methods or instrument to extract biomolecules in the propolis samples should be performed so that better comparison and identification of propolis components can be done. Furthermore, clinical trials using propolis on obese patients can help determine and evaluate the effect of propolis supplements on human health and its effectiveness when compared with other anti-obesity chemicals or weight loss drugs.

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PROPOLIS EXTRACT AS ADDITIVE IN BIO-PACKAGING FOR FOOD PRESERVATION

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Abstract: This study aimed to investigate the antioxidant activity, total phenolic content (TPC), total flavonoid content (TFC), antibacterial activity, and bioactive constituents analysis of propolis from three different Malaysian stingless bee species and characterize a biodegradable films by incorporating cornstarch with propolis extract to enhance the functional properties for potential use as active food packaging. The propolis samples were extracted with ethanol and analysed through UV-VIS spectrophotometer for the determination of antioxidant, TPC, and TFC. The antioxidant activity was then analysed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. Total phenolic and total flavonoid contents were then performed using Folin-Ciocalteu and aluminium chloride ($AlCl_3$) methods respectively. The individual bioactive compounds were identified by using Gas Chromatography–Mass Spectrometry (GC-MS) analysis. The antibacterial activity was investigated using disk diffusion method and the results show that Malaysian stingless bee propolis has significant antibacterial activity. The preservation properties of propolis-cornstarch films were evaluated by meat preservation. The presented results indicate that Malaysian stingless bee propolis are rich in phenolics, flavonoids and other active compounds which could potentially be an interesting alternative to existing chemical preservatives and can extend the shelf life of these products.
Keywords**:** *bioactive constituents, foodborne bacteria, meat products, natural preservative, stingless bee*

INTRODUCTION

The native stingless bees are widely distributed in Malaysia and are from *Melipona* sp. and *Trigona* sp., which are also locally known as “lebah kelulut”. According to Malaysian Agricultural Research and Development Institute (MARDI), in 2013, they claimed that stingless bee honey, propolis, and bee pollen were capable of providing numerous medicinal, cosmetic, and food preservative benefits.

Propolis is a complex resinous mixture collected by the stingless bees from plant secretions to protect their hive from invaders and prevent the microbial growth (Toreti et al., 2013). Moreover, the abiotic factors such as geographic origin, types of plant sources, time of collection, and season of the year could also influence the physical characteristic and natural chemical composition of the propolis (Anisava et al., 2019).

The bio-packaging materials manufactured from natural polysaccharides of fruits and vegetables origin would serve as potential material for biodegradable and edible packaging of meat products. Bio-based packaging films with natural additive help to improve the film’s properties and functionality as well as the meat product quality (Suriyatem et al. 2018). Hence, the aim of this research was (1) to address the great potential of stingless bee propolis extract as natural food preservatives to maximize the shelf life of perishable goods, (2) the antimicrobial properties of the propolis extract as biodegradable film for food packaging, and (3) the physical and biodegradability properties of the biodegradable film.

MATERIALS AND METHODS

Propolis ethanolic extract preparation

The samples were pried using a clean hive tool and placed in sealed bags. The crude propolis was crushed using pestle and mortar into powder form. 20 g of crushed propolis was extracted with 70% ethanol. Next, each mixture was heated on the hotplate (Jenway 1000 Series, UK) at 70°C for 5 min and left under dark condition for 24 hours. After extraction, each mixture was filtered through Whatman No. 1 filter paper (Devequi-Nunes et al., 2018).

2,2-Diphenyl- 1-picrylhydrazyl (DPPH) free radical-scavenging assay

The 1.0 mM DPPH stock solution was freshly prepared by mixing 4 mg of DPPH reagent with 100 mL of methanolic solution (0.004%). Next, 1.0 mL of each propolis extract was transferred into a test tube containing 3.0 mL of DPPH stock solution. The mixture was shaken gently to mix them and placed at ambient temperature under dark condition for 30 min. The absorbance measurements were fixed at 517 nm using UV-VIS Spectrophotometer (DU 730 Beckman Coulter). All samples were analyzed in triplicate. The percentage of DPPH scavenging activity was calculated using equation (Frezzini et al., 2019).

GC-MS analysis for bioactive constituents

The determination of alkaloid, terpenoid, lipid, carbohydrate, vitamin C, amino acid contents as well as TFC and TPC was performed using GC-MS analysis. Gas chromatography mass spectrometry was performed by using The Perkin Elmer Clarus 600 GC-MS coupled to Turbo Matrix Headspace Sampler 40, equipped with GC-MS column Elite 5MS (30m x 250mm) of 0.25 µm film thickness was used in this study. The compounds were identified by means of their retention time by comparison of their mass spectra with National Institute Standard and Technology (NIST) library data (Ramnath et al., 2015; Sudheeran et al., 2020).

Bio-packaging preparation

Cornstarch was purchased from TF Value Mart PD (Negeri Sembilan, Malaysia) and glycerol (Merck) plasticizer was purchased from Merck, Malaysia. The propolis-cornstarch films were prepared using solution casting technique as mentioned by (Abotbina et al., 2021). First, 30% ω/ω of plasticizer was added into a beaker containing 180 mL of distilled water. The mixture was then heated using a water bath at 85°C for 20 min. Next, 10 g of corn powder was introduced into prepared solution and heated again for 20 min at the same temperature. The slurry was left cool before casting on petri dish. Each dish was weighed at 45 g to ensure uniformity of film thickness. The dishes were left for 24-48 hours at room temperature, and the film was peeled after completely dried (Zabidi et al., 2022).

Characterization of bio-packaging

The physical properties of bio-packaging such as film moisture content (MC), film thickness, film solubility, and antimicrobial activity were determined as demonstrated by Cheng et al. (2021) and Marichelvam et al. (2019). Biodegradability testing of bio-packaging was then performed, according to Tarique et al. (2021).

Food storage analysis

The meat was bought from Pasar Awam Taman Sri Serdang, Selangor, Malaysia and transferred to the laboratory, at 4°C, on the day of its production. The samples were wrapped using prepared film and stored at 4°C for 14 days. The physical and chemical analysis such as firmness, color, weight, and pH as well as the biological analysis were investigated (Casquete et al., 2016; Natsir et al., 2017). Each trial was performed triplicate.

Statistical analysis

One-way analysis of variance (ANOVA) was carried out to determine significant differences within and between groups. Tukey's test was applied to compare the mean values. Statistical significance was set at $p < 0.01$ by using Statistical Package for Social Sciences (IBM SPSS Statistics Software, 22.0 Version).

RESULTS AND DISCUSSION

Table 1. ***Total phenolic contents (TPC), total flavonoid contents (TFC), DPPH scavenging activity, and IC_{50} of different propolis ethanolic extracts.***

Propolis	TPC (mg/mL)	TFC (mg/mL)	DPPH Scavenging Activity (%)	IC_{50} (mg/mL)
T. apicalis	58.48 ± 0.03	53.88 ± 0.04	33.50	7.17
T. binghami	31.95 ± 0.05	54.75 ± 0.07	31.21	11.72
H. fimbriata	45.35 ± 0.03	59.49 ± 0.56	50.34	5.06

No significant difference detected for both TPC, TFC, DPPH scavenging activity, and IC_{50} since $p>0.05$.

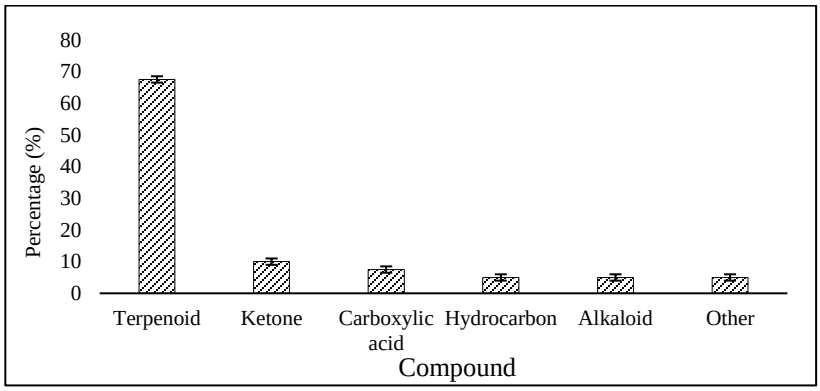

Figure 1. ***Percentage of major compounds detected in propolis ethanolic extracts.***

Table 2. The appearance of propolis-corn starch film.

Propolis	Film Appearance
H. fimbriata	Transparent, flexible, smooth surface, non-sticky, and easy to peel
T. apicalis	Transparent, flexible, small bubble appearance, and slightly sticky
T. binghami	Translucent, slightly elastic, rough surface, and non-sticky

Table 3. The thickness, moisture content, and solubility of propolis-corn starch film.

Propolis	Thickness (μm)	Moisture content (%)	Solubility (%)
H. fimbriata	0.110 ± 0.01	14.39 ± 1.41	12.14 ± 1.74
T. apicalis	0.110 ± 0.01	14.87 ± 1.88	12.97 ± 1.31
T. binghami	0.110 ± 0.01	17.85 ± 1.30	14.73 ± 1.66

The phenolic compounds found in the Malaysian *T. apicalis* propolis may also potentially be used to fight against oxidative stress, though further studies need to be investigated to ensure its potencies (Rosli et al., 2016). According to (Awang et al., 2018), they evaluated 10 species of Malaysian stingless bee propolis extracted using ethanolic solvent and found that hard propolis such as *H. fimbriata* extract contained high phenolic contents, followed by *T. apicalis* and *T. binghami* extracts with 16.2, 13.9 and 5.7 mg/mL, respectively.

Terpenoid was the major group found in the stingless bee propolis extracts, making up of approximately 67.5% of total compounds determined in the samples. According to Rufatto et al. (2017), α-cubebene and spathulenol possessed antimicrobial properties and were commonly used as food preservative agent as well as flavouring agent. Other than that, ketones were also identified in the propolis extracts which mostly used in the oil and gas industry while carboxylic acids could be acted as hypoglycemic agent (Balan et al., 2015). Hydrocarbons and alkaloids were also detected in the samples which responsible for healing therapy as well as other medical benefits (Santos et al., 2020). However, the compositions in propolis vary in each country according to the botanical origin, collecting season as well as the bee species (Mulyati et al., 2020).

Based on Table 3, propolis-corn starch film incorporated with 2% of *H. fimbriata* propolis extract showed lowest moisture content and solubility percentages followed by *T. apicalis* and *T. binghami*. In general, the hydrophilicity of starch-based films was increased with the addition of plasticizers. According to Mutmainna et al. (2019), glycerol contained hydroxyl groups with a strong attraction with water molecules, which enabled the film to hold water and form hydrogen bonds within their structure. However, the presence of beeswax in propolis offers hydrophobic properties that allow propolis to be fixed on various surfaces, decrease direct contact of food with air, and control food's respiration and transpiration rate. The moisture content and solubility percentages decrease when the beeswax concentration increases, which is essential in developing the bio-packaging.

CONCLUSION

This study had successfully showed that propolis collected from Malaysian stingless bee species, *T. apicalis*, *T. binghami* and *H. fimbriata*, are able to produce decent quantity of flavonoids, phenolics as well as antioxidant potential using ethanol as solvent. Besides, it was also noted that there is a great potential of propolis extract to be used as natural preservative in producing bio-packaging. Hence, this study may be able to encourage further investigations and studies on the bioactive constituents of stingless bee propolis which may lead to new discoveries of its composition and possible applications.

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DEVELOPMENT AND ACCEPTABILITY OF WHITE OYSTER MUSHROOM (*Pleurotus florida*)-MORINGA PILI TART

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Abstract: This study was developed to innovate and enhance the nutritional value of Pili tart by supplementing the tart shell with white oyster mushroom and moringa powder. Pili tart shell was prepared from a control recipe and three (3) treatments with varying proportions of flour, WOMP (25%, 20%, 15%), and MP (3%, 2%, and 1%). The physicochemical analysis and proximate analysis were determined through laboratory analysis. While, the organoleptic characteristics (color, texture, taste, and aroma), was evaluated by the group of respondents. In this study, moisture, ash, water activity, and pH content were 6.80%, 1.81%, 0.970 aw, and 5.65aw respectively. Likewise, 7.67% crude protein, 37.78% crude fat, 45.96% total carbohydrates, and 21410.5(J/g) calorie content were found in the product which represents the basic nutrients in innovated pili tart. While, the organoleptic evaluation concluded that the supplementation of 20% white oyster mushroom powder and 2% moringa powder was favorable by the respondents in all attributes. Moreover, supplementation of 15-25% of mushroom and 1-3% moringa powder in Pili tart shell was significantly acceptable by the consumers. Based on the Direct Method of Shelf-life Analysis, Pili tart with white oyster mushroom-moringa can store at room temperature for up to 14 days.
Keywords: White oyster mushroom, moringa, pili tart, organoleptic evaluation

INTRODUCTION

Oyster mushroom is commercially important in the world mushroom market, and several species are grown commercially on a large and small scale in many countries (Adebayo, et al., 2012). It is as one of the popular and valuable food in the century because they have a great nutritional value since they are quite rich in protein, with an important content of essential amino acids and fiber, poor fat but with excellent important fatty acids content (Valverde, et al., 2015). Besides, mushrooms are well-known containing bioactive compounds, such as ergosterol, β-glucans, lentinan, and peroxidase, which possess health functionalities. This shows that the incorporation of mushrooms into food products enhances the nutritional values, as well as the physical properties of the food product (Ho, et al., 2020).

Moringa (*Malunggay*) is one of the popular and abundant food plants in the Philippines that is available in nature. All plant parts are having a remarkable range of functional and nutraceutical properties (Singh et al., 2012). Moreover, leaves of this plant being rich in protein may serve in combating the protein-energy malnutrition for the undernourished population of the world. (Y. Singh et al., 2013). It is excellent source of many vitamins and minerals such, Vitamin B6, Vitamin C, Iron, Riboflavin (B2), Vitamin A (from beta-carotene), Vitamin A (USDA Food Composition Data Base 2019). It is also used as a potential antioxidant, anticancer, anti-inflammatory, antidiabetic, and antimicrobial agent. (Gopalakrishnan, et al., 2016).

The process of drying and powdering has been used in a different fresh food to prevent from early spoilage and can be easily incorporate significant ingredients in the food products. Particularly, Fresh mushrooms being perishable start deterioration instantly within a day after harvest. Because of the extremely delicate nature of fresh mushrooms, they have to be preserved (Majeed, et al. 2017). By this, the incorporation of mushroom and moringa has already been introduced in different bake products. The supplementation of mushroom powder has remarkably high moisture, protein, and total ash contents. The mineral composition increased with increasing mushroom supplementation except for magnesium, manganese, and calcium contents. The contents of all amino acids and B-vitamins analyzed were also increased (Ndung' u, et al., 2015). In terms of the product characteristics, the addition of 15% of mushroom powder level significantly improved the colour, flavor, and texture (Sheikh, et al., 2013). Meanwhile, supplementation of moringa powder significantly increased the fibre, ash, protein, and ether extract while decreasing moisture content (Sengev, et al., 2013). Additionally, the addition of Moringa oleifera leaf powder improves the nutritional quality substantial changes in the functional properties. Furthermore, the use of Moringa oleifera leaf powder, therefore, has the potential to combat protein-energy malnutrition and micronutrient deficiencies in developing countries (Karim, et al., 2015).

The aim of this study was to improve and innovate the pili tart by supplementing the tart shell with white oyster mushroom and moringa powder. This study also involves the processing of the mushroom and moringa into powder, evaluation of the physicochemical properties and nutrient contents (ash, crude protein, crude fat, total carbohydrates, and calorie), evaluation of organoleptic characteristics of each formulation. It was also done to find the best formulation that can be used in improving the nutritional value and quality of a carbohydrate-based food products.

MATERIALS AND METHODS

This study applied the descriptive-experimental method. The Research and Development (R&D) method was also used to meet the objectives of the study. Weighted mean, and ranking technique were used as the Statistical Tool of the study. Weighted mean and ranking techniques were used to determine the level of acceptability of the white oyster mushroom pili tart in terms of color, texture, taste, and aroma.

Collection and selection of Oyster Mushroom (P. florida) and matured Moringa

Harvesting of good quality White Oyster mushroom from CBSUA-Sipocot cultured Mushroom was facilitated to ensure high-quality Mushroom powder was produced. The supply of Moringa was collected from selected Barangays of the Municipality of Sipocot, where the abundant source of Moringa plant can be found. Harvesting of matured Moringa in the morning was facilitated ensuring the freshness of the leaves.

Preparation of Oyster Mushroom Powder

The mushroom was washed in clean running water to remove dirt, sand, and other undesirable materials before being used for the security of sanitation. The clean and fresh mushrooms were sliced into small pieces with a knife and blanched in hot water at 100°C for three minutes. Then was drained and mushrooms were spread in drying trays and dried in a sun for 3 days, 9 hours/day. After cooling to room temperature, the dried mushrooms were ground into powder in a grinder then they were sieved and packaged in polythene bags and stored at room temperature for further use in the preparation of pili tart.

Preparation of Moringa Powder

The harvested leaves were thoroughly washed in running tap water twice to remove the presence of dirt or surface impurities if any and place in a tray for drying. At last, dried leaves were milled and packed into the tight plastic container until further use.

Pili Tart making procedure

The experimentation of the Pili Tart was conducted by following the existing recipe. With the guide of the different studies related to the modification of bake products using white oyster mushroom and moringa powder, the tart shell was prepared from a control recipe and three (3) treatments with varying proportions of flour, white oyster mushroom powder, and moringa powder, other than the three manipulated ingredients, all of the remaining ingredients were constant. 100% All-Purpose Flour was used as the control recipe, and replaced with 25%, 20%, and 15% levels of white oyster mushroom powder, and 3%, 2%, and 1% level of moringa powder, respectively. However, all trials have a constant amount of salt, sugar, butter, and water. Chopped pili nuts were used for the filling of the tart.

Organoleptic Evaluation

Test questionnaires were facilitated to the respondents individually to gather data relevant to the study. Each respondent was given questionnaires together with the different trials to be evaluated based on the benchmarks for evaluation. The pili tart products were evaluated in terms of organoleptic evaluation (color, texture, taste, and aroma) with the use of a Five-point Likert scale.

Physicochemical Properties and Nutritional content analysis of White Oyster Mushroom *(P. florida) Moringa* Pili Tart

To assess the physicochemical properties and nutritional content of the developed white oyster mushroom pili tart, samples were submitted to Food Testing Laboratory, Shared Service Facility (SSF) of the Central Bicol State University of Agriculture – Pili Campus for food analysis. The sample was analyzed by using AOAC methods, IKA Bomb Calorimeter, and by computation.

Shelf-life analysis of White Oyster Mushroom-Moringa Tart shell at room temperature

To determine the shelf-life of the White Oyster Mushroom-Moringa Pili Tart, the Direct Method of Shelf-life analysis guided by the book of New Zealand Food Safety Authority (2005) was used. Five (5) replicates were prepared in the sterilized Petri plates and placed inside a chamber that is subjected for observations.

Tabulation, statistical analysis, and interpretation of data

With the use of weighted mean, ranking technique, and ANOVA, the results of this study were tabulated, analyzed, and interpreted.

RESULT AND DISCUSSION

Physicochemical properties of White Oyster Mushroom *(P. florida)* Moringa Pili Tart

Moisture Content

The moisture content of white oyster mushroom-moringa pili tart was found 6.80%, which considerably within the ranges of the required amount of moisture in bake products with the uses of mushroom powder with the ranges of 1.16 – 32.60% that Salehi (2019). The result indicates that the lower moisture content is due to the incorporation of white oyster mushroom and moringa powder in the preparation of pili tart. Besides, the findings of Farzan et al. (2016), validated the finding that adding mushroom and moringa powder decreases the moisture content. The moisture content of the food has a significant impact on the product's taste, texture, appearance, shape, and weight. More moisture encourages bacteria to proliferate quickly, it plays a crucial role in preserving good food quality and extending shelf life.

Ash Content

The ash content of white oyster mushroom-moringa pili tart was found at 1.81%, this also in close agreements in the study of Salehi (2019), were found the range of 0.84 – 3.47% of ash contents in different baked products containing 20% of oyster mushroom powder. This indicates that pili tart supplemented with mushroom-moringa powder can be a good source of minerals.

Water Activity

Water activity refers to water in food that is not observed in food molecules and can facilitate the growth of bacteria, yeast, and molds (Han et al., 2016). It can be controlled through drying and the addition of sugar, which become significant in the present study. The white oyster mushroom-moringa pili tart contains 0.970 aw. The result found that it is higher than the typical water activity of 0.95 for some foodstuffs stated by (Dairy Research & Information Center, 2017**)**. This indicates that the water activity of the present study may compromise the other components of the white oyster mushroom-moringa pili tart and affect the longevity of the shelf life.

pH level

The analysis found that the pH level of white oyster mushroom-moringa pili tart is 5.65. The value of pH can be a major factor affecting the appearance, texture, flavor, nutritional content, and even the safety of the food. pH values were greater than 5.6, which makes the food susceptible to bacterial spoilage and the possible growth of pathogens (Rahman, 2020). The interaction of water activity and pH on toxin production by *Clostridium botulinum* was found in the study of Rahman (2020) which stated that the toxin in food can be detected at day 14 with 0.973 aw and with a pH value of 5.50. A pH value of 4.0 to 5.8 is recommended for baked bread to prolong its shelf life (Sper Scientific Direct, 2021). This implies that the present study is within range of the recommended pH for baked products.

Organoleptic Evaluation of White Oyster Mushroom-Moringa Pili Tart

In the present study, the supplementation of 20% WOM powder and 2% Moringa powder was favorable of the respondents. In terms of color, the respondents were favored the brown color as compared to the darker and lighter color of the pili tart. It is obvious that the interactions of treatments showed a significant effect on the color of the crust from light brown to dark brown. This is consistent with Majeed et al. (2017), who reported that the color value varied significantly due to changes in mushroom powder supplementation amount, with crust color of light brown darkening progressively with increasing level of mushroom powder. In terms of texture, crumbly texture of the pili tart was favored by the respondents. The result proved that the 20% of mushroom powder supplementation was favorable texture of the respondents as the rates decreased when the supplementation of mushroom powder was more or less than the 20%. The score for texture gradually decreases as the supplementation level of mushroom powder increases in flour (Majeed, et al. 2017). Furthermore, in terms of aroma, respondent prefers the strong aroma of mushroom powder complemented with the sugary aroma of the filling. However, in terms of taste, it is been revealed that the increased supplementation of white oyster mushroom powder with the addition of the bitter taste of moringa powder will results in the strong woody-bitter taste of the pili tart.

Moreover, supplementation of 15-25% of mushroom and 1-3% moringa powder in Pili tart shell was significantly acceptable by the consumers.

Nutritional content of White Oyster Mushroom *(P. florida)* Moringa Pili Tart

The analysis presents the amount of ash, crude protein, crude fat, total carbohydrates, and calorie content.

The result reveals that crude fat (37.78) and total carbohydrates (45.96) are the major components of white oyster mushroom-moringa pili tart. While ash and crude protein was found 1.81 (Protein factor = 5.70) and 7.67 respectively. These results corroborate with the study of Salehi (2019) who summarized the effect of dried mushroom powder on the chemical properties of bakery products and found them ranging from 0.53 – 3.58 of ash, and 6.50 – 15.55 of protein, wherein the present study is within the results range. While fat found ranging from 1.68 - 23.08 which is lower than the result of the present study. And carbohydrates found ranging from 46.47 – 68.59 which is higher than the result of the present study. This implies that the addition of white oyster mushroom and moringa powder into carbohydrate-based food products improved the nutrients ingredients and quality of the product. The analysis also revealed that white oyster mushroom-moringa pili tart contains 21410.5 J/g of calories (5.11381 kcal).

This implies that the use of white oyster mushroom and moringa powder in a baked product can be a good source of energy.

Shelf life of White Oyster Mushroom-Moringa Pili Tart in room temperatures for storing

Using the Direct Method of Shelf-life Analysis guided by the book of New Zealand Food Safety Authority (2005), the shelf-life of White Oyster Mushroom-Moringa Pili Tart was analyzed in 25°C room temperature with an average total relative humidity of 51.33%. A total of five (5) replicates were undergone sterilization and are placed in Petri plates then placed in the chamber to ensure sanitation during the process of observation. All five replicates were observed in the chamber with 24 hours intervals for 20 data points (20 days). The observation stopped when the physical changes occurred.

Based on the ocular observation and data gathered, the White Oyster Mushroom-Moringa Pili Tart has been observed that will only last at least 14 days (2 weeks) at room temperature. On the 16th day of the observation period, the development of molds was seen on the surface of the tart of each sample.

The result was supported by the study of **Rahman (2020),** which found out that the toxin production by *Clostridium botulinum* can be found at day 14 in food having 0.973 aw and with a pH value of 5.50. This indicates that the growth of the bacteria can be seen in a minimum of 14 days. This implies that the amount of water activity (0.970), and pH (5.65) found in White Oyster Mushroom-Moringa Pili Tart with the addition of the amount of moisture (6.80) compromise the longevity of the shelf life of the product.

CONCLUSIONS

Supplementation of 15% - 25% white oyster mushroom and up to 3% of moringa powder in pili tart create significant impact in the physicochemical properties of the product. Moreover, the mixture of the powders contributes to improve the nutrients and the quality of the product. The study concluded that the level of supplementation of the two powder is highly acceptable by the consumers in terms of organoleptic characteristics. Further, 20% mushroom powder and 2% moringa powder in the ideal formulation of the product.

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ULTRASOUND-ASSISTED EXTRACTION AND CHARACTERIZATION OF PECTIN FROM JACKFRUIT (*ARTOCARPUS HETEROPHYLLUS*) WASTES

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Abstract: Jackfruit (*Artocarpus heterophyllus*) is a tropical fruit that is popular in many parts of the world, however, its peels and seeds are often discarded. Recent research has shown that jackfruit wastes (JFW) contain a significant amount of pectin. This study aimed to extract the pectin from different parts of JFW (JFW1 – rags; JFW2 – rinds; JFW3 – rags and rinds) using ultrasound-assisted extraction (UAE). The physicochemical and structural characteristics of JFW pectin were investigated and compared with commercial pectin. The yield of JFW pectin ranged from 22.80% to 30.01%. For ash content, JFW pectin reported values of 4.32% to 6.06%. The JFW pectin was classified as high methoxyl pectin (HMP) with a degree of esterification between 50.07% to 70.08%. Among the JFW pectin samples, only JFW2 exhibited the galacturonic acid content (68.29%), which met the criterion for food additive pectin. The gel strength of JFW pectin was reported between 63.5 g to 79.5 g, which was higher than the commercial pectin (58.67 g). Overall, the utilization of JFW as a source of pectin has the potential to provide a sustainable and eco-friendly solution to the growing demand for this valuable ingredient.
***Keywords*:** Jackfruit wastes, pectin, extraction, ultrasound, characterization

INTRODUCTION

Jackfruit (*Artocarpus heterophyllus*) is a popular fruit crop from the Moraceae family, which thrives in tropical countries. In Malaysia, it is also well-known as 'nangka' and its pulps are consumed either fresh or processed into various food products due to their high nutritional value and appealing flavor. However, approximately 60% of a jackfruit consists of non-edible portions (exterior rinds), and these unused parts are usually discarded as waste (Begum et al., 2017; Kalse & Swami, 2022). A significant amount of jackfruit waste (JFW) is generated during the jackfruit fruiting season, which could lead to an increase in waste being disposed of in landfills (Lim et al. 2016; Tahir 2012). However, there is an opportunity to minimize agricultural waste by exploring its potential transformation into value-added products.

The JFW have been identified as a valuable source of pectin in various studies (Koh et al., 2014; Leong et al., 2016; Sundarraj et al., 2018). Pectin finds common application in the food industry, where it is added to jams and jellies as a gelling agent, contributing to their gel-like structure. Additionally, it serves as a thickening agent, emulsifier, and stabilizer in various food products (Jong et al., 2023). The conventional commercial sources of pectin are citrus peels and apple pomace, which are not indigenous fruit crops to Malaysia. Therefore, the utilization of JFW in pectin production has the potential to decrease the production expenses associated with pectin-enhanced products.

The extraction methods used can significantly impact both the yield and quality of pectin. The prevailing conventional approach for industrial pectin production involves direct boiling in the presence of acid. However, this method is time-consuming and prolonged heat treatment can lead to pectin degradation. A variety of novel extraction methods, including ultrasound-assisted extraction (UAE) have been explored to address the limitations of the conventional techniques. UAE offers the advantages of higher extraction efficiency, shorter extraction time, and reduced solvent usage due to the ultrasonic cavitation (Grassino et al., 2016). This phenomenon enhances the solvent's penetration into the pectin matrix (Ganesh Moorthy et al., 2017; Koh et al., 2014; Zouambia et al., 2014). This study aimed to extract pectin from different parts of JFW (both rags and rinds) using UAE. The physicochemical and structural characteristics of JFW pectin were investigated and compared with commercial pectin.

MATERIALS AND METHODS

Raw materials

The jackfruit waste (JFW) of honey jackfruit J33 was collected from Fresh Boulevard, a grocery store located in Subang Jaya, Selangor. Control was the commercial pectin purchased from House of Ingredients, Bandar Sri Damansara.

Preparation of jackfruit waste powder

The JFW (JFW1 – rags; JFW2 – rinds; and JFW3 – rags and rinds) were initially washed, blanched, and cooled. Subsequently, the JFW were subjected to drying at 80 ℃ for 13 hours. The dried JFW was then milled into a powder form.

Extraction of pectin by ultrasound-assisted extraction method

Five grams of each sample were placed into a 500 mL Schott bottle. Subsequently, 0.1 N sulfuric acid was added at 1:35 (g/mL) solid-to-solvent ratio. The mixture was then heated in an ultrasonic bath (FB15055, Fisherbrand, UK) with frequency of 37 kHz at 80 ℃ for 35 minutes. The hot acid extracts were filtered through muslin cloth and the filtrate was left until cooled to the room temperature (Sundarraj et al., 2018).

Purification of pectin

Purification of pectin was done using the method of Koh et al. (2014) with slight modifications. The cooled filtrate was added with 95% ethanol with 1:2 filtrate to ethanol (v/v) ratio for pectin precipitation. The mixture was then stirred magnetically for 30 minutes under room temperature, followed by storage in chiller at 4 ℃ for 2 hours. Next, the coagulated pectin was filtered and cleaned twice with 75 mL of 70% ethanol followed by 75 mL of 95% ethanol until the pectin turned colorless. Lastly, the pectin was dried at 37 ℃ for 18 hours.

Pectin yield

The pectin yield was calculated according to the formula below:

$$\text{Pectin yield (\%)} = \frac{\text{Weight of dried pectin (g)}}{\text{Weight of JFW powder sample (g)}} \text{ x } 100\%$$

Ash content

The ash content of the extracted pectin was determined using dry ashing method (AOAC,1980).

$$\text{Ash content (\%)} = \frac{\text{Weight of crucible with ash (g)} - \text{Weight of empty crucible (g)}}{\text{Weight of JFW powder (g)}} \text{ x } 100\%$$

Galacturonic acid content

The galacturonic acid (GalA) content of the samples was conducted by using meta-hydroxy-diphenyl method (Begum et al., 2017). The absorbance of the samples was read at 525 nm against the control. GaLA content was determined by using a standard curve of galacturonic acid.

Equivalent weight

The equivalent weight (EW) of the extracted pectin was determined through titration with NaOH to pH 7.5 using phenol red indicator (Girma & Worku, 2018). The volume of 0.1 N NaOH titrated was recorded and the neutralized solution was kept for the following analysis. Equivalent weight was calculated using following formula:

$$\text{EW} = \frac{\text{Weight of pectin (g)}}{\text{Volume of NaOH titrated X normality of NaOH}}$$

Methoxyl content

The methyl (MeO) content of the samples was determined by saponification of the pectin sample and titration of the liberated carboxyl groups (Islam et al., 2023). The volume of 0.1 N NaOH titrated was recorded for the calculation using formula below:

$$\text{MeO (\%)} = \frac{\text{Volume of NaOH X Normality of NaOH X 3.1}}{\text{Weight of sample (g)}}$$

Anhydrouronic acid content

The EW and MeO content were used to calculate the anhydrouronic acid (AUA) content of pectin by using the following formula:

$$\text{AUA (\%)} = \frac{(176 \text{ X } 0.1\text{z X } 100) + (176 \text{ X } 0.1\text{y X } 100)}{Weight\ of\ sample\ (g) \text{ X } 1000}$$

Degree of esterification

Using the values of MeO content and AUA content, the DE of pectin sample was calculated as follows:

$$DE\ (\%) = \frac{176\ X\ MeO\%}{31\ X\ AUA\%} \text{X } 100$$

Gel strength

Gel strength of the pectin samples was conducted based on the method of Jiang et al. (2012) with some modification. Pectin gel was prepared by adding sucrose into 2% (w/v) pectin solution for 60% (w/v) sugar concentration under heating. Gel strength analysis was then performed by using a texture analyzer (Brookfield CT3, USA). The hardness of gel was derived from the analysis of the stress–strain curve.

Statistical analysis

All the data were analyzed using IBM Statistical Package for Social Science (SPSS) (Version 23.0, USA) software.

RESULTS AND DISCUSSION

Pectin yield, ash content, and galacturonic acid content

The pectin yields for JFW1, JFW2, and JFW3 were recorded as 30.01%, 22.80%, and 24.37%, respectively (Table 1). These findings indicate that the ultrasound-assisted extraction (UAE) employed in this study resulted in higher yields compared to the conventional acid extraction method for pectin extraction from jackfruit rinds (14.82% to 18.59%) and chempedak fruit rinds (17.62% to 20.50%), as reported by Leong et al. (2016). The ultrasound treatment induces a cavitation effect on the samples, leading to the expansion of pores. This phenomenon facilitates the entry of solvents into plant cells through diffusion, thereby releasing

pectin from the inner plant cells into the surrounding medium (Ganesh Moorthy et al., 2017). In comparison to other fruit wastes, optimal yield of 20.92% pectin was obtained by Yousuf et al. (2018) from citrus peel and Xu et al. (2014) reported the yield of pectin from grapefruit peel up to 26.74% when the optimal condition of UAE was applied.

The ash content indicates the inorganic impurities present in pectin, with lower ash content is preferable for gel formation (Islam et al., 2023). Based on Table 1, the ash content of control was lower than the JFW pectin with no significant difference ($p > 0.05$). Ismail et al. (2012) stated that ash content of a good quality pectin with desirable gel formation should not be more than 10%. All the JFW pectin samples produced in this study were in good agreement with the criterion. The galacturonic acid (GalA) is the fundamental component in pectin and the purity of pectin is defined by higher GalA and lower ash content (Koh et al., 2014). Table 1 shows that the GalA content of JFW3 and control was significantly different ($p < 0.05$). Among the samples, only JFW2 pectin and control achieved more than 65% of GalA content which is the criteria for pure pectin.

Table 1. Yield, ash content, and galacturonic acid content of JFW pectin and control

Samples	Pectin yield (%)	Ash content (%)	GalA content (%)
JFW1	30.01 ± 0.61^{a}	4.32 ± 1.50^{a}	50.40 ± 9.46^{ab}
JFW2	22.80 ± 0.60^{b}	6.06 ± 2.58^{a}	68.29 ± 11.26^{ab}
JFW3	24.37 ± 0.12^{c}	4.73 ± 1.97^{a}	46.71 ± 6.26^{b}
Control	ND	2.61 ± 0.80^{a}	74.44 ± 11.77^{a}

Mean value from triplicate mean±standard deviation. Values with different superscript^{abc} in the same column are significantly different ($p < 0.05$). ND = Not done.

Equivalent weight, methoxyl content, anhydrouronic acid content, degree of esterification

The equivalent weight (EW) of JFW pectin ranged from 705.26 to 1950.88 as presented in Table 2. JFW1 reported a significantly higher ($p < 0.05$) EW (1950.88) than JFW2, JFW3, and control. Pectin with higher EW would have better gel forming effect, whereas lower EW could indicate a higher partial degradation of pectin. The decreased or increased of the EW might also depend on the amount of free acid (Rose & Abilasha, 2016). The methoxyl (MeO) content of pectin was determined as it is a key factor in controlling the setting time of pectin and its ability in gel formation (Mohamed, 2016). Table 2 shows that the MeO content of JFW2 pectin (4.79) was the highest, followed by JFW1 and JFW3 pectin. However, these values were significantly lower ($p < 0.05$) than that of commercial pectin (6.93). Grassino et al. (2016) suggested that the high temperature applied during UAE may reduce the MeO content of pectin through thermal degradation. The maturity of the fruit is also a factor affecting the MeO content of pectin. An increase in maturity may lead to a decrease in MeO content due to ripening, which results in an increase in the sugar content of the fruits (Sirisakulwat et al. 2008).

The anhydrouronic (AUA) content of commercial pectin is suggested to be not less than 65%, calculated on an ash and moisture-free basis in meeting the legislative requirement of the Food and Agriculture Organization (FAO) and Food Chemical Codex 1996 (FCC) for food application (Jong et al., 2023). The AUA of both the control and JFW pectin shown in Table 2 was lower than 65%, as the weight included moisture and ash. In this finding, the AUA content of JFW2 pectin and control was significantly higher ($p < 0.05$) than that of JFW1 and JFW3 pectin, which was approximately consistent with the results of GalA determination. Ismail et al. (2012) stated that low AUA content indicates that the pectin might contain a high amount of protein or sugar. Pectin can be categorized into two types based on the degree of esterification (DE), which are high methoxyl (HMP) (DE > 50%) and low methoxyl (LMP) (DE < 50%) (Jong et al., 2023). In this study, the DE of JFW pectin was reported to range from 52.07% to 70.08%, which are classified as HMP (Table 2). Thus, JFW pectin is suitable for food applications such as making jam or marmalade, which require high sugar content and a low pH for gel formation. Similar DE ranges of jackfruit rind pectin were also reported by Liang et al. (2016), ranging from 72.82% to 75.82%.

Gel strength

As shown in Table 2, the gel strength of pectin extracted from different JFW did not differ significantly ($p > 0.05$). Possessing HMP properties, both the sampled pectin and the control formed a gel successfully under conditions of low pH (pH 2.5), low water activity, and high sugar concentration (60%, w/v). The stability of the gel structure is maintained by hydrophobic interactions and hydrogen bonding. However, the gel strength of the commercial pectin was the lowest, despite its higher DE. This disparity may be attributed to the pH conditions during gel formation. Yurliati (2011) reported that weaker gels were produced at pH levels above 2.5, and the maximum gel hardness was achieved at pH 1.7 for HMP. A lower pH can reduce the quantity of negative charges, thereby increasing attraction and decreasing the repulsive force between pectin molecules. This promotes the formation of hydrophobic interactions between pectin's ester groups and hydrogen bonds (Jiang et al., 2012).

Table 2. Equivalent weight, methoxyl content, anhydrouronic acid, degree of esterification (DE), and hardness of JFW pectin and control

Samples	EW	MeO (%)	AUA (%)	DE (%)	Hardness (g)
JFW1	1950.88 ± 494.82^{a}	3.88 ± 0.45^{bc}	31.43 ± 0.45^{b}	70.08 ± 7.18^{a}	79.50 ± 7.05^{a}
JFW2	705.26 ± 10.97^{b}	4.79 ± 0.42^{b}	52.15 ± 2.80^{a}	52.07 ± 1.79^{c}	63.00 ± 31.19^{a}
JFW3	1149.62 ± 328.51^{b}	3.47 ± 0.21^{c}	36.79 ± 5.53^{b}	56.55 ± 6.41^{bc}	65.17 ± 35.56^{a}
Control	933.78 ± 21.26^{b}	6.93 ± 0.69^{a}	58.19 ± 3.65^{a}	67.51 ± 2.45^{ab}	58.67 ± 10.02^{a}

Mean value from triplicate mean±standard deviation. Values with different superscript[abc] in the same column are significantly different ($p < 0.05$).

CONCLUSIONS

In conclusion, this study focused on the extraction and characterization of pectin from three different JFW using the UAE method. The results highlighted that JFW could serve as promising sources for industrial pectin production, given their high yield and comparable quality to commercial pectin. All the JFW pectin exhibited HMP characteristics, meeting the criteria for good-quality pectin with ash content of less than 10%. Moreover, these samples demonstrated superior gelling properties compared to commercial pectin. For future research, it is advisable to explore pectin extraction from JFW at lower temperatures using the UAE method to minimize the pectin degradation and reduced MeO content. Overall, the utilization of JFW as a pectin source holds the potential to offer a sustainable and eco-friendly solution to the increasing demand for this valuable ingredient.

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ASSESSMENT OF FLAVOR CHARACTERISTICS OF NEW OKINAWAN PINEAPPLE BREEDING LINES BY GC-MS-BASED ELECTRONIC NOSE, ALCOHOL-ACYLTRANSFERASE ACTIVITY, AND GLYCOSYLATED-VOLATILE MEASUREMENTS

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Abstract: The recent increase in demands for pineapple obliges development of new varieties with superior flavor quality. The study aimed to evaluate flavor characteristics of new Okinawan pineapple breeding lines by GC-MS-based electronic-nose (e-nose), alcohol-acyltransferase (AAT) activity, and glycosylated-volatiles measurements. Volatile profiles of fruit edible parts of five new breeding lines from experimental farm were observed by GC-MS-e-nose, and their total scanned ions data were visualized through multivariate statistical plots. Crude enzymes of fruit flesh were extracted, and their *in-vitro* AAT activities were then determined by solid-phase microextraction (SPME)-GC-MS. The glycosides were collected through solid-phase extraction, hydrolyzed by *β*-glucosidase, and the released volatiles were measured by SPME-GC-MS. As the results, three biological replicates of each line with closed volatile profile were identified via GC-MS-e-nose principal component analysis, and further compositional examination confirmed their constituents. Moreover, AAT activity of 2-methylbutyl acetate varied among lines. Additionally, twelve hydroxyl-group compounds, including chavicol (medicinal-herbal aroma), geraniol (sweet-floral), and linalool (floral-citrus), were released from the glycosides. These results provide important information regarding volatiles profiling, esterification potential, and bounded-aroma resources in Okinawan pineapple for fresh consumption and agroindustrial processing.
***Keywords*:** pineapple, GC-MS-e-nose profiling, alcohol-acyltransferase activity, glycosidically bound volatile, glycoside

INTRODUCTION

Pineapple is a highly nutritious tropical-subtropical fruit with unique flavor characteristics. Volatile components greatly contribute to the aroma quality of pineapple fruit by providing distinct sensory properties that are sweet, fruity, pineapple-like, coconut-like, and so on (Asikin et al., 2022). Esters are important volatile aroma components in many fruits, including pineapple, and understanding mechanism underlying ester formation has been assigned as a key interest in breeding studies for fruit flavor improvement (Kamiyoshihara et al., 2020). On the other hand, glycosylated volatiles, which are present in pineapple, can be considered as hidden supplies of bounded-aroma resources, and releasing these aglycones can potently alter the aroma profiles of the fruits (Asikin et al., 2022).

Okinawa, the most southern prefecture of Japan, is the only subtropical region that largely farms pineapple in the country, and its produce supports local agroindustry businesses and attracts ecotourism activities. The recent increase in demands for Okinawan pineapple obliges development of new varieties with superior flavor quality that meets consumer acceptances, and understanding flavor characteristics of newly developed breeding lines are key factors for agrobusiness players in determining their potential applications. The study aimed thus to evaluate flavor characteristics of new Okinawan pineapple breeding

lines by GC-MS-based electronic-nose (e-nose), alcohol-acyltransferase (AAT) activity, and glycosidically bound volatile compounds measurements.

MATERIALS AND METHODS

The ripen fruits of Okinawan pineapple cultivars and new breeding lines were collected during 2020–2021 harvest seasons from a farm at Okinawa Prefectural Agricultural Research Center, Nago, Japan. Upon arrival at the laboratory, the fruits were immediately and cut, and the fruits' edible parts were stored at −30 °C prior to analysis. Volatile profiles of fruit edible parts of 'N67-10' cultivar (control cultivar; Hawaiian Smooth Cayenne clone) and five new breeding lines, namely, 'Okinawa No. 22', 'Okinawa No. 25', 'Okinawa No. 26', 'Okinawa No. 27', and 'Okinawa No. 28' were determined by GC-MS-e-nose analysis (Asikin et al., 2018). Volatiles of fruit flesh (2 g) were extracted using G1888 headspace sampler (Agilent J&W, Santa Clara, CA, USA) and their total scanned ions were assessed by GC-MS (Agilent 7890A GC-5975C MS). The acquired MS data was converted to a chemometric dataset, and then was visualized through multivariate statistical plots. The volatile compounds were isolated using solid-phase microextraction (SPME)-Arrow (120 µm DVB/PDMS, Restek, PA, USA), and the volatile composition was analyzed using GC-FID/MS analysis (Agilent 7890B GC/7890A GC-5975C MS).

Crude enzymes of pineapple fruit flesh (2 g) were extracted using PD-10 desalting column (Cytiva, MA, USA), the enzyme extract was collected in a buffer solution containing glycerol, Tris-HCl, and β-mercaptoethanol (Kamiyoshihara et al., 2020). The enzyme was then reacted with acetyl-CoA and alcohol precursor, i.e. 2-methyl butanol, at 30 °C for 30 min. Afterward, the AAT activities were determined by SPME-GC-MS (Agilent GC 7890B-5977A MSD). The SPME fiber (50/30 µm DVB/CAR/PDMS, Supelco, PA, USA) was used to extract the esters at 30 °C for 30 min, and nonyl acetate (0.003 mg/mL, 10 µL) was used as internal standard. The protein content was determined using Lowry method (RC DC™ Protein Assay, Bio-Rad, CA, USA).

Glycosidically bound volatile compounds of pineapple juice (4 mL) were extracted using Oasis HLB 3cc/60 mg Vac cartridge (Waters, Milford, MA, USA), and methanol was applied to elute glycosides from the sorbent (Asikin et al., 2022). The methanol extract was then evaporated using a centrifugal evaporator at 40 °C and dried under a gentle nitrogen stream. Afterward, the glycosides were hydrolyzed by Rapidase Revelation Aroma solution (20 mg/mL in citric buffer, containing β-glucosidase, enzymatic activity ≥ 4000 u/g) at 40 °C for 2 h. The released volatiles from were extracted using SPME fiber at 40 °C for 30 min, and were then measured by GC-MS (Agilent GC 7890B-5977A MSD). 2-Methyl-1-pentanol (0.03 mg/mL, 20 µL) was used as internal standard.

RESULTS AND DISCUSSION

GC-MS-e-nose provided the differentiation of volatile profiles of 'N67-10 cultivar' and new breeding lines via multivariate statistical analysis, i.e., principal component analysis plot (Figure 1). The score plot can be effectively used as a selection tool to indicate three biological replicates of fruits from each cultivar or breeding line with comparable volatile profiles for further compositional investigation. The composition of volatile aroma compounds varied among the five breeding lines, and they were much different from 'N67-10' cultivar (Table 1). Thoroughly, 'Okinawa No. 22' contained more esters and ketones, while 'Okinawa No. 27' possessed a noteworthy mixture of esters, alcohols, terpenes, and ketones. These distinct volatile proportions might affect the aroma profiles of the pineapple fruits; thus, each new breeding line could have different potent practical uses, yet further sensory evaluation is required.

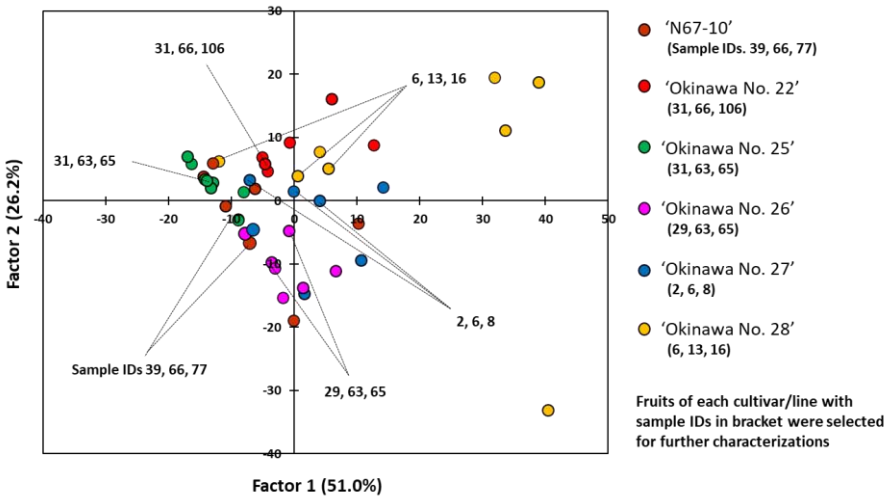

Figure 1. Principal component score plot of the volatile profiles of 'N67-10' cultivar and new breeding lines, obtained by GC-MS-e-nose analysis.

Table 1. Relative concentration (%) of volatile component groups of 'N67-10' cultivar and new breeding lines

Component group	'N67-10'	'Okinawa No. 22'	'Okinawa No. 25'	'Okinawa No. 26'	'Okinawa No. 27'	'Okinawa No. 28'
Ester	26.04	65.41	31.73	39.94	29.43	68.79
Alcohol	32.31	3.67	16.24	18.90	11.74	5.95
Aldehyde	6.43	3.65	12.69	1.94	2.77	1.68
Terpene	11.20	3.27	1.79	1.14	17.12	6.17
Ketone	2.47	11.82	2.33	6.43	10.56	5.18
Hydrocarbon	nd	0.48	nd	nd	nd	0.28

Each value is expressed as the mean of three replicates; nd.: not detected.

The *in-vitro* esterification activities of AAT, particularly for 2-methylbutyl acetate, varied among lines (Figure 2a). There was a positive association between AAT activity and ester content (Figure 2b), indicating that biochemical reactions had occurred during ester formation; thus, more studies are needed to reveal aroma development in pineapple. Additionally, twelve hydroxyl-group compounds, including chavicol, eugenol, geraniol, and linalool, were released from the glycosides (Table 2). The amounts of these substances were higher in new breeding lines than 'N67-10' cultivar; thus, careful consideration must be given when releasing the aglycones in food products.

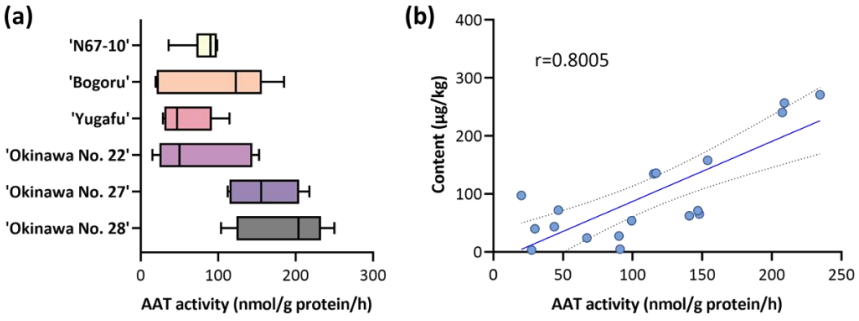

Figure 2. a) Box plot of AAT activity of 2-methylbutyl acetate of Okinawan pineapple cultivars and new breeding lines; b) Pearson's correlation plot between AAT activity and 2-methylbutyl acetate content.

Table 2. Released volatiles (µg/100 mL) from glycosides of 'N67-10' cultivar and new breeding lines

Compound	Aroma traits[1]	'N67-10'	'Okinawa No. 22'	'Okinawa No. 25'	'Okinawa No. 26'	'Okinawa No. 27'	'Okinawa No. 28'
Chavicol	Medicinal, herbal (–)[2]	38.95	104.72	441.55	274.08	237.79	37.32
Eugenol	Spicy, woody (1)	nd	5.83	19.46	9.26	14.98	0.44
Geraniol	Sweet, floral (7.5)	0.60	3.19	3.22	3.11	14.20	1.58
Isoeugenol	Spicy, clove (10)	nd	tr	13.16	tr	17.39	nd
Linalool	Floral, citrus (1.5)	nd	2.93	1.44	tr	3.10	0.74
Benzyl alcohol	Rose, phenolic (5500)	tr	5.12	tr	25.36	44.21	nd
4-Methylphenol	Phenolic, smoky (20)	nd	0.97	1.50	3.67	5.47	1.01
2-Methyl-1-butanol	Fusel, ethereal (6000)	nd	1.53	4.11	1.52	4.53	0.49
3-Methyl-1-butanol	Fusel, alcoholic (250)	nd	1.99	15.03	8.71	24.31	2.93
3-Methyl-2-butene-1-ol	Fruity, lavender (–)	nd	0.61	nd	nd	1.63	nd
1-Hexanol	Fusel, oily (200)	tr	0.33	2.41	tr	tr	5.72
2-Ethyl-1-hexanol	Citrus, fresh (–)	1.97	0.57	0.43	tr	tr	0.26

Each value is expressed as the mean of three replicates; nd.: not detected; tr.: trace amount (<0.01 µg/100 mL).
[1]Retrieved from The Good Scents Company Information System (2023).
[2]Odor threshold in µg/kg (van Gemert, 2011).

CONCLUSIONS

GC-MS-e-nose effectively screened pineapple fruits from a cultivar or breeding line with comparable volatile profiles. Some of the breeding lines possessed distinct aroma proportions that could promote their unique practical applications compared to the current available pineapple cultivars. Moreover, the new breeding lines had different levels of esterification activities that reflect variations in the esters amount; thus, aroma composition. However, careful consideration must be given to the usage of the released aglycones with undesirable strong odors in processed pineapple, which may alter the overall flavor quality of the final products. The outcomes of this study, thus, provide important information regarding volatiles profiling, esterification potential, and bounded-aroma resources of new Okinawan pineapple breeding lines for fresh consumption and processing into various foods and beverages.

This research was supported by the research program on development of innovative technology grants from the Project of the Bio-oriented Technology Research Advancement Institution (BRAIN), grant number JPJ007097.

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PROFESSIONAL COMPETITIVENESS ROADMAP FOR THE FOOD TECHNOLOGY PROFESSION IN THE PHILIPPINES CY 2023-2028

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Abstract:Several higher education institutions in the Philippines offer food science and technology courses with the goal of producing a critical mass of graduates who can serve in the food industry. However, there is a growing concern about whether these institutions are generating graduates with adequate skills and knowledge necessary to meet the demands of society, particularly given the rapidly evolving food systems and environment. An act regulating the practice of Food Technology (FT) in the Philippines was signed into law in 2018 and the first licensure examination will be conducted in 2023. Hence, it is essential to gauge the career advancement of Professional Food Technologists as Licensure Examination and Continuing Professional Development programs are implemented. This paper outlines the Professional Competitiveness Roadmap for the FT Profession in the Philippines. The proposed plan period is 2023-2028, aligned with the Medium-Term Philippine Development Plan (MTPDP) of the current administration. The MTPDP will provide the platform and mechanisms for the generation of resources that will support the implementation of the roadmap. This paper details the objectives, key results, actions to be undertaken, responsible entities and timelines, and the indicators to assess performance in the process of enhancing the competitiveness of FT professionals.

Keywords: Competitiveness Roadmap, Food Technology, Licensure Examination, Philippines, Professional Food Technologists

INTRODUCTION

The UNESCO prescribes 380 researchers, scientists, and engineers per million population to support the socio-economic development of a country. For the Philippines, with a population of 110 million in 2020, this translates to a necessity of 41,800 professionals. Targeting 10% of this figure for food scientists and technologists (FSTs), given that the food and beverage processing industry contributes 43% to the country's total manufacturing output (Philippine Statistics Authority, 2019), yields an estimate of 4,000 FSTs. With 110M population, the Philippines requires 4,400 FSTs.

Several higher education institutions (HEIs) in the Philippines offer bachelor's degree in Food Technology (BSFT). However, there is a growing concern about whether these institutions are adequately preparing graduates to meet societal demands. Licensing is a significant concern for food technologists, as it restricts their ability to sign important technical documents and limits access to valuable job assignments. Recognizing the need for professionalizing the field, the Philippine Food Technology Act (RA No. 11052) was enacted in 2018, regulating the FT practice and establishing the Board of FT (PRBFT).

The Acting Chairperson of the Professional Regulation Commission (PRC) emphasized the need to develop and implement Professional Competitiveness Roadmaps as a crucial tool for development. This paper presents the goals, strategies, and timelines to enhance the competitiveness of FT professionals. It aligns with the Medium-Term Philippine Development Plan (MTPDP) of the new administration, which supports resource generation for the Roadmap's implementation. It is important to gauge the career advancement of Professional Food Technologists as Licensure Examination and Continuing Professional Development (CPD) programs are implemented.

Following the Strategic Planning framework and Process developed by the Department of Science and Technology Region XI (DOST XI), this study developed the Professional Competitiveness Roadmap for the FT Profession in the Philippines through a Situational Analysis utilizing SWOT and DEEP LIST Analysis tools. Based on the analysis of results, the Roadmap was constructed, defining the goal and major steps to achieve it.

MATERIALS AND METHODS

This study employed a mixed-methods approach: data collection and analysis. The data utilized in this study was obtained through inspections and monitoring performed by PRBFT as of February 2023.

1.1 Situational Analysis:

The Philippines' food and beverage (F&B) processing industry has surged, quadrupling to $27.1 billion from 2009 to 2013, and contributing 50% to the country's manufacturing output. The sector consists of various segments including beverages, coffee, condiments, dairy, fats, bakery, fruits, meats, seafood, snacks, and sugar products. Notably, both micro and medium-sized businesses, as well as large corporations, operate within the approximately 500 F&B processors registered under the Philippine Food and Drug Administration (PSA, 2019).

1.2 Undergraduate Education:

There are 57 HEIs offering BSFT, consisting of 48 state universities and colleges (SUCs) and 9 private HEIs. Each HEI produces 10 graduates on average, resulting in approximately 500 BSFT graduates annually nationwide. Among these inspected HEIs, only 11 are fully compliant with the policies, standards, and guidelines (PSGs) for the BSFT as promulgated in CHED Memorandum Order No. 07 series of 2019. Less than half of the HEIs have the required physico-chemical, microbiological, sensory, and processing laboratory facilities.

In terms of the qualifications of Program Chair/Coordinator and Faculty, only 69% and 55%, respectively, are compliant with standards. Many of the Faculty members teaching professional courses do not have the required qualifications i.e., BSFT and Master of Science in FT/Food Science (FS) or an allied field. Only 75% of faculty teaching professional courses are BSFT graduates. Key informant interviews revealed challenges in attracting BSFT graduates to academia due to industry competition, limited supply of graduates, and gaps in availability of BSFT programs in high-demand regions.

1.3 Postgraduate Education:

There are 7 HEIs offering advanced degrees in FT/FS. The number of MS/Ph.D. graduates from these HEIs is still very minimal. Data from the Science Education Institute (SEI) of the DOST indicate that only 3-5 scholars graduate in a year under the MS FS Programs of the University of the Philippine (UP) System. The specialization of most BSFT graduates pursuing post-baccalaureate degrees in local HEIs are in FS or Food Engineering.

1.4 PRC Licensed Food Technologists:

To date, the PRBFT has approved 88 applications for registration without examination. Notably, 61% of these approvals are from the National Capital Region (NCR). Additionally, 37% of applications come from regions such as Davao, Northern Mindanao, Central Luzon, and Calabarzon. This trend is echoed in the distribution of members in the Philippine Association of Food Technologists (PAFT, Inc), with concentrations in urban areas like NCR, Calabarzon, and Mindanao. Most of the approved applicants (81%) are employed in the industrial sector, while 8% work in government and 9% in academia.

The preference for the industrial sector among FT graduates is clear, but shifts might occur due to higher salaries in government and academia. Moreover, a significant 83% of approved registrants are female. A substantial 72% of applicants are from public higher education institutions. This may have been brought about by the fact that there is now free tuition policy in all SUCs.

It can be observed that graduates of other fields (i.e., BS Chemistry, BS Nutrition, BS Chemical Engineering) are also interested to obtain licenses to practice FT. This may be since these professionals are already occupying positions in food establishments and performing the tasks of food technologists, and therefore, would want to legitimize their positions. Professionally, many graduates engage in the food processing sector, handling tasks such as quality control, product innovation, and marketing. On advanced degrees, only 17% have pursued master's degrees, and few have Ph.D. qualifications, indicating the current post-baccalaureate landscape.

1.5 Assessment of Competitiveness

In the industry sector, potential metrics for evaluating the competitiveness of FT graduates include recognition of technical expertise, educational attainment, and demonstrated leadership skills, encompassing effective communication, teamwork, and result-driven approaches. Furthermore, transitioning from rank-and-file to supervisory or managerial roles is another notable marker. Data from PAFT, Inc. highlights that 29% of its members occupy such positions, with a correlation between years of experience and role progression. Perhaps it is safe to conclude that for highly competitive graduates, it takes about 5-10 years to transition from being a rank-and-file employee to the time that they assume a supervisory or managerial role. It was also observed that among those in supervisory/managerial roles, only 29% possess MS/Ph.D. degrees. The distribution of professionals with advanced degrees shows 69% in academia, while 19% and 12% are in industry and government respectively. Notably, 93% in academia have graduate degrees, compared to 47% in government and 15% in the industry.

Merely 20% of PAFT, Inc. members are in the top 20 food manufacturing companies in the Philippines. This suggests the competitiveness of BSFT graduates regardless of their originating institutions. Indicators such as membership in reputable national and international professional organizations, like the National Research Council of the Philippines, further contribute to assessing competitiveness. Involvement in international bodies as experts or consultants adds another layer of competitive evaluation.

As further basis for the Professional Competitiveness Roadmap for FT professionals, the collected data were analyzed through a Situational Analysis utilizing SWOT and DEEP LIST Analysis tools. Quantitative data, such as the number of HEIs, graduates, and licensed food technologists, were analyzed using descriptive statistics to determine trends and patterns. Qualitative data, including information from interviews and open-ended survey questions, were thematically analyzed to identify key themes and insights.

RESULTS AND DISCUSSION

Taking cognizance of the Vision of PRC "to be the instrument of the Filipino people in securing a progressive system of determining the competence of professionals by credible and valid licensure examinations and standards of professional practice that are globally recognized", and having undertaken the assessment of the planning environment, the Vision for the FT profession can be expressed as follows: A critical mass of competent, virtuous, and productive FT professionals whose standards of practice and service are excellent, globally competitive, and attuned to the development imperatives of the country.

The Vision is composed of two elements, one quantitative and the other qualitative. The former element speaks about "a critical mass" which is indicative a specific quantity or number of professionals. The second element focuses on shaping FT professionals by 2028, aligned with the 6-year MTPDP. The aim is to develop "competent, virtuous, and productive FT professionals" in accordance with the law.

An implementation plan was developed to outline specific strategies, major initiatives, and activities aimed at realizing the vision. Complementing this, a risk management plan that highlights potential challenges in the implementation process and the corresponding strategies devised to effectively mitigate these risks was also developed. Aligning with the Vision 2028, the Roadmap (Table 1) was constructed and will serve as the checklist for determining gaps and action steps to be taken.

Table 1. Professional Competitiveness Roadmap for the FT Profession, Roadmap and Major Milestones

KRA/Indicators	Base Year, 2022	2023	2024	2025	2026	2027	2028
KRA 1: Licensure Examination							
Number of HEIs with Certificate of Compliance	10	25	50	57			
Number of industry partners in internship/immersion programs		285	285	285	285	285	285
Guidelines developed and deployed	Developed	Deployed	Deployed	Deployed	Deployed	Deployed	Deployed
Number of successful examinations conducted		1	1	2	2	2	2
Number of successful examinees/Professional Food Technologists (PFT)		250	300	350	400	450	500
Percentage passing rate		25%	30%	35%	40%	45%	50%
KRA 2: CPD/Career Progression and Specialization Program (CPSP)							
Number of accredited CPD/CPSP Providers		5	5	10	10	15	15
CPD/CPSP Programs		Developed/ deployed	Deployed	Deployed	Deployed	Deployed	Deployed
Number of PFTs serving as mentors/subject matter experts		10	20	30	40	50	60
KRA 3: Internationalization / Mutual Recognition Agreement (MRA)							
Number of MRAs developed and executed			1	2	3	4	5
Number of MRAs reviewed and enhanced				1	3	6	10
Number of partner countries with MRAs			1	2	3	4	5
PSG reviewed for compliance with MRAs				1	1	1	1
Number of HEIs compliant with MRAs				10	25	50	57
Number of foreign professionals issued special temporary permits	As needed	As needed	As needed	As needed	As needed	As needed	As needed
KRA 4: Registration Without Examination							
Number of food technologists registered without examination	150	300	450				
Guidelines reviewed and enhanced		1	1				
KRA 5: Inspection and Monitoring							

Number of establishments inspected and monitored for compliance		10	10	10	10	10	10
Number of food technologists trained/oriented on quality management systems through CPD Programs		10	10	10	10	10	10
Number of trainings/seminars on quality management systems conducted in collaboration with CPD Providers		1	1	1	1	1	1
Directory/database of establishments employing food technologists		Developed	Deployed	Deployed	Deployed	Deployed	Deployed
KRA 6: Research and Development (R&D)							
No. of R&D Institute (RDI) with R&D, Innovation, and Extension agenda on food		10	20	30	40	50	60
No. of RDIs with a well-established ecosystem for R&D, Innovation, and Extension on food		1	2	3	4	5	6
No. of food technologies successfully transferred to the private sector and other technology users			1	2	3	4	5
No. of food enterprises assisted in terms of knowledge/technology transfer services			1	2	3	4	5

CONCLUSION AND RECOMMENDATIONS

The Roadmap sets forth a comprehensive strategy to enhance the competitiveness of professionals in the field of FT. It outlines a meticulously designed framework of goals, strategies, and timelines aimed at fostering the growth and proficiency of FT practitioners. By aligning with the aspirations of the MTPDP and Ambisyon Natin 2040, this Roadmap envisions a future where food technologists play a pivotal role in propelling the nation's progress. Drawing upon meticulous analysis and incorporating the expertise of stakeholders, this roadmap serves as a dynamic guide to elevate the capabilities and impact of FT professionals across the nation.

The Roadmap shall be monitored periodically i.e. at the end of each calendar year to keep track of progress and identify gaps in the implementation. An annual review of the action steps taken shall be carried out. Results and analysis should be indicative of the progress of the Roadmap and the necessary implementation of interventions. Depending on the results of the yearend assessment, the Roadmap may be recalibrated and reformulated to address new developments or changes in the planning environment. Impact analysis will be conducted at Midterm (after three years) to determine the outcomes of the implementation of the Roadmap. Existing tools such as the UNESCO SETI Scorecard may be used to determine contributions of the Roadmap to the attainment of the Sustainable Development Goals (SDG) and the MTPDP and Ambisyon Natin 2040.

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THE EFFECT OF PRETREATMENTS ON THE MOISTURE CONTENT, FAT CONTENT AND ACCEPTABILITY OF VACUUM FRIED WHITE OYSTER MUSHROOM (*Pleurotus ostreatus)* CHIPS

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Abstract: The study investigated the effect of pretreatments on the moisture content, fat content and acceptability of vacuum fried white oyster mushroom chips. Control and four different pretreatments (1) freezing, (2) osmotic dehydration with maltodextrin solution and freezing, (3) steam blanching and freezing (4) steam blanching, osmotic dehydration and freezing were used in the study. All samples were fried in coconut oil at 80°C with vacuum pressure of −700 mmHg for 15 min., followed by spinning at 1420 rpm for 10 min. The results showed that pretreatments significantly ($p < 0.05$) affected the moisture content, fat content, and acceptability of the vacuum fried oyster mushroom chips. Vacuum fried oyster mushroom chips pretreated with steam blanching, osmotic dehydration and freezing was found to have the lowest moisture content (0.86%). Other pretreatments resulted to vacuum fried oyster mushroom chips with higher moisture contents, still below the standard level for microbial growth. The osmotic dehydration with maltodextrin solution and freezing pretreatment minimized the oil uptake (22.24% fat content) of the vacuum fried oyster mushroom chips and is the most acceptable in sensory evaluation. Therefore, the osmotic dehydration and freezing before vacuum frying was the most suitable pretreatment for vacuum fried white oyster mushroom chips.
***Keywords*:** vacuum fried, white oyster mushroom, chips, moisture content, fat content, acceptability

INTRODUCTION

The word “mushroom” refers to the fruit body of a certain fungi. They belong to the class *Basidiomycetes*, order; *Agaricales* (Gamaje & Ohga, 2018). Mushrooms are edible fungi of commercial importance and their cultivation has emerged as a promising agro-based land-independent enterprise (Shivhare et al., 2014). They are increasingly being utilized as important food products for their significant role in human health, nutrition and disease control (Chang & Miles, 1989). With their flavor, texture, nutritional value and high productivity per unit area, mushrooms have been identified as an excellent food source to alleviate malnutrition in developing countries (Pathmashini et al., 2009).

At present, oyster mushrooms (*Pleurotus species*) are the world’s third most common species of cultivated mushrooms after button and shitake (Fernandes et al., 2015; Josephine, 2015). Oyster mushrooms got their name from their fan-shaped cap, which resembles an oyster shell, and has pronounced gills on the underside (Motoviloff, 2014). They are rich of large amount of essential nutrients such as carbohydrates, proteins, vitamins, amino acids, fiber, organic elements, lipid and volatile compounds. Thus, are considered as one of the richest well-balanced sources for human nutrition and is widely used in food and nutraceutical industries (Maftoun et al., 2015).

Despite the high nutritional value of mushrooms, they are very perishable and can be preserved only if properly processed (Martinez-Soto et al., 2001). The mushrooms of the *Pleurotus genus* are delicate and sensitive, and start deteriorating within 1 day after harvest (Apati et al., 2010). Thereby, causing difficulties in their distribution and marketing as fresh products (Ren et al., 2018). Value adding it is a must to reduce post-harvest losses (Gamage & Ohga, 2018). One way of value adding food is by vacuum frying. It is well known that fried products have consumer appeal in all age groups and in virtually all cultures. The process is quick and can easily be made continuous for mass production, and the food appears sterile and dry, with a relatively long shelf life (Ren et al., 2018).

Vacuum frying is the processing of fruits and vegetables under pressures well below atmospheric levels, preferably below 8 kPa that can lower the boiling points of frying oil and moisture in food. Moisture can thus be removed from the fried food rapidly once the oil temperature reaches the boiling point of water (Ren et al., 2018). Vacuum frying reduces the oil content in the fried product. Moreover, it can preserve the natural color and flavors of the product and has less adverse effect on oil quality (Tagalpallewar, 2015). It is a cost-effective process and provides high quality attributes of fried foods. As a result, vacuum frying is a feasible option for the processing of white oyster mushroom into chips. Inevitably, some quality deteriorations could also take place during the vacuum frying process. To improve the quality of vacuum fried products, several pretreatment methods, such as blanching, pre-drying, osmotic pre-treatment, coating, and freezing have been applied to the frying of foods (Fan et al., 2006). Hence, the

aim of this study is to investigate the effect of pretreatments on the quality, specifically on the moisture content, fat content, and acceptability, of vacuum fried white oyster mushroom chips.

MATERIALS AND METHODS

Materials

Fresh white oyster mushrooms were purchased from a local mushroom cultivator from Legazpi City, Albay. Maltodextrin was purchased from a food ingredient supplier in Quezon City, Manila. Coconut oil was purchased from a local supermarket in Legazpi City, Albay and aluminum foil pouch was purchased from a food packaging supplies store in Ermita, Manila. All other chemicals used were analytical grade.

The vacuum fryer used equipped with a spinner (*model PD120S-220, Department of Science and Technology (DOST))*, with a capacity of 5 KG and a maximum temperature and vacuum degree of 130°C and −700 mmHg**,** respectively, for an oil capacity of 64 L is from the Bicol Regional Food Innovation and Commercialization Center, Bicol University - East Campus, Legazpi City, Albay.

Pretreatment of white oyster mushroom

Fresh white oyster mushrooms were cleaned by wiping with a clean white damp cloth. This was followed by the removal of the stipes of the cleaned oyster mushrooms. White oyster mushrooms free from stipe were treated as follows: (1) untreated as control, (2) freezing, (3) osmotic dehydration with maltodextrin solution (50% w/v) at 25°C for 60 min and freezing at -18°C for 24 hours, (4) steam blanching (95°C) for 5 min and freezing at -18°C for 24 hours (5) steam blanching (95°C) for 5 min, osmotic dehydration with maltodextrin solution (50% w/v) at 25°C for 60 min and freezing at -18°C for 24 hours. Pretreatments used were adapted from the study of Ren et al. (2018), with modifications.

Vacuum frying

A batch of 2 KG of white oyster mushrooms, after the pretreatment, was fried in 64 L of coconut oil. The oil temperature used was 80°C, vacuum pressure of -700 mmHg, and frying time of 15 min. After the frying process, residual frying oil was removed from the product using a spinner (1420 rpm for 10 min). Spun mushrooms were packed in aluminum foil pouches under vacuum condition before analysis. Vacuum frying process was based on the study conducted by Charoen et al. (2015), with modifications.

Determination of moisture and fat contents

To determine the effect of the pretreatments on the moisture and fat contents of the vacuum fried white oyster mushroom chips, moisture content and fat content were determined according to AOAC 930.04, 21st Ed. and AOAC 948.15, 21st Ed. methods, respectively.

Sensory evaluation

Nine-point hedonic scale was used to determine the acceptability of the white oyster mushrooms vacuum fried at 80°C. The panelists were asked to assess the acceptability of the vacuum fried white oyster mushroom chips using the following rating: nine (9) as Likely extremely, eight (8) as Like very much, seven (7) as Like moderately, six (6) as Like slightly, five (5) as Neither like nor a dislike, four (4) as Dislike slightly, three (3) as Dislike moderately, two (2) as Dislike very much, and one (1) as Dislike extremely. Fifty (50) consumer-type panelists were utilized in the sensory evaluation and were asked to evaluate the level of acceptability of the vacuum fried white oyster mushroom chips in terms of appearance, color, aroma, texture, taste, and general acceptability.

Statistical analysis

Data were analyzed using a one-way Analysis of Variance (ANOVA) and Tukey's Range Test. Mean values were considered significantly different when $p < 0.05$.

RESULTS AND DISCUSSION

Effects of pretreatment on the moisture content of the vacuum fried white oyster mushroom chips

The moisture contents of the vacuum fried white oyster mushroom chips are presented in Table 1. Steam blanching, osmotic dehydration and freezing pretreatment resulted to vacuum fried white oyster mushroom chips with the lowest moisture content, .86%. Steam blanching and freezing pretreatment resulted to vacuum fried white oyster mushroom chips with the highest moisture content, 2.01%, followed by the control (1.79%), vacuum fried white oyster mushroom

chips with freezing as pretreatment (1.62%), and vacuum fried white oyster mushroom chips with osmotic dehydration and freezing as pretreatment (1.60%).

Steam blanching disrupts the cells and makes it easy for moisture to evaporate (Wickramasinghe, 2020). Similarly, freezing could increase cell membrane penetrability of the material and can cause water to more easily evaporate (Fan et al., 2006). Despite this, vacuum fried white oyster mushroom chips with steam blanching and freezing as pretreatment obtained the highest moisture content. Moisture may have been absorbed by the white oyster mushrooms during the steam blanching process resulting to a higher initial moisture content prior vacuum frying. However, in combination with osmotic dehydration, it resulted to vacuum fried white oyster mushroom chips with the lowest moisture content. From the result, it can be inferred that pretreatments with osmotic dehydration yielded to vacuum fried white oyster mushroom chips with the lower moisture contents. Osmotic dehydration is the removal of water by immersing food in salt or sugar solution of high osmotic pressure. The water is transferred from the food to the solution by virtue of the difference in osmotic pressure (Berk, 2018). Moisture contents of the vacuum fried white oyster mushroom chips is lower than the moisture content (2.15%) of vacuum fried gray oyster mushroom chips developed in the study of Charoen et al. (2015).

Table 1. Moisture content of the vacuum fried white oyster mushroom chips

Control	Freezing	Osmotic dehydration with maltodextrin solution + freezing	Steam blanching + freezing	Steam blanching + osmotic dehydration with maltodextrin solution + freezing
1.79±.06[a]	1.62±.10[b]	1.60±.05[b]	2.01±.04[c]	0.86±.03[d]

Effects of pretreatment on the fat content of the vacuum fried white oyster mushroom chips

Fat contents of the vacuum fried white oyster mushroom chips are presented in Table 2. Osmotic dehydration and freezing pretreatment caused the highest reduction in fat content, 22.24%. This was followed by the vacuum fried white oyster mushroom chips with steam blanching, osmotic dehydration and freezing as pretreatment, 41.61%. This suggests that pretreatment with osmotic dehydration, just like in moisture content, resulted to vacuum fried white oyster mushroom chips with lower fat contents. This is in agreement with the study of Song et al. (2007), low initial moisture content resulted to low final fat content, as seen in tables 1 and 2. Untreated vacuum fried white oyster mushroom chips obtained the highest fat content (63.87%). This shows that pretreatment helps in the reduction of fat content. Typical potato chip has a fat content of 35-40% (Riaz, 2016). Vacuum fried white oyster mushroom chips with osmotic dehydration with maltodextrin and freezing pretreatment is 12.76-17.76% lower in fat content.

Table 2. Fat content of the vacuum fried white oyster mushroom chips

Control	Freezing	Osmotic dehydration with maltodextrin solution + freezing	Steam blanching + freezing	Steam blanching + osmotic dehydration with maltodextrin solution + freezing
63.87±3.70[a]	55.46±.13[b]	22.24±.26[c]	57.91±2.56[ab]	41.61±.11[d]

Effects of pretreatment on the acceptability of the vacuum fried white oyster mushroom chips

A sensory evaluation was performed to determine the acceptability of the vacuum fried white oyster mushroom chips. Figure 1 shows that the panelists find all samples as acceptable as indicated by the scores in the overall acceptability. Significant differences ($p < 0.05$) were observed in the attributes, appearance, color, texture, taste, and overall acceptability, except in aroma. Vacuum fried white oyster mushroom chips with osmotic dehydration with maltodextrin solution as one of the pretreatments resulted in higher acceptability in all attributes. Maltodextrin is a polysaccharide used as a food additive and flavor enhancer (Marcus, 2019). It is made from the hydrolysis of starch and comes in the form of white powder and has a sweet taste (Wang & Wang, 2000). This explains the 8.24 (*like very much*) rating of the vacuum fried white oyster mushroom chips with osmotic dehydration with maltodextrin solution and freezing pretreatment, in terms of taste. As seen in Figure 1, osmotic dehydration with maltodextrin solution and freezing pretreatment had the highest value in appearance, color, aroma, texture, taste, and overall acceptability, indicating that this pretreatment was the most suitable for the vacuum fried white oyster mushroom chips.

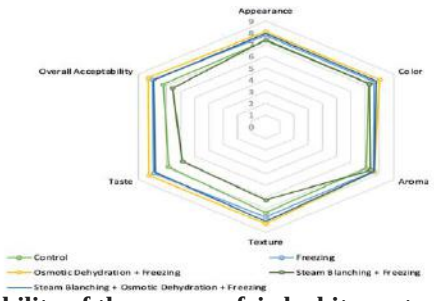

Figure 1. Acceptability of the vacuum fried white oyster mushroom chips

CONCLUSIONS

The osmotic dehydration with maltodextrin solution and freezing pretreatment minimized the oil uptake (22.24% fat content) of the vacuum fried oyster mushroom chips and is the most acceptable in sensory evaluation. Therefore, the osmotic dehydration and freezing before vacuum frying was the most suitable pretreatment for vacuum fried white oyster mushroom chips. Shelf-life analysis may be done to determine its stability.

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POTENTIAL OF NYONYA DESSERT WASTES AS THE SUBSTRATE FOR SINGLE CELL PROTEIN PRODUCTION

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Abstract: Nyonya desserts have short shelf life due to their high moisture and fat content, hence the unsold desserts end up as waste. *Kueh talam* (KT) and *kueh lapis* (KL) were the selected substrates for this study. Potential of these wastes as the substrate for single cell protein (SCP) production was investigated. The wastes were hydrolysed to produce sugar hydrolysate using amylase. The effects of lipase-pretreatment and polyvinylpyrrolidone (PVP)-post-treatment on the crude fat, crude protein and sugar content of the hydrolysates were determined, then the effects of these components on the yield of SCP were investigated using Baker's yeast. Molasses was used as the positive experiment control. Results of the study revealed that combined lipase-pretreatment and PVP-post-treatment(L-PVP) significantly reduced crude fat content and increased sugar content of both hydrolysates. SCP produced from KT and KL hydrolysates were increased by 23% and 39%, respectively after L-PVP treatment. Although the SCP yield from molasses fermentation was 19% and 11% higher than KT and KL hydrolysates, respectively, the process was still economically feasible due to the expensive price of molasses. Crude protein content of SCP produced from both KT and KL were significantly higher than those produced from molasses. This study provides new insight into a novel food waste recycling strategy to achieve the goal of sustainable food production with minimum wastage, aligned to SDG 12.
Keywords: Nyonya dessert, lipase-pretreatment, single cell protein, amylase hydrolysis, polyvinylpyrrolidone-post-treatment.

INTRODUCTION

'*Nyonya kueh*' is a popular type of Chinese *Peranakan* desserts that emerged from the cultural borrowing and innovation through the contact with local ingredients and non-Chinese principles of food preparation. It is innovated by combining the Chinese cooking techniques with Malaysian ingredients and Indonesian spices and flavours. In the local market, diverse types of *kuehs* such as *angku kueh*, *kueh dodol*, *kueh bangkit*, *kueh koci*, *kueh seri muka*, *kueh kosui*, *huat kueh*, *kueh talam*, *kueh lapis* and many more can be found in both Chinese and Malay food stalls (Ng & Karim 2016). However, these desserts have a short shelf life of approximately 1 to 3 days due to high moisture and fat content. Consumer demand for freshness restricts the use of chemical preservatives in these perishable products. Furthermore, re-processing of these products is almost impossible due to the abrupt change of texture (Seow et al. 1995). Therefore, discarding the unsold *kuehs* is the common practice among the *Nyonya* dessert manufacturer. This unwise practice not only generates substantial amounts of food wastes in the industry, but it also contributes to resource depletion, financial loss and environment pollution (Mulya et al. 2022). In this study, *kueh talam* and *kueh lapis* were selected as the substrate of investigation.

Single cell protein (SCP) is a nutrient dense source of high-quality protein with approximately 50 – 80% of protein content in dry basis. SCP is obtained from the dried cells of microorganisms. Besides protein, SCP also contains other nutrients such as essential carbohydrates, vitamins and minerals which originate from the biomass of the microorganisms. SCP can be obtained from different microbial sources such as microalgae, fungi, and bacteria. Among these sources, bacterial SCP is the most welcome due to its fast-growing capability under varying conditions and well-balanced essential amino acids profile (Malav et al. 2017). However, Vicente et al. (2023) proposed that yeast *Saccharomyces cerevisiae* is also an excellent candidate for SCP production due to its ability in metabolizing simple sugars such as glucose and fructose through alcoholic fermentation. Furthermore, Khan et al. (2022) suggested that producing SCP using food waste is a highly promising approach in waste management. Food wastes, such as banana peel, citrus peel, carrot pomace and potato peel were among the substances that had been reported to be used in SCP production. Hence, *S. cerevisiae* was selected as the model to study the SCP production using *Nyonya* dessert wastes.

In the study by Parapouli et al. (2020), fat was proven to slow down the metabolism and hinder the growth of *S. cerevisiae*. Besides, Barnett & Entian (2005) reported that yeast cannot metabolize complex starch polymers, thereby hindering biomass production. In addition, a study by Pande & Mead (1968) reported that fat reduced activity of amylase enzyme. Therefore, fat removal and saccharification of *Nyonya* dessert waste is crucial before fermentation. Therefore, the efficiencies of lipase-pretreatment and polyvinylpyrrolidone (PVP)-post-treatment on fat removal were investigated. Lipase is a versatile enzyme widely used in lipolysis to break down triacylglycerol into fatty acids and glycerol, whereas PVP is a versatile polymer which has a unique characteristic in forming complex with both hydrophobic and hydrophilic substances (Chandra et al. 2020; Teodorescu & Bercea 2015).

In general, this study was conducted to compare the efficiency of lipase-pretreatment and PVP-post-treatment in oil removal from the sugar hydrolysates of *kueh talam* (KT) and *kueh lapis* (KL). Besides, the effects of fat and sugar content in the sugar hydrolysates of KT and KL on the production of *S. cerevisiae* cell biomass were also determined. Lastly, the yield and total crude protein of *S. cerevisiae* cell biomass produced from the KT and KL sugar hydrolysates were compared. This study is expected to provide new insight into the novel strategy in *Nyonya* dessert waste recycling for food ingredient production.

MATERIALS AND METHODS

The KT and KL were gifted by Usaha Maju Kini Sdn. Bhd. (Seri Kembangan, Selangor). Initially, the collected KT and KL were ground using a kitchen blender (Model PB-3203L, Pensonic, Malaysia) into homogenous paste. Next, distilled water was added to the paste at a ratio of 1:1, then homogenized using a high-speed homogenizer at a speed of 10 000 rpm for 3 min. The suspension was centrifuged at 8000 ×g for 15 min at ambient temperature. Oil layer was observed on the top of the water layer. Then, the supernatant was discarded and distilled water was added (at 1:1 ratio). The homogenization and centrifugation processes were repeated for 3 cycles to remove the excess oil from the KT and KL. The KT and KL obtained were labelled as untreated samples (UKT and UKL).

Lipase-pretreatment was carried out according to the method by Omar et al. (2016) with slight modification. Briefly, 20 % w/v of the *kueh* paste was suspended in 0.2 M phosphate buffer (pH 7), then 1.5 % w/w of lipase (Sigma Aldrich, U.S.) was added. The pretreatment was conducted at 45ºC for 1 h in a water bath. The suspension was then centrifuged at 8000 ×g for 15 min to recover the lipase-pretreated *kueh* paste (labelled as LKT and LKL).

To produce sugar hydrolysate, the UKT, UKL, LKT and LKL at 20 % w/v, respectively were suspended in 0.2 M phosphate buffer (pH 6), then 1.5 % w/w of amylase (Sigma Aldrich, U. S.) was added. The hydrolysis was carried out at 55ºC for 1 h in a water bath. The hydrolysate was recovered through centrifugation (8000 ×g, 15 min). All hydrolysates were boiled for 5 min to terminate enzyme activity.

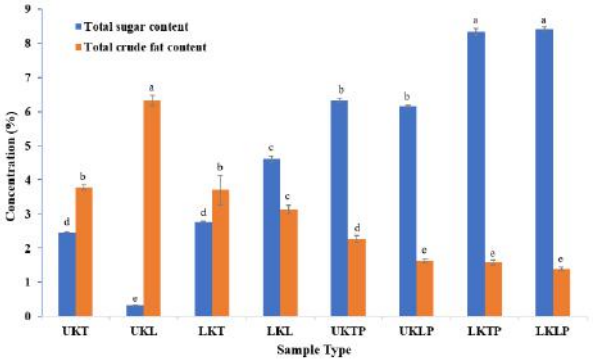
To investigate the efficiency of PVP-post-treatment in oil removal, the hydrolysate was filtered through the PVP-treated cotton, according to procedures proposed by Tang et al. (2016). The hydrolysates that went through the PVP-post-treatment were labelled as UKTP, UKLP, LKTP, and LKLP. UKT and UKL which did not go through lipase-pretreatment and PVP-post-treatment served as experiment control.

All hydrolysates were sterilized at 121ºC for 15 min before inoculated with 4 % w/v *S. cerevisiae* dry yeast (Mauripan, Malaysia). Molasses (contains 9.2±0.1 % total sugar) was used as positive control for the fermentation experiment. The fermentation progressed at 37ºC for 24 h in an incubator (Chinma et al. 2014). Total sugar content of the hydrolysates before and after fermentation by *S. cerevisiae* were determined using phenol-sulfuric acid method (Zhang et al. 2020). Total crude fat content of the hydrolysate was determined by extracting the fat using petroleum ether (at a ratio of 1:1), then determined gravimetrically after dried the petroleum ether to dryness using a rotary evaporator. The yield of cell biomass was determined based on dry cell weight, whereas total crude protein content of the cell biomass was determined through Kjeldahl method (AOAC, 2000).

RESULTS AND DISCUSSION

Figure 1 shows the effects of lipase-pretreatment, PVP-post-treatment and their combination on the total sugar and crude fat content of sugar hydrolysate produced from KT and KL. Based on the results, hydrolysate of UKL contained the highest crude fat content (6.33 ± 0.15 %), followed by UKT and LKT (~3.7 %). Among the hydrolysates, UKL hydrolysate contained the lowest total sugar content (0.32 ± 0.01 %), followed by UKT and LKT (about 2.5 – 2.8 %). These findings suggest that crude fat content negatively impacted enzymatic hydrolysis of the starchy waste of *kueh* in releasing total sugar content. This finding is in accordance with the study by Pande & Mead (1968), whereby amylase activity was proven to be inhibited in the presence of fat. Besides, results in figure 1 also indicate that lipase-pretreatment alone did not significantly ($p>0.05$) reduce crude fat content of KT, but it did significantly reduce ($p<0.05$) crude fat content of KL by about 50% to 3.13 ± 0.12 %. Significant crude fat reduction had contributed to the substantial increase of total sugar content in LKL hydrolysate by about 4 folds to 4.61 ± 0.08 %. However, PVP-post-treatment was proven as the most efficient fat removal method for both untreated and lipase-pretreated *kueh*

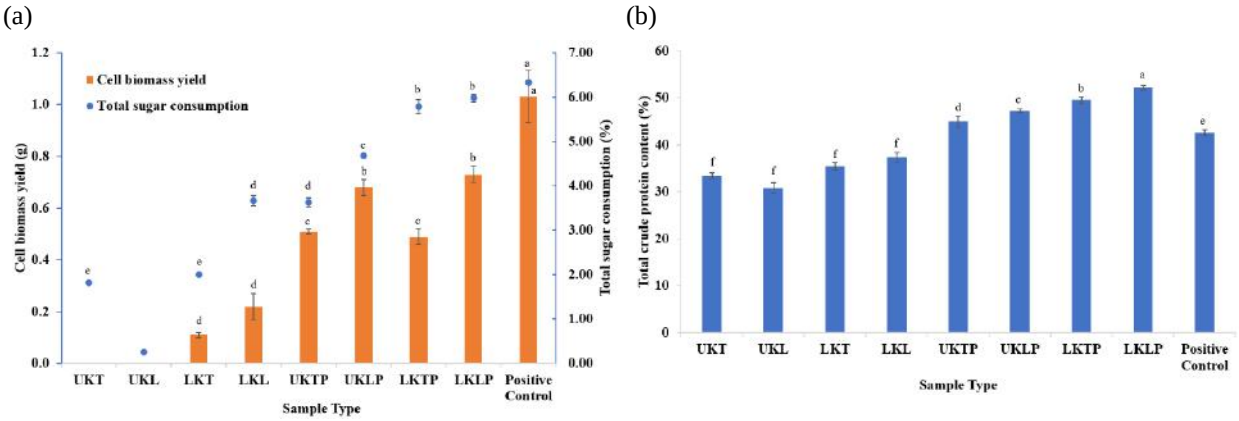
wastes. The highest total sugar content (8 – 9 %) was detected in the hydrolysate of LKTP and LKLP, followed by UKTP and UKLP. Total crude fat content in UKLP, LKTP and LKLP hydrolysates was the lowest (1.4 – 1.6 %) among the samples. The applications of PVP in oil and dye removal were also reported in the study by Teodorescu & Bercea (2015). This study proved that combined lipase-pretreatment and PVP-post-treatment was the best method to remove crude fat and enhance the rate of sugar released from amylase saccharification of starchy *kueh* waste.



Note: Different alphabets indicate there is significant difference ($p<0.05$) between the mean of the same chemical component.

Figure 1. Total sugar and crude fat content of different sugar hydrolysates of KT and KL (n=3)

Figure 2 demonstrates the yield of *S. cerevisiae* cell biomass and total sugar consumption by the yeast during fermentation of different hydrolysates of KT and KL. Besides, total crude protein of yeast cell biomass produced from different hydrolysates was also compared.



Note: Different alphabets indicate there is significant difference ($p<0.05$) between the mean of the same chemical component.

Figure 2. Effect of different feeding substrates on the (a) total sugar consumption and yield of cell biomass, and (b) total crude protein content of cell biomass in *S. cerevisiae* fermentation (n = 3).

According to figure 2 (a), yeast *S. cerevisiae* consumed the highest total sugar (6.33 ± 0.04 %) in the molasses (positive control) for producing the highest cell biomass yield (1.0 ± 0.1 g). Among the hydrolysates, fermentation of UKLP and LKLP produced the highest yield of cell biomass (0.7 g), followed by UKTP and LKTP (0.5 g). Sugar consumption by yeast in the combined lipase-pretreated and PVP-post-treated hydrolysates (LKTP and LKLP) was the highest (approximately 6%). Compared to KT, hydrolysate of KL was proven to be a more efficient substrate for yeast cell biomass production, whereby cell biomass yield of LKL, UKLP and LKLP was significantly higher ($p<0.05$) than LKT, UKTP and LKTP, respectively. Furthermore, figure 2 (b) shows that total crude protein of yeast cell biomass produced from LKLP was the highest (52.15 ± 0.46 %). Moreover, there was no significant cell growth observed in UKT and UKL. Hence, it is hypothesized that high crude fat content in the hydrolysate remarkably inhibited *S. cerevisiae* growth. This finding is in accordance with the finding reported by Parapouli et al. (2020), whereby fat was reported to adversely impact the yeast cell growth.

In addition, results in figure 2 also unveils that PVP-post-treatment was more efficient than lipase-pretreatment in increasing the yield of cell biomass and crude protein content in the biomass produced. Besides, figure 2 (b) also shows that crude protein content of yeast biomass produced from PVP-post-treated hydrolysates (UKTP,

UKLP, LKTP and LKLP) was significantly higher than positive control. Wang et al. (2017) reported that PVP-modified cotton could efficiently separate both surfactant-free and stabilized oil-in-water emulsion. The study revealed that PVP possesses both superhydrophilic and superoleophilic properties, which makes it an excellent material for effective separation of water rich immiscible oil droplets. Based on these results, it was strongly believed that the *kueh* wastes might contain nutrients which could enhance the crude protein content of the yeast cell biomass, while crude fat content in the waste is one of the limiting factors in the bioconversion process.

CONCLUSIONS AND RECOMMENDATIONS

The study proposes a novel green approach to utilise the oily and starchy *Nyonya* desserts (*kueh talam* and *kueh lapis*) waste as the substrate for single cell protein production. In this study, the Baker's yeast *S. cerevisiae* was used as the model of investigation. The study proved that *kueh lapis* was a better substrate than *kueh talam* for producing cell biomass with a significantly higher crude protein content. Besides, the combined lipase-pretreatment and PVP-post-treatment has also been proven to be the best technique to reduce crude fat content of the waste, subsequently turning the waste into an enzymatically-readily hydrolysed and fermentable substrate for single cell protein production. This study provides new insights into a novel food waste recycle approach to reduce food wastage in the food supply chain. The study was conducted to align with the call of SDG 12 (Responsible consumption and production) by the United Nations. Nonetheless, further study to explore the type of nutrients in the *kueh talam* and *kueh lapis* that contributed to a higher crude protein content in the yeast cell biomass is needed. Besides, the experiment should also be extended to more varieties of *Nyonya* desserts so that the feasibility of the proposed bioconversion process can be thoroughly assessed.

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EFFECTS OF THE ADDITION OF RED ONION BULB AND SKIN EXTRACTS ON THE BIOACTIVE PROPERTIES OF COTTAGE CHEESE

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Abstract: The increased consumer interest in cottage cheese because of its high protein and low calories content is projected to positively impact the functional food market. This study aims to investigate the effects of incorporating red onion bulb (ROB) and skin (ROS) extracts on the bioactive properties of cottage cheese. The bioactive compounds of ROB and ROS were extracted using ultrasound-assisted hot water extraction, then encapsulated with kappa-carrageenan. The antioxidant, anti-inflammation and anti-obesity activities of the extracts were determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH), albumin denaturation inhibition (ADI), and pancreatic lipase inhibition (PLI) assays, respectively. Lastly, 1% w/w of extracts were added into the cheese. The ROB and ROS extracts contained 18.14%–41.10%, 22.43%–34.29%, and 7.78%–26.28% of DPPH, ADI and PLI activities, respectively. Incorporating ROB into cheese significantly increased ($p<0.05$) its DPPH, ADI and PLI activities to 9.03%, 20.36%, and 7.50%, respectively. The addition of ROS remarkably increased DPPH by 49.60%, ADI by 26.60 folds, and PLI by 9.71 folds in ROS-cheese, compared to the plain cheese. The DPPH, ADI and PLI activities of ROS-cheese were 1.04–2.03 folds higher than ROB-cheese. This study recommends ROS as a better substrate than ROB in enhancing the bioactive properties of cottage cheese.

Keywords: Cottage cheese, red onion bulb and red onion skin extracts, antioxidant activity, anti-inflammatory activity, anti-obesity activity.

INTRODUCTION:

Red onion (*Allium cepa*) is a vegetable with multiple uses, which can be consumed fresh or used in cooking in various form of recipes and processed products (Sidnu et al. 2019). The consumption of onions has shown to provide various pharmacological properties and therapeutic effects such as antioxidant, anti-inflammatory, anti-cancer, and other benefits (Marefati et al. 2021). These health benefits are attributed to the large amounts of bioactive compounds such as phenolics, flavonoids, anthocyanins and its derivatives in the onions, nonetheless these compounds are more abundant in red onion compared to other species (Chadorshabi et al. 2022). Besides the onion bulb, previous studies also reported that onion skin is a rich source of bioactive compounds, and the quantity of bioactive compounds in the skin is more than in the bulb (Chadorshabi et al. 2022). Benítez et al. (2011) reported that more than 500,000 tonnes of onion wastes were discarded annually in European Union, primarily Spain, United Kingdom, and Holland. Large volume of onion skin disposal poses environmental issues because of limited utilization due to its strong aroma, and rapid development of phytopathogenic agents. Because of the copious amount of bioactive compounds available in the onion skins, it is foreseen to be a potential recycled health food ingredient for the fortification of food products.

The objective of this study is to investigate the effects of red onion bulb (ROB) and red onion skin (ROS) extracts on the antioxidant, anti-inflammatory and anti-obesity activities of the cottage cheese. Cottage cheese was chosen as the carrier because of its growing consumer interest due to its high proteins, minerals, vitamins and low total calorie content (Khatun et al., 2019). The ROB and ROS extracts were prepared using ultrasound-assisted hot water extraction technique. Then, the extracts were encapsulated using kappa-carrageenan before being added into the cottage cheese. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay, albumin denaturation inhibition (ADI) assay, and pancreatic lipase inhibition (PLI) assay were conducted to determine the antioxidant, anti-inflammatory,

and anti-obesity activities, respectively. The addition of ROB and ROS extracts was expected to impart beneficial effects to improve the bioactive properties of cottage cheese.

MATERIALS AND METHODS:

Red onions, low fat milk, citric acid, calcium chloride, powdered rennet, salt, 2,2-diphenyl-1-picrylhydrazyl (Sigma Aldrich, Germany), methanol (Supelco, Germany), bovine serum albumin (Merck, USA), sodium chloride (Supelco, Denmark), potassium chloride (Bendosen, Malaysia), disodium phosphate (ChemSoln, Malaysia), monopotassium phosphate (ChemSoln, Malaysia), lipase from porcine pancreas (Sigma Aldrich, USA), dipotassium phosphate (ChemSoln, Malaysia), and 2,4-dinitrophenyl butyrate (Biosynth, United Kingdom).

Preparation ROB and red onion skin ROS extracts

The extraction was performed in accordance with the method as described by Tang et al. (2020) with minor modifications. Initially, the ROS was separated from the ROB. Then, both ROS and ROB were milled separately using a knife mill. To prepare the ROS and ROB extracts, a 10% (w/v) ROB in distilled water was extracted using ultrasound-assisted water extraction method in a 40 kHz ultrasound bath. After 1 hour of extraction, the solid residues were separated from the extract through filtration using a cheese cloth, then re-immersed into the distilled water to reach a final concentration at 10% (w/v). The solid residues were re-extracted in hot water at 130 °C in an autoclave for 1 hour. Next, the solid residues were filtered through the cheese cloth. The extracts obtained from both extractions were combined and centrifuged at 20 °C and 11180 × g for 5 minutes to obtain clean liquid extract. The liquid extracts were then freeze-dried and ground into powder. To encapsulate ROB extract, about 10% (w/v) of ROB extract powder was homogenized in 2% (w/v) kappa-carrageenan solution at 6000 rpm for 5 minutes, then freeze dried and ground into powder. The encapsulated ROB extract powder was stored at 4 °C for further usage. The same procedures were repeated for ROS.

Preparation of cheese

The cheese making process was performed in accordance to the method as described by Souza and Saad (2009) with minor modifications. Approximately 2 L of commercial, pasteurized low fat milk was mixed with 1.6 g/L of citric acid and heated up to 35 °C. Then, about 0.2 g/L of calcium chloride and 0.16 g/L of rennet were added into the milk and mixed for 30 seconds. The milk was left for 1 hour to coagulate. After coagulation, the curds were cut into cubes and heated up to 50 °C, followed by whey draining and addition of 6 g of salt. Next, about 1% (w/w) of powdered ROB extract and ROS extract was folded into the cottage cheese, respectively. A plain cheese without ROB and ROS was prepared as the experiment control for comparison. All the cheese samples were prepared in triplicates and were stored at 4 °C before further analysis.

DPPH scavenging assay

The DPPH scavenging activity of the red onion extracts (ROB and ROS) and cheese samples were evaluated following the method as described by Fernández-Agulló et al. (2013). All samples were prepared at a concentration of 100 µg/mL in methanol. An aliquot of 0.3 mL sample was mixed with 2.7 mL of 0.06 mM DPPH in methanol solution. The mixture was vortexed and kept in dark for 1 hour. The DPPH scavenging activity by the sample was measured by determining absorbance at wavelength 517 nm. The DPPH scavenging activity was expressed as the percentage of DPPH discolouration using the following equation:

DPPH scavenging activity (%) = $[(A_{DPPH} - A_S) / A_{DPPH}] \times 100$

where A_S is the absorbance of the solution with samples and A_{DPPH} is the absorbance of the DPPH solution.

Albumin denaturation inhibition (ADI) assay

Protein denaturation assay was conducted according to the method as described by Gunathilake et al. (2018) with some modifications. Samples at the concentration of 1 mg/mL in phosphate buffered saline were prepared. About 0.2 mL of 1% bovine serum albumin, 2.0 mL of phosphate buffered saline (pH 6.4), and 0.5 mL of sample solution were mixed. Then, the mixture was incubated in a water bath at 37 °C for 15 minutes, followed by heating at 80 °C for 45 minutes. After cooled down to room temperature for 15 minutes, absorbance of the mixture at wavelength 280 nm was measured. Phosphate buffer solution was used as the control. The percentage of inhibition of albumin denaturation was calculated by using the following equation:

Albumin denaturation inhibition activity (%) = $\left[1 - \frac{(S - S_b) - A}{B - A}\right] \times 100$

where S is the absorbance of the solution with sample after reaction, S_b is the absorbance of sample blank, A is the absorbance of control before reaction, and B is the absorbance of control after reaction.

Pancreatic lipase inhibition assay

The pancreatic lipase activity of samples was determined based on the method described by Zheng et al. (2010) with some modifications. About 0.1 mL of sample (1 mg/mL) was mixed with 0.5 mL of pancreatic lipase enzyme (at the concentration of 100 U/mL in 0.1 mM potassium phosphate buffer pH 6.0) and 1.9 mL of potassium phosphate buffer (0.1 mM, pH 7.2, combined with 0.6 mL/100mL Tween 80). Next, the reaction was started by adding 0.5 mL of 25 mM 2,4-dinitrophenyl butyrate (DNPB). After incubation at 37 °C for 30 minutes, the amount of 2,4-dinitrophenol released in the reaction was measured at a wavelength of 360 nm. The inhibitory activity was calculated using the following equation:

Pancreatic lipase inhibition activity (%) = $[1 - (B - b) / (A)] \times 100$

where A is activity of the enzyme without sample, B is the activity of the enzyme with sample, and b is the control with sample.

Statistical analysis

The statistical analysis was performed using IBM SPSS Statistics Version 26 (IBM Corp., Armonk, NY, USA). Sample t-test was performed to compare means between the ROB and ROS extracts, whereas one-way analysis of variance (ANOVA) was used to compare the means of cheese samples. Post-hoc test to determine the significant difference between means was evaluated using Tukey's test at 95% confidence interval.

RESULTS AND DISCUSSION:

Table 1 shows the DPPH scavenging activity, albumin denaturation inhibition activity and pancreatic lipase inhibition activity of ROB and ROS extracts.

Table 1. DPPH scavenging, albumin denaturation inhibition, and pancreatic lipase inhibition activities of ROB and ROS extracts (n=3).

Samples	DPPH Scavenging Activity (%)	Albumin Denaturation Inhibition Activity (%)	Pancreatic Lipase Inhibition Activity (%)
Red Onion Bulb Extract	18.14 ± 1.26^{b}	22.43 ± 1.32^{b}	7.78 ± 1.17^{b}
Red Onion Skin Extract	41.10 ± 0.20^{a}	34.29 ± 1.50^{a}	26.58 ± 1.13^{a}

Note: Different superscript lowercase letters in each column of extracts are significantly different ($p < 0.05$).

The results show that the DPPH scavenging, albumin denaturation inhibition (ADI), and pancreatic lipase inhibition (PLI) activities of ROS extract were significantly higher ($p < 0.05$) than ROB extract. Red onions generally contain higher amount of flavonoids than white and yellow onions, in which anthocyanins and flavonols are the two major groups of flavonoids in the red onions (Vian et al., 2011). The ROS has been reported to contain 2-10 g/kg of flavonoids, which is higher than the ROB with approximately 0.03-1 g/kg (Chadorshabi et al. 2022). The ROS extract exhibited higher bioactivity because of the large amounts of anthocyanins that are highly concentrated in the skin of red onions; whereas, the anthocyanins in the bulbs are only present in a single layer of cells of the epidermal tissue (Celano et al., 2021). Besides, Takahama and Hirota (2000) reported that 3,4-dihydroxybenzoic acid and 2,4,6-trihydroxyphenylglyoxylic acid, which are the oxidation products of quercetin, are concentrated in the dry onion skin to protect the bulb from soil microbes. Perhaps, this is the cause of a higher total flavonoids content in the onion skin. Besides, Lee et al. (2008) also reported that onions tend to synthesize flavonoids, which are mainly distributed in the onion skin to protect the onion against damage from UV radiation and intracellular hydrogen peroxide Therefore, it can be concluded that ROS extract is a better antioxidant, anti-inflammatory, and anti-obesity additive than the ROB extract.

Table 2 shows the effects of the addition of ROB and ROS extracts on the DPPH scavenging activity, albumin denaturation inhibition activity and pancreatic lipase inhibition activity of cottage cheese. Based on the results in Table 2, the control cheese had the lowest ($p < 0.05$) DPPH, ADI and PLI activities. The addition of ROB and ROS extracts had respectively increased the DPPH scavenging activity of ROB- and ROS-cheeses by 1.44 folds and 1.50 folds, compared to the control. However, the DPPH scavenging activity of ROB- and ROS-cheeses was no significant difference ($p > 0.05$). This observation may be due to the complex interaction between proteins in the cheese and the flavonoids. The formation of protein-flavonoid complexes, either soluble or insoluble, limit the antioxidant functionality of the compounds, causing the decrease in the bioavailability of flavonoids (Kamiloglu et al., 2021). However, anti-inflammatory and anti-obesity properties of ROB- and ROS-cheeses were significantly different ($p <$

0.05) as the ADI and PLI activities of ROS-cheese were significantly higher ($p < 0.05$) than the ROB-cheese, the ADI and PLI activities of ROS-cheese is 1.62 folds and 2.03 folds of ROB-cheese, respectively.

Table 2. DPPH scavenging, albumin denaturation inhibition, and pancreatic lipase inhibition activities of control cheese, ROB-cheese and ROS-cheese (n=3).

Samples	DPPH Scavenging Activity (%)	Albumin Denaturation Inhibition Activity (%)	Pancreatic Lipase Inhibition Activity (%)
Control Cheese	6.27 ± 0.18^{b}	1.24 ± 0.25^{c}	1.57 ± 0.55^{c}
Red Onion Bulb-Cheese	9.03 ± 0.26^{a}	20.36 ± 0.63^{b}	7.50 ± 0.50^{b}
Red Onion Skin-Cheese	9.38 ± 0.15^{a}	32.98 ± 0.75^{a}	15.25 ± 0.65^{a}

Note: Different superscript lowercase letters in each column of cheeses are significantly different ($p < 0.05$).

This study proves that ROS extract is superior to ROB extract in enhancing the bioactive properties of cottage cheese.

CONCLUSION:

In this study, ROB and ROS extracts were added into the cottage cheese to determine their effects on the bioactive properties in terms of antioxidant, anti-inflammatory, and anti-obesity activities. ROS extract exhibited significantly higher bioactivity than ROB extract. Both ROB- and ROS-cheese had significantly higher bioactive properties compared to the control cheese. The ROS-cheese exhibited significantly higher anti-inflammatory and anti-obesity properties than ROB-cheese. It is recommended that red onion skin extract is a better substrate as compared to red onion bulb extract as the bioactive additive in cottage cheese. This study provides a preliminary insight into the novel concept of zero-waste processing by recycling the onion skin in health food production.

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OPTIMISATION OF ENZYME HYDROLYSIS CONDITIONS ON THE CHEMICAL AND BIOACTIVE PROPERTIES OF LOW-GRADE EDIBLE BIRD'S NEST (EBN) USING NEUTRASE

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Abstract: Low-grade EBN is broken EBN pieces that are highly contaminated with bird feathers, nonetheless contain almost equivalent nutritional value as the premium grade. This by-product is usually discarded due to its low market value even after tedious and laborious cleaning process. This research was conducted to maximise the chemical (viscosity, degree of hydrolysis, total soluble protein, glycoprotein content), and bioactive (antioxidant, anti-inflammation, hypoglycemic activities) properties of the low-grade EBN through enzymatic hydrolysis using Neutrase enzyme. The five-levels central composite design response surface methodology (CCD-RSM) with three parameters, namely EBN concentration (X_1: 1-7%w/v), enzyme concentration (X_2:1-3%w/v) and hydrolysis time (X_3: 1-4 hr) were employed. Through the optimization process, EBN hydrolysate (EBNH) produced via the hydrolysis of 5.55% of EBN using 3.0% of Neutrase for 1.02 hours contained 222.83±6.61mg/g of total soluble protein and 142.61±4.51mg/g of glycoprotein with 2.99±0.10% degree of hydrolysis and low viscosity at 3.06±0.13mPa·s. Besides, the EBNH was also proven to exhibit 65.72±1.34% ABTS scavenging activity (at 1.39 %w/v EBNH), 21.14±4.19% DPPH scavenging activity (at 2.78 %w/v EBNH), FRAP (450.67±49.06µmol/L $FeSO_4$), 17.65±1.11% of α-amylase inhibition activity and 87.43±3.2% anti-lipoxygenase activity (at 2.78 %w/v EBNH). The results were validated at 95% confidence interval. This study provides basic data for further scale-up processing to transform low-grade EBN into value-added EBN glycopeptides.
***Keywords*:** Optimization, Enzyme Hydrolysis, Edible Bird's Nest, Neutrase

INTRODUCTION

EBN is the secreted saliva of swiftlet species *Aerodramus* during reproduction, which is usually highly contaminated with impurities like feathers and dirt. EBN rich in glycoprotein, sialic acid, minerals, and other nutrients. The health-promoting properties of EBN includes anti-aging, anti-oxidative, anti-influenza viral properties, promoting cell proliferation, enhancing complexion, neuroprotective effect, strengthening bone, immune system and overall general health. A large amount of EBN by-products, which is difficult to be effectively utilized, are generated during the cleaning process, eventually turning into waste (Cao et al., 2022). Previous studies have shown that there is no significant difference in the nutritional composition of EBN by-products and EBN, and the hydrolysate of EBN (EBNH) has been proven to display a better protein efficiency ratio. These findings indicate that EBNH is a better protein source than raw EBN. Besides, the study also proved that DPPH scavenging activity and FRAP of EBNH were almost doubled of the raw EBN (Jin Wei Alvin et al., 2020). These finding suggests that hydrolysis potentially improve bioactivity of EBN.

Since decades, hydrolysis is widely employed to modify physicochemical and bioactivity properties of food proteins. Enzymatic hydrolysis using commercial food-grade enzymes is the preferred option because of easy control in process to produce hydrolysates with consistent desirable properties, such as in terms of the peptide chain length. Besides, previous study also proved that enzymatic hydrolysis increases total polysaccharide, reducing sugar, peptide and amino acids contents of EBN solution. Therefore, enzymatic hydrolysis process is adopted as the highly potential technology in the EBN industry to recover the valuable bioactive glycopeptides from the low-grade EBN and its by-products (Ng et al., 2020). Furthermore, degree of hydrolysis (DH) was also reported to be influenced by hydrolysis time and enzyme concentration, at where a longer hydrolysis time led to a higher DH (Mat Amin, X.X, et al., 2019). Therefore, optimization of EBN enzymatic hydrolysis process is important to regulate the enzymatic hydrolysis process as the foundation to comprehend the structure-function relationship of EBNH.

Due to the proven nutritional values of the low-grade EBN, this research was conducted to optimise the enzyme hydrolysis process using Neutrase enzyme to transform the low-grade EBN into EBNH with enhanced bioactive properties. The five-levels central composite response surface methodology (CCD-RSM) was employed to optimize the EBN concentration, enzyme concentration and hydrolysis time to produce EBNH with the maximum

antioxidant, anti-inflammatory and hypoglycemic activities. Data of this study is expected to serve as the fundamental information for the future scale-up production.

MATERIALS AND METHODS

Materials: Clean low-grade edible bird's nest (*Aerodramus fuciphagus*) was supplied by Nestlin Malaysia Sdn. Bhd., Tangkak, Malaysia. The chemicals used include hydrochloric acid, Bradford reagent, trolox, methanol (Merck, Germany), Neutrase (0.8U/g), leucine, picrylsulfonic acid, 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), 2,2-Diphenyl-1-picrythydrazyl(DPPH), α-amylase, lipoxidase, linoleic acid, catechin hydrate, peroxidase, periodic acid, Schiff's fuchsin-sulfite reagent (Sigma, U.S), sodium hydroxide, sodium dihydrogen phosphate, disodium hydrogen phosphate, bovine serum albumin, sodium acetate 3-hydrate, iron (III) chloride anhydrous, ferrous sulphate, potassium persulphate, 3,5-Dinitrosalicyclic acid, potassium sodium tartrate-4-hydrate, starch solution, boric acid, ascorbic acid (Chemsoln, Malaysia), glacial acetic (Bendosen, Malaysia) acid were all in analytical grade.

Method:

The low-grade EBN was ground using a knife mill (GX-10B, Gao Xin). The particle size of the EBN powder was determined at the average of ≤150 µm using a sieve shaker (Analysette 3 Spartan, Fritsch). The EBN powder at a specific concentration (as shown in table 1) was soaked in water for 16 hrs at refrigerated temperature, then boiled for 30 mins before subjected to enzymatic hydrolysis. The pH of the boiled EBN was adjusted to 8 before enzyme was added. The EBN hydrolysis was carried out according to the parameters stated in table 1 using Neutrase enzyme in a shaking incubator shaker (N-Biotek) at 150 rpm.

The effect of three variables, namely EBN concentration (X_1: 1 – 7 %w/v), enzyme concentration (X_2: 1 – 3 %w/v) and hydrolysis time (X_3: 1 – 4 hrs) on the viscosity (Y_1), total glycoprotein content (Y_2), total soluble protein content (Y_3), DH (Y_4), ABTS scavenging activity (Y_5), DPPH scavenging activity (Y_6), FRAP assay (Y_7), α-amylase inhibition activity (Y_8) and anti-lipoxygenase activity (Y_9) of the EBNH produced were investigated through a five-levels face-centered central composite response surface methodology (CCD-RSM). The independent experimental variables were fixed in the range of 1 – 7 %w/v for X_1, 1 – 3 %w/v for X_2, and 1 – 4 hrs for X_3. A total of 20 runs were carried out according to experimental design created by the Design Expert 13.0.5 software (Stat-Ease Inc., USA).

Table 1: Experimental design of five-levels CCD-RSM for the optimization of EBN concentration (X_1), enzyme concentration (X_2) and hydrolysis time (X_3) with nine response variables (Y_1: Viscosity, mPa·s; Y_2: Total glycoprotein content, mg/g; Y_3: Total soluble protein content, mg/g; Y_4: DH, %; Y_5: ABTS scavenging activity, %; Y_6: DPPH scavenging activity, %; Y_7: FRAP, µM; Y_8: α-amylase inhibition activity, %; Y_9: Anti-lipoxygenase activity, %).

Run	X_1	X_2	X_3	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7	Y_8	Y_9
1	1.00	2.00	2.50	1.26	948.45	131.54	1.59	23.11	31.84	148.00	0.86	1.04
2	5.78	1.41	1.61	2.63	227.39	191.62	3.32	64.73	59.18	647.00	13.56	78.87
3	2.22	1.41	1.61	1.41	791.59	346.31	2.56	35.63	46.82	296.00	10.14	9.64
4	4.00	2.00	2.50	1.64	567.01	181.79	3.23	55.79	64.23	570.00	16.19	57.18
5	4.00	2.00	4.00	1.75	482.33	176.94	3.24	52.04	57.18	437.50	16.47	59.35
6	5.78	1.41	3.39	3.76	227.39	181.63	3.27	65.81	58.41	603.50	23.26	75.28
7	4.00	2.00	2.50	1.74	564.07	169.31	3.30	54.62	63.78	561.00	13.84	56.11
8	4.00	2.00	2.50	1.71	552.65	174.52	3.40	52.68	65.17	558.00	14.55	56.62
9	5.78	2.59	1.61	3.66	227.64	168.54	3.45	62.34	63.13	867.00	22.83	89.29
10	7.00	2.00	2.50	6.25	157.80	187.25	3.51	69.96	57.81	665.50	13.78	84.47
11	4.00	3.00	2.50	1.93	508.84	175.59	3.42	56.58	65.64	576.50	14.10	56.96
12	4.00	1.00	2.50	1.44	449.19	194.26	3.07	54.57	69.02	491.00	12.87	55.20
13	5.78	2.59	3.39	3.35	251.32	156.62	3.47	63.33	58.23	656.50	7.27	83.73
14	2.22	2.59	3.39	1.20	777.29	159.29	2.65	31.27	38.43	293.00	9.54	23.00
15	4.00	2.00	2.50	1.66	550.81	172.19	3.33	53.57	63.89	543.00	16.72	55.72
16	4.00	2.00	2.50	1.70	576.95	169.04	3.30	53.24	62.20	518.50	13.76	57.36
17	2.22	1.41	3.39	1.21	792.27	155.39	2.53	33.67	47.36	334.00	4.63	10.03
18	2.22	2.59	1.61	1.10	761.62	187.93	2.55	32.93	50.29	226.00	7.39	20.43
19	4.00	2.00	2.50	1.64	549.71	180.98	3.25	51.33	62.70	552.00	15.88	55.54
20	4.00	2.00	1.0	1.90	493.74	209.24	3.25	52.75	68.80	591.50	6.49	64.69

After enzyme hydrolysis, the EBNHs were boiled on hot plate for 5 mins to terminate enzyme activity before subject to analysis. The viscosity of the EBNH was measured using a rheometer equipped with DG26.7/T200/AL double-gap measuring geometry (MCR102, Anton Paar). The DH, total soluble protein (TSP) and total glycoprotein content (TGP) were determined by 2, 4, 6 – trinitrobenzenesulfonic acid (TNBS) assay, Bradford assay and Periodic Acid/Schiff (PAS) assay, respectively. Antioxidant activity was assessed by ABTS scavenging, DPPH scavenging and FRAP assays. Anti-inflammatory activity was evaluated based on anti-lipoxygenase activity whereas α-amylase inhibition activity was determined to assess the hypoglycemic activity (Hui Yan et al., 2022; Kazeem et al., 2013; Torres et al., 2018).

The correlation among 9 tested responses was determined through Pearson correlation analysis at 95% confidence level ($p<0.05$) by using SPSS software version 26 (IBM SPSS Statistics, USA). The multivariate analysis of CCD-RSM optimization and the prediction of optimum point were carried out using Design Expert 13.0.5 software (StatEase Inc., USA). The predicted optimum process parameters were validated by carrying out the experiment in triplicates at the predicted optimum point. The predicted parameters were validated at 95% confidence level ($p<0.05$).

RESULTS AND DISCUSSION

For optimization purposes, the response variables were chosen based on the significancy, goodness of the model and Pearson correlation coefficent between the response variables. All the response variables were significant ($p<0.05$) at quadratic model except TSP significant at 2FI model and inhibition of α-amylase activity significant at linear model. For Pearson correlation coefficient show significant correlation ($p<0.05$) between response variables, DH was highly correlated with ABTS scavenging (r=0.905), anti-lipoxygenase activity(r=0.894), FRAP assays (r=0.882), DPPH scavenging (r=0.857) and α-amylase inhibition activity (r=0.687). Among the three antioxidant assays, ABTS scavenging had the highest correlation (r=0.966) with anti-lipoxygenase activity, followed by FRAP assay (r=0.941) and DPPH scavenging activity (r=0.737). Anti-lipoxygenase activity was also highly correlated with α-amylase inhibition activity (r=0.653) while viscosity was positively correlated with ABTS scavenging activity (r=0.542) and anti-lipoxygenase activity (r=0.502). In addition, TGP had high negative correlation with anti-lipoxygenase activity (r=-0.954), ABTS scavenging (r=-0.949), FRAP assays(r=-0.880), DH (r=-0.809), inhibition of α-amylase activity (r=-0.625), viscosity (r=-0.618) and DPPH scavenging (r=-0.611). Thus, it could not be chosen as criteria for optimization process. Therefore, the response variables that were chosen for optimization of enzyme hydrolysis are viscosity, DH, ABTS scavenging activity and anti-lipoxygenase activity.

The quadratic models that explain the relationship between the independent variables (X_1, X_2, X_3) and responses (Y_1, Y_4, Y_5, Y_9) are as follows:

Y_1: $1.46 + 2.08X_1 + 0.141X_2 + 0.047X_3 + 0.325X_1X_2 + 0.32X_1X_3 - 0.4013X_2X_3 + 1.95X_1^2 - 0.126X_2^2 + 0.025X_3^2$ (R^2=0.96)

Y_4: $3.29 + 0.78X_1 + 0.13X_2 + 0.009X_3 + 0.081X_1X_2 - 0.038X_1X_3 + 0.07X_2X_3 - 0.737X_1^2 - 0.044X_2^2 - 0.052X_3^2$ (R^2=0.966)

Y_5: $53.9 + 24.52X_1 - 0.858X_2 - 0.453X_3 + 0.111X_1X_2 + 1.98X_1X_3 + 0.075X_2X_3 - 8.75X_1^2 + 0.354X_2^2 - 2.73X_3^2$ (R^2=0.985)

Y_9: $56.81 + 49.25X_1 + 5.61X_2 - 1.98X_3 - 1.79X_1X_2 - 4.23X_1X_3 + 0.076\ X_2X_3 - 16.5X_1^2 - 2.97X_2^2 + 3.04X_3^2$ (R^2=0.975)

By integrating the regression models, the optimum EBN hydrolysis conditions were predicted at 5.55 %w/v EBN concentration, 3.0 %w/v Neutrase concentration and 1.02 hrs hydrolysis time. Under this optimum condition, the EBNH produced is predicted to show its viscosity at 3.75 mPa·s, DH at 3.51%, 60.56 % ABTS scavenging activity (at 1.39 %w/v EBN) and 86.63% anti-lipoxygenase activity (at 2.78 %w/v EBN). These optimum parameters were successfully validated at 95% confidence interval ($p<0.05$), whereby the experimental values had no significant differences with the predicted values. The EBNH produced in the validation experiment was found to have viscosity at 3.06 ± 0.13 mPa·s, 2.99 ± 0.10 % DH and exhibited 65.72 ± 1.34 % ABTS scavenging activity (at 1.39 %w/v EBN) and 87.43 ± 3.20 % anti-lipoxygenase activity (at 2.78 %w/v EBN). Under this optimum condition, the EBNH produced was also found to contain 222.83±6.61mg/g of total soluble protein and 142.61±4.51mg/g of glycoprotein with 21.14±4.19% DPPH scavenging activity (at 2.78 %w/v EBNH), FRAP (450.67±49.06μmol/L FeSO4) and 17.65±1.11% of α-amylase inhibition activity.

Based on the results of ANOVA, the main factor that affects the chemical and bioactive properties of EBNH was EBN concentration. Only the term X_1 was significant ($p<0.05$) in all four selected quadratic models. For EBNH with higher EBN concentration, glycoprotein content is lower. This indicates enzyme can access to cleave the glycosidic bonding to produce glycopeptides and shorten the chain length which result in higher degree of hydrolysis. However, high viscosity was observed in EBNH with high EBN concentration. This indicates that there are more intermolecular interactions between the protein molecules, causing the aggregation in EBNH with high EBN concentration (Hui Yan et al., 2022). Besides, there was no interaction effect among the three independent variables (all interaction terms of

X_1X_2, X_1X_3, X_2X_3 were not significant with $p>0.05$) that significantly influence the response variables. Higher bioactivity is observed in the EBNH with higher EBN concentration. This shows that enzymatic hydrolysis on EBN produced EBNH in the form of bioactive glycopeptides which can enhance the bioactivity of hydrolysate. This is probably related to the unfolding and breaking down of the insoluble macro-glycoprotein, thus increased the bioaccessibility of the hydrophobic bioactive peptides exists within the EBN molecules (Hui Yan et al., 2022). Furthermore, DH and anti-inflammation activity increased significantly when enzyme concentration increased. This proved that higher concentration of enzyme will increase the accessibility of enzyme to cleave the protein bonding and resulting in higher amount of bioactive peptides that can catalyze anti-inflammation activity. Upon enzyme hydrolysis, more active amino acid R groups will be generated and exposed that leads to increased bioactivity (Mat Amin, Khuzma, et al., 2019).

CONCLUSIONS

This research successfully optimized the enzymatic process using Neutrase enzyme to produce EBNH with high antioxidant, anti-inflammatory and hypoglycemic activities. By hydrolysing 5.55% w/v of EBN using 3.0% w/v of Neutrase enzyme for 1.02 hrs, EBNH with 65.72 ± 1.34% ABTS scavenging activity (at 1.39 %w/v EBN), 21.14 ± 4.19% DPPH scavenging activity (at 2.78 %w/v EBN), 450.67±49.06 µM $FeSO_4$ of FRAP, 17.65 ± 1.11 % α-amylase inhibition activity and 87.43 ± 3.20 % anti-lipoxygenase activity (at 2.78 %w/v EBN) was produced. Finding of this study recommended low-grade EBN as an economically feasible nutraceutical for diverse future applications, such as in the development of functional foods, natural cosmetics, and potent pharmaceuticals. Future study to investigate the bioaccessibility of EBNH, particularly its bioactive peptide along the gastrointestinal tract is needed to verify its efficacy in application.

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APPLICATION OF BOX-BEHNKEN DESIGN WITH RESPONSE SURFACE METHODOLOGY IN THE OPTIMIZATION OF THERMALLY PROCESSED "*LECHON PAKSIW DE LEYTE*"

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Abstract: *Lechon* is a widely known delicacy that is always present in a Filipino home at every event and celebration. The Tacloban City Litson Industry Association (TACLIYA) is a group of micro-entrepreneurs producing "*lechon*" (roasted pig), that has an average daily excess production of 30 kilograms and surges up to 200 kilograms during off-peak season. These unsold products triggered problems of storage, storage cost, spoilage and wastage, and eventually profit loss. Retort technology was employed through screening variables and optimization through Plackett-Burman and a three-level, two-factor Box-Behnken Design, respectively. Response Surface Regression results revealed that the variables and levels used had linear and quadratic effects on the sensory parameters evaluated which produced significant differences in the acceptability ratings. The optimum combination was obtained with the highest possible acceptability as the boundary at $\geq$6.5 on the 9-point Hedonic scale. The optimum product was commercially sterile with Fo value of 5 minutes and an estimated shelf life of 1.5 years. The reworking of unsold *lechon* is a value addition generating more income for lechon producers for business sustainability. Moreover, other lechon producers in the country can benchmark the technology for inclusive economic growth.
Keywords: Box-Behnken Design, *Lechon*, Response Surface Methodology

INTRODUCTION

The Tacloban City Litson Industry Association (TACLIYA) is a group of micro-entrepreneurs producing '*Lechon*' (roasted pig) in Tacloban City. On the daily production, an average excess '*lechon*' of 30 kilograms is collated and surges up to 200 kilograms during off-peak season. These unsold products triggered problems of storage, storage cost, spoilage and wastage, and eventually profit loss. Thus, the need for processing techniques to rework the unsold 'lechon' is imperative. Reworking will introduce additional preservation methods, create convenience, and generate value addition to the product that will ultimately increase income for the association.

Eastern Visayas Food Innovation Center (EVFIC) proposed a method to allow the excess products to be reworked/reprocessed into a shelf-stable product that will perform well in the local market and expand eventually to the global market. One (1) reworked product identified is the "*Lechon Paksiw de Leyte*''. It can be noted that the term "*Leyte*" is used since "*lechon paksiw*' in Leyte is quite different from that of the other parts of the country. Lechon paksiw in Leyte is cooked with vinegar, garlic, and a little bit of soy sauce.

Value addition is presented through two (2) hurdle technologies namely acidification and thermal process or retort technology, and the use of more convenient packaging materials such as flexible pouches and jars. These technologies and materials will guarantee shelf stability and longer shelf life that could last for more than six months. The hurdle technologies will ensure the safety and quality of the product while the packaging materials will act as a barrier to maintaining product integrity.

Hurdle Technology uses the intelligent combination of different preservation factors or techniques to achieve multi-target, mild, but reliable preservation effects (Featherstone, 2015). More than one hurdle is implemented in order to create safe meat and meat products as well as to achieve the desired shelf life. The sum of several hurdles in place acts in a synergistic way to result in a safe product, with no negative impact on the quality of the product (Feiner, 2006). In modern meat processing, the effect of heat treatment can be supported by the application of "hurdles", which have the potential to slow down microbial growth. Such "hurdles" allow keeping the heat treatment of sterilized products at lower temperature levels, so that the product quality is less affected. Frequently used hurdles are lowering water activity or acidity in a product, or utilization of chemical preservatives (Heinz and Hautzinger, 2007).

Acidification will lower the pH level thus inhibiting the growth of microorganisms in food. After the process of acidification, the product will be subjected to a thermal treatment process using the water retort system of the Eastern Visayas Food Innovation Center.

Thus, developing a convenient, shelf-stable, value-added product would eliminate the problems identified by the Tacloban City Litson Industry Association (TACLIYA) and at the same time increase their product lines as well as their income. Aside from its longer shelf life, it can be distributed as a disaster food, considering that the Eastern Visayas is a disaster-prone area, with its expedient packaging and can be displayed well with an engaging label. Other 'lechon' producers in the country can also adapt this technology and benchmark this research towards economic growth.

MATERIALS AND METHODS

Raw Material and Product Preparation

The delivered unsold '*lechon'* was cooked according to formulations. Mixing was done using a spiral-type mixer and pre-heating in a kettle. The pre-formulated mixtures were then hot-filled in jars and/or retortable film/pouches, sealed, and thermally processed using the EVFIC Vertical Water Immersion Retort.

Factorial Experiment: Box-Behnken Experimental Design

In order to optimize the product formulation, a Box-Behnken Experimental Design (Montgomery, 2017) was employed with 15 treatments for experimental combinations with meat cut (1, 2, and 3 cm), sugar (100, 200, and 300 grams), and vinegar (75, 150, and 300 grams) as experimental variables. Such variables were identified from preliminary experiments conducted (Plackett-Burman Screening Experiment)

pH Determination

The equilibrium pH of the different treatments was determined using a calibrated bench-top pH meter (Thermo Scientific™ Orion Star™ A112 and/or Hanna Instruments HI5521). Calibration was performed using two (2) buffer solutions and a minimum slope of 92%. Three (3) readings were taken and the mean of the three values was recorded as the pH of the product.

Commercial Sterility Testing

Commercial Sterility Testing was conducted based on the methods of the Bacteriological Analytical Manual (BAM), online (BAM 21A) for low-acid foods. Samples were first incubated at 30^{o}C for ten (10) days, then undiluted 1 – 2 grams of each sample were inoculated to prepare Cooked Meat Medium and Bromcresol Purple Dextrose Broth and incubated at 35^{o}C and 55^{o}C with positive and negative controls.

Product Sensory and Quality Evaluation

The different commercially sterile treatments were subjected to sensory evaluation to determine the acceptability of the product in terms of Appearance (Meat Size), Aroma, Taste, Texture, and General Acceptability using a 9-point Hedonic scale labeled from "dislike extremely" to "like extremely (Fu and Labuza, 1997). An Incomplete Block Design (IBD) as laid out by Cochran and Cox (1957) was used during the presentation of the different treatments since (15) treatments were too many for each panelist to evaluate. The set plan of t=15, k=3, r=7, b=35, λ=1, E=0.71, Type I was followed with seven (7) observations per treatment with two (2) runs from 35 recruited, screened, selected, and trained panelists

Statistical Analysis

The response surface regression (RSREG) analysis was employed in the analyses of the sensory qualities and the acceptability for all formulations of the product using StatSoft STATISTICA 6.0 online. The same software was used for the graphical presentation of the response surface plots, predicted response of each treatment, mode of descriptive scores, and product desirability profiling using the sensory acceptability results.

Thermal Validation

The heat distribution trials were done using dummy products" in starch solution. Heat penetration test on the other hand was conducted at 121.1^{o}C and 116^{o}C as the reference and processing temperatures, respectively. Since the product was low-acid, the target microorganism was Clostridium botulinum with a death rate or z-value of 10^{o}C, the process calculation used was Fo=5.0 minutes, using Ball's Formula Method using the Valsuite Pro ver. 6.2.2.0 software (Ellab A/S, Trollesmindealle 25, DK-3400 Hillerød, Denmark)

Accelerated Shelf-life Testing

Accelerated Shelf-Life Testing (ASLT) was conducted using test temperatures of 35ºC, 45ºC, and 55ºC. The target longevity was one (1) year for the product in cans and six (6) months in pouch. Evaluations or tests performed were pH determination, commercial sterility testing, and sensory evaluation employing a Focus Group Discussion (FGD) on perceptible changes in the products through time.

A mathematical model using the Arrhenius equation, allowed the estimation of the shelf-life of the products at 30ºC using first-order reaction kinetics at elevated conditions, plotted by reporting the changes of the natural logarithm of pH (ln pH) as a function of the storage time (1/T-1/Tref, measured in K).

$$k = k_{ref}\, exp\left[-\frac{E_a}{R}\left(\frac{1}{T^*}-\frac{1}{T_{ref}}\right)\right]$$

Where $\boldsymbol{k_{ref}}$ and $\boldsymbol{E_a}$ were substituted to the corresponding estimates and T* was the temperature at which to predict shelf-life at 30ºC using the formula:

$$SL = \frac{lnI_0 - I_{lim}}{k_{ref}\, exp\left[-\frac{E_a}{R}\left(\frac{1}{T^*}-\frac{1}{T_{ref}}\right)\right]}$$

Where $\boldsymbol{I_o}$ was the experimental value of $\boldsymbol{I}$ at time zero (0) or the initial pH of the product, and $\boldsymbol{I_{lim}}$ was the limit of the pH value for low-acid food, which is **4.6**

RESULTS AND DISCUSSION

Product pH and Commercial Sterility

pH values ranged from 4.63 - 5.31 with a mean pH value of 4.91, indicating that the samples are Low-Acid foods with the target microorganism *Clostridium botulinum*. Moreover, no observed bubbling or gas production was exhibited with the anaerobic tubes at different incubation temperatures and no color change and growth were observed with aerobic tubes; indicating commercial sterility of the products produced.

Optimization Results

The summary of F-ratios from the Analysis of Variance (ANOVA) and parameter estimates using response surface regression are presented in **Tables 1** and **2**, respectively. In terms of the significant differences between the treatments, Meat Size was with high significance at p<0.001 except for the texture acceptability. As expected, sugar was significant for the taste response. However, with the regression coefficient results, the appearance attribute for the meat size variable was the only significant with a positive linear coefficient, suggesting the bigger size of meat is preferred. Other attributes were not significant even though their ANOVA results were, indicating a joint effect of variables.

Table 1. Summary of the ANOVA F ratios for all Sensory Parameters

Regression	Appearance	Aroma	Taste	Texture	General
(1) Sugar L+Q	0.089	0.791	4.802**	1.241	1.366
(2) Vinegar L+Q	0.334	0.885	0.837	0.150	0.291
(3) Meat Size L+Q	3.213*	5.904**	6.920**	1.254	6.430**
Error	518.98	368.00	457.39	443.73	367.31
Total SS	537.53	401.28	514.02	466.20	396.49

*- significant at P<0.05 **- significant at P<0.01 ***- significant at P<0.001

Table 2. Summary of the Regression Coefficients for all Sensory Parameters

Regression	Appearance	Aroma	Taste	Texture	General
(1) Sugar L	-0.075	0.776	0.887	-1.434	1.018
Sugar Q	0.143	0.712	-0.389	1.513	-0.702
(2) Vinegar L	0.696	0.104	0.084	0.380	1.234
Vinegar Q	-0.494	0.187	-0.299	0.517	-0.640
(3) Meat Size L	2.299*	0.082	0.833	1.522	1.799
Meat Size Q	-1.615	0.450	-0.214	-0.517	-0.160

*- significant at P<0.05 **- significant at P<0.01 ***- significant at P<0.001

Contour plots were integrated and superimposed (**Figure 1**), identifying a sensory acceptability rating of ≥6.5 as the cut-off point for all the sensory responses in selecting the region that is most desirable. At constant (a) 200

grams sugar, (b) 150 grams vinegar, and (c) 2 cm meat size. The limiting response was the texture acceptability of the products which was greatly affected by the joint effect or interaction of vinegar and meat size variables. This was very evident in the ANOVA and regression coefficient results. Examining visually the overlaid contour plots, taking into consideration the cost of the ingredients and acceptability of the product; the optimum region was at 260 grams sugar, 290 grams vinegar, and 2.8 cm meat size at ≥6.5 hedonic sensory acceptability.

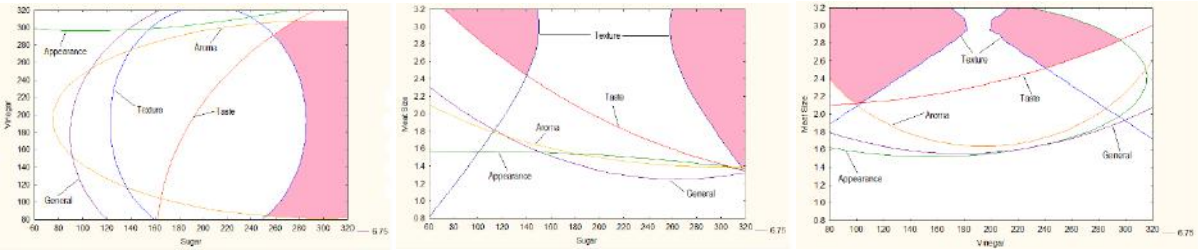

Figure 1. Optimum Region and Formulation of Lechon Paksiw de Leyte

Calculated Process Schedule

The calculated processing schedule at a processing temperature of 250.0 ºF (121.1 ºC) and assumed Fo value of 5.0 minutes were: a) 35min and 4.20sec at 45.0ºC; b) 37min and 25.20sec at 35.0ºC; c) 39min and 31.20sec at 25.0ºC; and d) 41min and 24.60sec at 15.0ºC;

Estimation of Shelf-life

Based on the pH values as well as the commercial sterility test results, shorter longevity was depicted on packaged products in a pouch. The End-of-Shelf-life (ESOL) was established when the Cooked Meat Medium exhibited bubbling or gas formation, indicative of microbial positive growth. The shelf life plots (**Figure 2**) indicate the estimated shelf-life of the products as 448 days and 337 days, for cans and pouches, respectively.

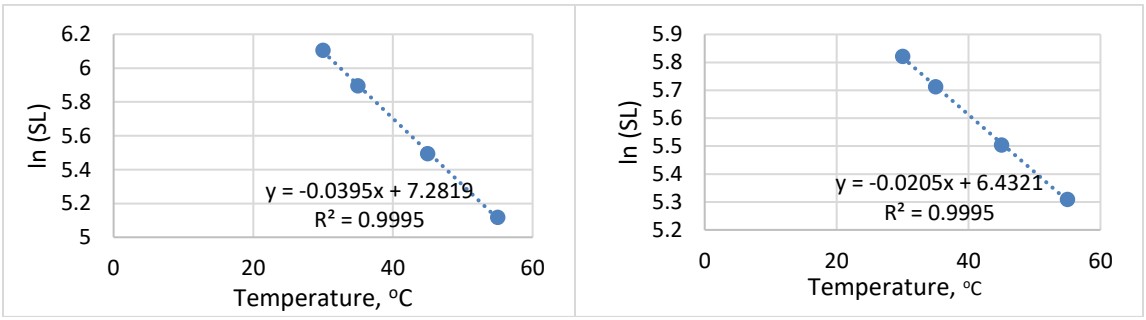

Figure 2. Shelf-life plot (left) can, (right) pouch

CONCLUSIONS

Though considered waste, unsold lechon can be reprocessed using retort technology and is acceptable when packaged with convenient materials such as tin cans and flexible pouches. Additionally, processing at elevated temperatures and pressurized equipment increases products keeping quality to (6) months to one (1) year.

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IMPACT OF SUSTAINABLE AGRICULTURAL CULTIVATION SYSTEM POLICY ON FOOD SECURITY AND WELFARE OF RICE FARMERS IN SOUTH SUMATRA PROVINCE, INDONESIA TITLE

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Abstract: Ensuring Food Security has become an issue for a critical country at various levels of economic development, but the agricultural sector plays a strategic role in improving food availability. Sustainable food production and environmental protection should be government policy. This research aims to analyze the impact of the sustainable agricultural cultivation system (SACS) policy on food security and the welfare of rice farmers. The area of the research, Tanjung Lago District, of South Sumatra province, were selected purposively. The research method used is the survey method, and the sample withdrawal method is a sample random method with 30 farmers in 2021. The results showed that with the implementation of SACS, land productivity increased to 6,5 tons/ha, previously 4,5 tons/ha. Therefore, the farmers' income also increased, amounting to Rp1,647,464.00/ha. Food availability increased the impact of production, and the rice stock was steadier than before. Better food affordability due to distribution, stabilization of supply and price, stock management, access to markets and information are raising the usability of food, including improving consumption patterns and food safety and quality more stadily. The welfare of farmers is feeling prosperous because of the SACS. So, SACS could improve food security and farmers' welfare.
***Keywords*:** agriculture, food security, sustainable, welfare.

INTRODUCTION

El Niño and La Nina as implications of climate change can have a negative impact on agriculture production (Li et al., 2020). These two extreme climatic conditions have at least caused a drastic decline in rice productivity in Indonesia by -0.50% and -0.65% respectively for 40 years from 1970-2010 (Khairullah et al., 2021). In addition to quantity, changes in the price and quality of agricultural commodities are indicators that show that climate change significantly impacts agricultural production (Anderson et al., 2020) Extreme climate change also causes the emergence of pests and diseases that attack crops and have an impact on decreasing agricultural production (Gomez-Zavaglia & Mejuto, 2020). Climate change is becoming an unavoidable natural phenomenon. If allowed to continue, it will indirectly become a threat to food security and farmers' welfare (Lenderking L. et al., 2021).

Nevertheless, food security has a strong relationship to the economic growth of a region. At least 76.92% of 26 empirical studies conducted throughout 2004-2021 show that food security and economic growth have a positive relationship (Fernandes & Samputra, 2022). This opinion is also reinforced by research results from(Ceesay & Ndiaye, 2022) which states that food security is correlated with economic growth. Adequate food security is a basic human need, being a source of human nutrition and energy to carry out daily activities (Fanzo et al., 2018). Meeting sufficient food needs can participate in encouraging one's behavior in carrying out economic activities. Moreover, the background of Indonesian people who are mostly farmers, there needs to be a special policy made by the government and oriented towards food security and farmers' welfare.

In several Southeast Asian countries such as Malaysia, the establishment and implementation of specific policies in the agricultural sector is carried out as a form of climate change mitigation and adaptation (Tang, 2019). Other studies show that sustainable agricultural policies that focus on increasing agricultural production and technology are positively able to increase agricultural productivity and farmer welfare (Shikur, 2020). Other sustainable agricultural policies at the local/regional level that focus on improving infrastructure and providing clean water have proven to be able to contribute to food security and economic growth (Chemin et al., 2019). On the other hand, the state also has an obligation to ensure food availability and achieve food welfare for the community (Mukadar et al., 2019).

In Indonesia itself, sustainable agriculture cultivation system (SACS) policy is present as an effort to realize food sovereignty and security. Tanjung Lago is one of the regions in South Sumatra that has implemented this policy since 2015. Tanjung Lago is one of the regions in South Sumatra which has an area of tidal rice fields reaching 15.59 Hectare. Tidal land can be used as an effort to support the success of SACS through various technological innovations. The forms of realization of the sustainable agriculture cultivation policy that have been carried out include the following: 1) Improvement of t irrigation technic; 2) assistance with driving equipment in the form of a water pump

machine; 3) seed feeding and the fertilizers; 4) The placement of extension workers who are ready to assist farmers in overcoming various problems faced.

According to the problem description above, thus this paper presents a comprehensive review of how sustainable agriculture cultivation systems can fill the gap between the threat of climate change to food security and the welfare of farmers which is one of the pillars of regional economic development.

MATERIALS AND METHODS

The study was conducted in Tanjung Lago District, South Sumatra Province, which is located between latitude 2°39'6.17"S and longtitude 104°42'9.79"E was deliberately chosen as the research location because it has a large tidal rice field area and is one of the food granary locations in South Sumatra. The research was conducted directly through an interview method of 30 farmers who were randomly selected from a total of 105 farmers who had implemented sustainable agricultural cultivation policies. The data collection method used in this study is the survey method, in order to obtain opinions or opinions from farmers through direct interaction with the observed objects. All variables in the questionnaire are valid and reliable. The data processing method is carried out using descriptive statistical analysis and multiple linear regression formulated with the following model:

$$LS_i = (\alpha_1 He\alpha_i\circ) + (\alpha_2 Eco_i\circ) + (\alpha_3 Job_i\circ) + (\alpha_4 Fam_i\circ) + (\alpha_5 Fri_i\circ) + (\alpha_6 Per_i\circ) + \mu i$$

RESULTS AND DISCUSSION

Socio-Demographic Profile of Rice Farmers

Rice farmers' socio-economic factors are known to affect the impact of sustainable agricultural cultivation system policy on food security and welfare, namely gender, age, farming experience, education, farm size and household size. In accordance with previous research several variabels above can explain the socio-demographic of farmers. This study shows that based on the gander category, all respondents are men who have a dominant role in running the sustainable agriculture cultivation system policy. In contrast to research conducted by (Satama et al., 2022) which shows that women actually have a more dominant role, especially in decision making, knowledge and adoption of sustainable measures. Based on the age category, farmers can be classified into productive age categories (Issahaku et al., 2020) and have had long independent farming experience, which is an average of 22 years. Age maturity and experience of farming independently will influence the attitude of individuals towards agriculture and the environment (Euriga, 2008). The more mature the age and the longer the experience of farming, the more concerned a person will be about agricultural activities and the environment (Mulyaningsih et al., n.d., 2018). Based on the education categories, most farmers get access to education until junior high school. (Van Thanh & Yapwattanaphun, 2015) in their research mentioned that the higher a person's level of education, the more they understand that the natural environment will affect human survival. Based on the category of land area, most farmers can be classified as large farmers (Assan, 2019) because they have an average land area of more than 1 hectare. Finally, based on age categories, most farmers have a fairly large number of family members, which is an average of 4 people. One of the advantages of having large family members is that farmers can involve family members in farming activities carried out (Oyewole et al., 2022)

The Impact of Sustainable Agricultural Cultivation System (SACS) Policy on Food Security

The impact of the sustainable agriculture program policy on food security in Tanjung Lago District is analyzed by comparing the values of food security indicators. Several indicators of food security index that will be analyzed including food availability, food accessibility, and food utilization and stability. These indicators are measured by comparing the conditions before and after the sustainable agricultural cultivation system policy is implemented. Based on the availability indicators, shows that the implementation of sustainable agricultural cultivation policies can be increase rice production by 20% and total production increase by 4.5%. It was happened because the implementation sustainable agricultural cultivation policies focus on improving agricultural production facilities, such as seed assistance, fertilizers and improving irrigation systems for rice fields. Thus, with the improvement of irrigation, farmers are able to carry out farming activities twice a year. This is also in line with the results of the research by (Materu et al., 2018)which states that one of realization of sustainable agricultural policies in the form of the application of irrigation techniques in rice farming can increase production by 1.5 tons /hectare/year while saving water by 33%. Nevertheless, it does not show a significant change in the amount of food stock by farmers. An increase in rice production will indirectly increase farmers' income. Generally, economic theory states that the higher the family income, the higher the quantity of food. This study shows is different from the research conducted by (Kansiime et al., 2021)which shows that income affects the quality of household food consumption, such as during the Covid-19 pandemic which caused a shock in household income and had an impact on increasing the proportion of food insecurity by 38% and 44% in Kenya and Uganda. Based on the accessibility

indicators, the implementation of sustainable agriculture policies has no influence on the marketing channels. This is not in line with the results of research conducted by (Saleh & Endang S., 2023) which states that sustainable agriculture policies allow farmers to be able to produce superior commodities. While the superior commodities should have a bargaining position to be able to directly distribute agricultural products efficiently to consumers. A sufficient distribution and good marketing infrastructure is considered a dissemination of agricultural change in sustainable agriculture (Adenle et al., 2019). Based on the utilization and stability indicator, shows that there is no change in the consumption pattern of farming families and farmers' food adequacy. However, in terms of quantity, farmers' food sufficiency increased in line with the increase in rice production which originally could only be harvested once a year into twice a year. It shows that sustainable agricultural cultivation system (SACS) policies as an effort to mitigate food insecurity have been successful implemented.

The Impact of the Sustainable Agricultural Cultivation System (SACS) Policy on Rice Farmers' Welfare

This study shows that the variables of health, work, family and friendship partially have a significant relation to farmer's welfare with the SACS policy. The existence of technical assistance, one of which is by providing agricultural equipment such as machines, can help reduce the level of work of farmers where usually worked on traditionally and require lighter and more efficient effort (Khodijah et al., 2022). This indirectly makes farmers feel prosperous in terms of health, family and work. This is also in line with the research conducted by which in research states that the purpose of implementing this SACS policy is to improve the standard of living and health of farmers. (Guth et al., 2022) also stated that technical efficiency through time, effort and costs emerged as a positive impact from the existence of the SACS policy. Furthermore, this SACS policy does not only provide technical assistance to farmers, but also non-technical assistance such as extension efforts. Extension activities are usually carried out massively to members of farmer groups. In extension activities, social capital acts as a tool that be able to create harmonious interactions both between extension agents and farmers and among the farmers themselves (Prayitno et al., 2022).

CONCLUSIONS

Food security and farmers' welfare are important issues that need attention because they are related to the economic development of a country. The existence of a sustainable agriculture cultivation system (SACS) policy comes as a mitigation effort to achieve food security and farmer welfare needs. The results of the study of the three food security indicators, namely availability, accessibility and utilization and stability, are proven that the SACS policy in Tanjung Lago District is able to meet the family food needs. Especially, in the availability of food productivity which has increased dramatically due to the assistance of various kinds of technical and non-technical assistance received by farmers. In line with this, another finding of this study shows that the implementation of the SACS policy can created farmers welfare which is generally indicator measured by economic satisfactions variables, but there are other variables such as health satisfactions, job satisfactions, family satisfactions and friendships satisfactions which are other welfare indicator findings. Overall, the implementation of SACS policy in Tanjung Lago District has succeeded fill the enhancement food security and farmers' welfare needs.

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DEVELOPMENT AND CHARACTERIZATION OF ELECTROSPUN ZEIN-BASED COATINGS FUNCTIONALIZED BY CAFFEIC AND *P*-COUMARIC ACID FOR ACTIVE PACKAGING APPLICATIONS

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Abstract: This study focused on the fabrication of zein-based coatings functionalized by two phenolic acids, namely caffeic acid (CA) and *p*-coumaric acid (*p*CA) using electrospinning for active food packaging applications. The objectives of the study were to determine the antioxidant and antibacterial properties of zein electrospun fibers incorporated with CA and *p*CA and evaluate their physicochemical properties. The electrospun fibers were fabricated with three different concentrations (5, 10 and 20% w/w with respect to zein) of CA and *p*CA individually. The average fiber diameter increased due to the addition of phenolic acids. While new weight loss stages occurred corresponding to CA and *p*CA thermograms, degradation temperatures of the zein electrospun fibers were not significantly affected ($p > 0.05$) as revealed by thermogravimetric analysis. CA loaded zein electrospun fibers exhibited increasing DPPH radical scavenging activity ranging from 23.42 to 71.40% with increasing CA concentration. Both phenolic acids loaded zein electrospun fibers displayed favourable antibacterial activities against the Gram positive *Staphylococcus aureus* and Gram negative *Escherichia coli* foodborne pathogens. Nevertheless, these zein-based electrospun fibers also exhibited rapid release profiles of the incorporated phenolic acids. Overall, zein electrospun fibers with 20% w/w CA demonstrated the most desirable properties for potential active food packaging application among all the formulations. An application study with actual food product can be conducted as future studies to determine the system's performance over a period time.
Keywords: electrospinning; zein; phenolic acids; coatings; active performance.

INTRODUCTION

The growing demand for improved food safety and quality has driven the continuous research and development of functional packaging system. Active food packaging is an example of innovative packaging approach that has been designed to extend shelf life of products and provide functions beyond barrier properties. More recent advances in food packaging development have focused on the application of nanomaterials, particularly the use of nanofibers as coatings for active food packaging applications due to improved functional and structural properties (Zhang *et al.*, 2020). Electrospinning is one of the most versatile, cost effective and simple techniques for fabrication of nano to sub-micron fibers (Shishir *et al.*, 2018). The electrospun fiber mats can serve as coatings that may be functionalized through addition of active compounds to create an active food packaging system (Zhang *et al.*, 2020). Additionally, their unique properties such as large surface area, sensitivity to surrounding environment and suitability to encapsulate thermally labile active ingredients provide advantages to support such applications (Vega-Lugo and Lim, 2009). Zein, a major storage protein found in corn, is a biopolymer with high thermal resistance, great barrier properties and ability to form films (Shukla and Cheryan, 2001; Torres-Giner, Gimenez and Lagaron, 2008). Due to these properties, zein has been used as coating for candies, fresh and dried fruits, and nuts (Lai and Padua, 1997; Bai *et al.*, 2003). The incorporation of phenolic compounds including phenolic acids from plants as bioactive agents in packaging system has gained interest as they are known for their potent antioxidant and antimicrobial properties, which are preferred among consumers as natural ingredients (Singh, Kim and Lee, 2022). Hence, the objectives of this study were to determine the antioxidant and antibacterial properties of zein-based electrospun fibers loaded with caffeic (CA) and *p*-coumaric (*p*CA); and to evaluate the physico-chemical properties of the developed zein-based electrospun fibers loaded with CA and *p*CA.

MATERIALS AND METHODS

Zein powder (Z 3625), CA and *p*CA standards and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Aldrich (St Louis, USA). Absolute ethanol, methanol, tryptic soy broth were purchased from Merck KGaA (Dermstadt, Germany). Nutrient agar and peptone were purchased from OXOID (Basingstoke, England). Deionised water was used for the preparation of all chemical solutions throughout the study and was obtained from the Micromeg deionizer water filtration system (Elga, High Wycombe, United Kingdom). *Escherichia coli* ATCC 2922 and *Staphylococcus aureus* ATCC 29737 used for the antibacterial activity assay in present study were obtained from Taylor's University Lakeside Campus School of Biosciences' microbiology laboratory.

PREPARATION OF ELECTROSPINNING SOLUTIONS

Zein and zein-phenolic acid solutions were prepared based on method reported by Neo *et al.* (2013) with minor adjustments. For zein electrospun fibers containing CA, various concentrations were prepared at 5%, 10% and 20% (w/w), with respect to zein and were denoted as Ze-CA5%, Ze-CA10% and Ze-CA20%, respectively. They were prepared by first dissolving zein powder in 80% ethanol aqueous solutions to obtain 25 wt.%, w/w zein solutions followed by the addition of CA at different concentrations individually. Zein electrospun fibers with 5%, 10% and 20% w/w of *p*CA, denoted as Ze-*p*CA5%, Ze-*p*CA10% and Ze-*p*CA20%, respectively were prepared in the same manner.

ELECTROSPINNING PROCESS

The zein and zein-phenolic acids solutions were placed in 5 mL plastic syringe (Terumo, Leuven, Belgium) with 22-gauge metallic needle (Terumo, Leuven, Belgium) separately. The solutions were driven by syringe pump (NLS20, NLi, Nanolab Instruments Sdn Bhd, Selangor, Malaysia) at 0.5 mL/h flow rate and a voltage of 16 kV was applied to the needle using a high voltage power supply (PS35-PV, NLi, Nanolab Instruments Sdn Bhd, Selangor, Malaysia). The distance from needle tip to ground collector was set at 13 cm. The electrospun fibers deposited on an aluminium foil covering the ground collector were collected and stored in air tight bags until further analysis.

AVERAGE FIBER DIAMETER

The morphologies and average fiber diameter (AFD) of neat zein, Ze-CA and Ze-*p*CA electrospun fibers were observed using scanning electron microscopy (SEM). Approximately 100 fibers were randomly measured using image analysis software (Image J, NIH, Maryland, USA) to determine the AFD.

THERMAL ANALYSIS

Thermal properties of the fiber mats were determined using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). TGA was conducted using TGA-8000 (Perkin Elmer, Waltham, USA) where a 10 $^{\circ}$C/min heating rate from 40 ^{o}C to 600 ^{o}C was applied under nitrogen flow rate of 25 mL/minute. Meanwhile for DSC, sample was placed in a aluminium pan (BIO16-9321, Perkin Elmer, Waltham, USA), sealed and heated from 25-200 $^{\circ}$C under nitrogen gas flow at 10 $^{\circ}$C/min, scanning speed using DSC-8000 (Perkin Elmer, Waltham, USA). All measurements were carried out in triplicates on separate occasions.

ANTIOXIDANT ACTIVITY ASSAY

The DPPH antioxidant activity was performed according to the method described by Neo *et al.* (2013). The absorbance was recorded at 516 nm using a microplate reader (Epoch2, BioTek, Winooski, USA). The theoretical concentrations of pure CA and *p*CA corresponding to loaded amounts in the electrospun fiber mats were prepared following the same procedures. The prepared solutions were assayed to compare differences in antioxidant activity before and after electrospinning.

ANTIBACTERIAL EFFICACY

The antibacterial activity of zein electrospun fibers as well as CA and *p*CA loaded zein electrospun fibers were evaluated against Gram negative bacteria *E. coli*, and Gram positive bacteria *S. aureus* according to the Japanese Industrial Standard (JIS Z 2801:2000).

RELEASE ASSAY

Release assay was conducted to study release behaviour of the phenolic acids from the electrospun zein fiber mats. Briefly, 10 mg of the electrospun fiber mat was submerged in 10 mL aqueous solution at room temperature. Samples were withdrawn every 5 min for 1-hr and placed in 96 well cell culture microplate (F-type, SPL LifeSciences Co. Ltd, Korea) individually. Fresh medium was replaced after every withdrawal. The amount of released phenolic acids was determined using the Folin-Ciocalteu procedure. The measurements were carried out in triplicates on separate occasions.

STATISTICAL ANALYSIS

Statistical analysis were performed using SPSS software, version 23.0 (IBMÒ SPSS Statistics, Chicago, USA) and the results were analysed using one-way analysis of variance (ANOVA) where appropriate at 5% significance level.

RESULTS AND DISCUSSION

FIBER MORPHOLOGY

The Ze-CA and Ze-*p*CA electrospun fibers were found to retain similar morphologies as neat zein fiber with no apparent aggregates of the phenolic acid particles. Nevertheless, the AFD varied after the introduction of the phenolic acids. The AFD of zein, Ze-CA and Ze-*p*CA electrospun fibers are tabulated in Table 1. The AFD of the electrospun fibers was found to range from 428.24 to 526.59 nm. Neat zein fibers exhibited the smallest AFD (428.24 ± 106.56 nm) among all the formulations. The addition of CA resulted in a significant increase ($p < 0.05$) in AFD compared to neat zein electrospun fibers. Overall, smooth and defect-free fibers were obtained for both phenolic acids, confirming that no phase separation occurred during the electrospinning process.

Table 1. Average fiber diameter (nm) and dehydration temperature (T_D)of the zein-based electrospun fibers.

Sample	Diameter (nm)	T_D (°C)
Neat zein	428.24 ± 106.56^{d}	95.24 ± 6.84^{a}
Ze-CA5%	493.03 ± 101.91^{abc}	96.08 ± 5.68^{a}
Ze-CA10%	526.59 ± 119.51^{a}	98.87 ± 3.56^{a}
Ze-CA20%	505.62 ± 108.64^{ab}	95.18 ± 5.54^{a}
Ze-*p*CA5%	456.13 ± 133.94^{cd}	97.44 ± 3.66^{a}
Ze-*p*CA10%	478.38 ± 93.72^{bc}	98.82 ± 2.84^{a}
Ze-*p*CA20%	446.85 ± 109.26^{cd}	99.53 ± 2.27^{a}

Results are expressed as mean ± standard deviation

Values labelled with different superscript letters in the same column are significantly different ($p < 0.05$)

THERMAL PROPERTIES

The DSC thermograms of neat zein, Ze-CA and Ze-*p*CA electrospun fibers exhibited broad endothermic peaks around 100 °C due to the evaporation of water and volatile components. These characteristic peaks are termed as the dehydration temperature (T_D) and have often been observed in the thermograms of zein electrospun fibers (Wang *et al.*, 2016, 2017; Hosseini *et al.*, 2021). The T_D of neat zein, Ze-CA and Ze-*p*CA electrospun fibers are shown in Table 1and was found to be insignificantly different ($p > 0.05$) among all electrospun fibers. For neat zein electrospun fibers, degradation temperature (T_d) was found to be at 321.73 ± 3.09 °C. Interestingly, despite new weight loss stages occurred corresponding to CA and *p*CA were determined through TGA, the T_d for CA loaded zein electrospun fibers were not significantly different ($p > 0.05$) from the neat zein electrospun fibers (Table 3). The T_d of pure *p*CA powder was found to be at 231.54 ± 2.82 °C and decreased with increased *p*CA concentrations. The addition of both phenolic acids did not affect the thermal stability of zein fibers and no significant difference ($p > 0.05$) was observed between the T_d of neat zein fibers compared to the Ze-*p*CA electrospun fibers.

Table 3. Degradation temperatures of the zein-based electrospun fibers

	Degradation temperature (C°)	
Sample	1st peak	2nd peak
Neat zein	-	321.73 ± 3.09[b]
Ze-CA5%	186.15 ± 12.76[b]	323.60 ± 9.77[b]
Ze-CA10%	187.60 ± 4.24[b]	321.39 ± 7.85[b]
Ze-CA20%	184.80 ± 4.04[b]	318.09 ± 5.28[b]
CA powder	233.36 ± 11.54[a]	331.06 ± 8.10[b]
Ze-*p*CA5%	214.26 ± 4.02[a]	326.15 ± 5.97[b]
Ze-*p*CA10%	218.74 ± 4.66[a]	323.79 ± 4.81[b]
Ze-*p*CA20%	220.79 ± 13.15[a]	326.24 ± 6.14[b]
*p*CA powder	231.54 ± 2.82[a]	366.63 ± 14.45[a]

Results are expressed as mean standard deviation of three replicates

Values labelled with different superscript letters in the same column are significantly different ($p < 0.05$)

ANTIOXIDANT ACTIVITY

Figure 1 shows the antioxidant activity of pure CA powder and after incorporation into zein electrospun fibers at their corresponding concentrations. The antioxidant activity was found to increase with increasing CA concentrations. However, this increment in antioxidant activity was only significant ($p < 0.05$) as the CA concentration doubled from 10% to 20%. Neat zein electrospun fibers did not exhibit any DPPH scavenging activity in present study. Hence, the antioxidant activity exhibited by the Ze-CA electrospun fibers can be solely attributed to the addition of CA. Pure *p*CA powder and Ze-*p*CA electrospun fibers did not exhibit DPPH radical scavenging activity at the incorporated percentages in present study. The lack of antioxidant activity exhibited by Ze-*p*CA electrospun fibers is attributed to structural difference of the phenolic acids rather than interactions arising after the electrospinning process in present study.

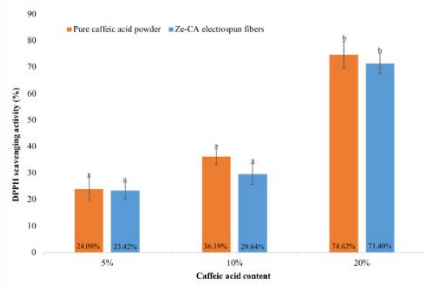

Figure 1. Antioxidant activity of the zein-based electrospun fibers and their corresponding theoretical loaded concentrations of phenolic acids.

ANTIBACTERIAL EFFICACY

Figure 2 shows the efficiency of Ze-CA and Ze-*p*CA electrospun fibers in inhibiting bacterial growth against *S. aureus* and *E. coli* bacteria. Neat zein electrospun fibers did not exhibit any antimicrobial activity against either of the bacteria. This is in agreement with several studies that reported the ineffectiveness of zein electrospun fibers to reduce the growth of *S. aureus* and *E. coli* before the incorporation of antimicrobial agents (Wang *et al.*, 2017; Hosseini *et al.*, 2021). Ze-CA and Ze-*p*CA electrospun fibers showed increasing antimicrobial activities against *S. aureus* with increasing concentrations as seen in Figure 2. It is notable that Ze-CA5% and Ze-*p*CA5% electrospun fibers displayed significantly better ($p < 0.05$) antimicrobial activities against *E. coli* compared to *S. aureus*. The reason could be attributed to different cell membrane constituents of Gram positive versus Gram negative bacteria.

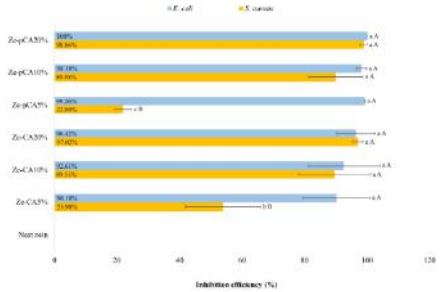

Figure 2.Inhibition efficiency (%) of zein-based electrospun fibers against S. aureus and E. coli.

RELEASE BEHAVIOUR

The in vitro release profiles of CA and pCA from zein electrospun fibers are shown in Figure 3. The release profile assay was performed in water in order to resemble a model food simulating solvent. An initial burst release was observed within the first 5 minutes in all the Ze-CA% and Ze-pCA% electrospun fibers, which is attributed to the release of loaded components on or near the surface of the fibers (Wang et al., 2017; Hosseini et al., 2021). The overall release profile of Ze-CA% and Ze-pCA% electrospun fibers was very rapid as 80-100% of the total loaded phenolic acids was released within one hour of submersion in the water release medium.

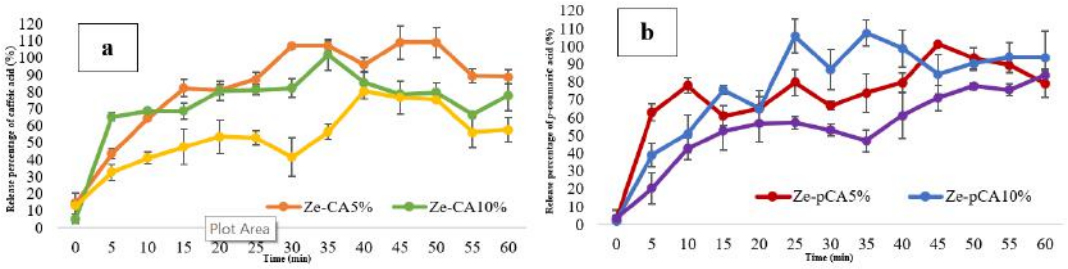

Figure 3. Release percentage (%) of (a) caffeic acid (CA) and (b) p-coumaric acid (pCA) from zein electrospun fibers.

CONCLUSIONS

Zein fibers incorporating CA and *p*CA as food coating that demonstrates active properties were developed using electrospinning. Ze-CA exhibited larger AFD compared to Ze-*p*CA electrospun fibers. Electrospinning has proven its feasibility to functionalize zein biopolymers as CA and *p*CA were well loaded into its matrices effectively as determined by TGA in the present study. While there was a decrease in degradation temperature of incorporated CA and *p*CA compared to their pristine counterparts, the overall thermal stability of the zein electrospun fibers was maintained. When it came to functional properties, Ze-CA electrospun fibers showed a better overall performance. An increase in antioxidant activity was observed with increasing CA concentration while Ze-*p*CA electrospun fibers did not exhibit any DPPH scavenging activity. As for antibacterial activity, Ze-CA5% had significantly higher inhibition efficiency against *S. aureus* than Ze-*p*CA5%. Nevertheless, these zein-based electrospun fibers also exhibited rapid release profiles of the incorporated phenolic acids. Given that the highest concentration of CA did not adversely affect the physicochemical properties of the electrospun fibers compared to lower concentrations, Ze-CA20% can be used as the preferred formulation to obtain the highest antioxidant and antimicrobial properties to achieve the targeted development of food coatings with active properties. A real-time study on applications of this system as packaging material can be conducted in future to determine its performance over a period time.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
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TUNİNG THE THERMAL AND RHEOLOGİCAL ATTRİBUTES OF EMULSİON GEL HYBRİD TEMPLATES WİTH PHENOLİC COMPOUNDS

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Abstract: Consumers' increasing demand for plant-based fatty meat alternatives and health concerns regarding animal fats have spurred worldwide interest in developing edible oil structuring techniques that mimic animal fats' texture and functionality. Based solely on natural food fibres and vegetable oil high in unsaturated fat, we have previously developed naturally-processed emulsion gel hybrid templates with 50-82% lipid content. These templates had storage modulus (G', at 1Hz, 1% strain) from $<10^2$ to $>10^3$ Pa in order of magnitude, bearing rheological resemblance to selected references of animal-based fat. In our latest development, we investigate the use of phenolic compounds—from phenolic acid to flavonoid categories— to attain emulsion gels with higher G'. Our preliminary results from the crosslinking action of laccase-oxidised phytochemicals on curdlan have been promising, providing technical basis in our subsequent quest for textural- and thermal property-tailoring in plant-based emulsion gel hybrids toward industrial adoption.

***Keywords*:** Emulgel; plant-based fat; phenolic acids; cross-linking; citrus fibre; curdlan

INTRODUCTION

The escalating global demand for plant-based alternatives to fatty meat, coupled with growing health concerns related to animal fats, has driven a surge in research interests around development of edible oil structuring methods that can effectively replicate the texture and functional properties of animal fats. In our prior work, our primary focus was directed toward development of naturally derived emulsion gel hybrid templates, characterised by lipid content ranging from 50% to 82%. These templates were exclusively formulated with natural food fibres and high-unsaturated vegetable oils. To expand the structural versatility that could be imparted by natural food fibres, we looked toward the modification of fibres by natural phenolic compounds as a green-tech and efficient approach. Numeral natural phenolic compounds derived from plants have been documented for their reactivity with polysaccharides (Bozic et al., 2012; Karaki et al., 2016). Broadly speaking, the incorporation of phenolic compounds into polysaccharides typically revolves around four techniques: carbodiimide-based coupling, enzyme-catalyzed reactions, free-radical-mediated grafting, and electrochemical approaches (Cai et al., 2019; Liu et al., 2018). Employing enzymes to graft polyphenols onto polysaccharides emerges as a sustainable alternative to methods that might be toxic, environmentally detrimental, or not food-grade compliant (Kim et al., 2017; Sousa et al., 2009). Specifically, the enzyme laccase can convert phenolic compounds into quinones (Adelakun et al., 2012), which possess electrophilic characteristics to engage in Michael addition or Schiff base reactions with nucleophilic amines such as amino acids. This reactivity occurs from as low as 15°C and upward in temperature, following second-order rate constants for the reactions (Liu et al., 2023). In this ongoing study, we intend to leverage the crosslinking action of laccase-oxidised phytochemicals on trace protein components that might be found in fibres, to boost the thermo-mechanical strength of our emulsion gels. The phytochemicals reported here include ferulic acid (phenolic acid), catechin (flavonoid), and proanthocyanidin (flavonoid). The fibres tested were citrus fibre and curdlan, which served as emulsifying and structuring fibres, respectively, in the emulsion gel matrix.

MATERIALS AND METHODS

2.1. Materials

Commercial citrus fibre "CFN" was purchased from CPKelco (Atlanta, Georgia, U.S.) and curdlan from Natural Colloids Industries Pte. Ltd. (Singapore). Sunflower oil with 85% (w/w) average total unsaturated fat co\tent was bought from a local supermarket. All food-grade chemicals and their sources were as follows: ferulic acid (purity ≥99%) from Penta International Corporation (West Caldwell, New Jersey, U.S.); catechin hydrate (purity ≥98%) from Henan Alfa Chemical Co. Ltd. (Zhengzhou, Henan, China); proanthocyanidin (purity ≥98%) from Wellgreen Technology Co. Ltd. (Xi'an, Shaanxi, China); laccase (*Aspergillus oryzae*) from Sunson Industry Group Co. Ltd. (Yinchuan, Ningxia, China). Chemical solvents used for analytical purpose include formic acid from Sigma-Aldrich (St.Louis, Missouri, U.S.) and acetonitrile from Merck (Darmstadt, Germany). All other common laboratory chemicals were from major chemical producers. Distilled water was used in sample preparation.

2.2. Preparation of aqueous phase and emulsion gel hybrid templates

Aqueous phases and emulsion gels with 50% sunflower oil load, based on a fixed percentage of activated CFN and 10% curdlan, were prepared based on modifications from the methodology outlined by Tan and Phoon (2023). Modifications include the use of laccase oxidised or non-oxidised phenolic compounds—namely ferulic acid (FA), catechin hydrate (CH), and proanthocyanidin (PC)—at 1 mM aqueous concentration, pH 4.5-5.0. Oxidation was carried out via incubation with 300 U/g of laccase at 45°C for a duration of 16 hours in the dark. In the process sequence of preparing different samples of both aqueous phase and emulsion gel templates, variations were introduced with respect to whether the phytochemical oxidation with laccase took place in the presence/absence of fibres and oil. Consequently, three sequences (A, B, C) were designed—A: Oxidation of phytochemical in the

absence of both fibres and oil; B: Oxidation of phytochemicals in the presence of fibres, without oil; C: Oxidation of phytochemicals in the presence of both fibres and oil

2.3. Temperature sweep rheological measurement

For the aqueous phase and emulsion gel hybrid templates, rheometer measurements were conducted within one day of their preparation. After their preparation, they were kept in ice water bath prior to rheological measurements on a MCR 302 rheometer controlled by a RheoCompass software (Anton Paar GmbH, Graz, Germany). A parallel plate geometry (diameter 19.965 mm) was used, with the measurement gap set at 1mm. Heat-setting of aqueous phase and emulsion gel products was induced by rapid heating from 20ºC to 95ºC at 25 ºC /min, followed immediately by a cooling ramp at 10.7 ºC /min back to ~21 ºC, the temperature at which G' value (0.2% strain, 1 Hz) was measured.

2.4 Liquid chromatography-mass spectrometry (LC-MS)

300 U/g of laccase was dispersed into 1mM of phenolic compound (FA, CH, PC) aqueous solutions (pH 4.5-5.0). The solutions were incubated at 45°C for 16h in the dark. An aqueous solution of laccase without any phenolic compound was prepared as a control. These solutions were injected into an Agilent UHPLC 1290 Infinity unit connected to an Agilent 6540 quadrupole time-of-flight (QTOF) mass spectrometer, equipped with an electron spray ionization (ESI) source and a splitter (Santa Clara, California, U.S.). LC-MS analysis was carried out using a HSS T3 column 2.1 × 50 mm, 1.8 µm, with these settings (i) flowrate: 0.4 mL/min; (ii) solvent A: water + 0.1% formic acid; (iii) solvent B: acetonitrile + 0.1% formic acid; (iv) gradient conditions: isocratic at 100% solvent A for 0.5 min, 0 to 50% solvent B over 4.5 min, followed by 50 to 100% solvent B over 2 min, and finally isocratic at 100% solvent B for 2 min.

RESULTS AND DISCUSSION

3.1 Laccase-catalysed oxidation of phenolic compounds verified by LC-MS

LC-MS revealed the emergence of distinct reaction products resulting from laccase-catalysed oxidation, as highlighted by the boxes in Fig. 1. For FA, various structural arrangements are feasible, wherein dimeric, trimeric, and tetrameric products from laccase-catalysed oxidation (Adelakun et al., 2012) could undergo subsequent modifications such as decarboxylation (Perna et al., 2018). LC-MS identified masses that corresponded to the presence of dimeric products (Fig. 1A). For PC, which essentially is a condensed form of tannin (Rauf et al., 2019), LC-MS detected masses that coincides with monomer, dimer, and oligomers of proanothocyanidin (Fig. 1B). As for CH , laccase-catalysed oxidation showed the generation of complex tannins (Fig. 1C). While specific compounds within the range of peaks in Figs 1B-C have not yet been identified, they nonetheless serve as indicative evidence of the oxidation of PC and CH during laccase-catalysed oxidation. Taken altogether, these LC-MS observations offer a valuable entry point for investigating how these oxidised phenolic compounds could modify fibres to bring about consequential changes in the rheological and thermal characteristics of the resultant emulsion gels.

3.2 Effect of phenolic compounds (native/oxidised) on the G' of heat-set emulsion gels and their aqueous phases

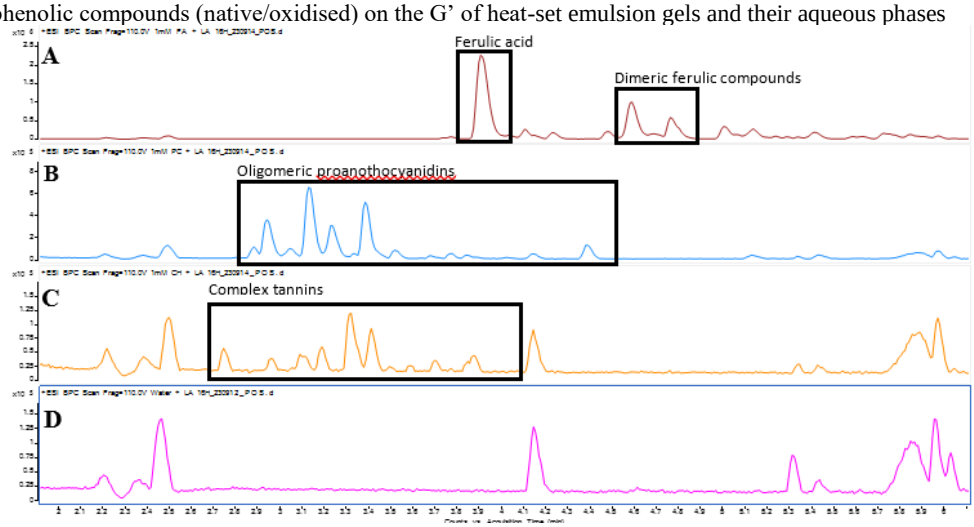

Fig. 1. Liquid Chromatography- Mass Spectrometry (LC-MS) base peak chromatogram (BPC) results. (A) 1 mM ferulic acid with 300 U/g laccase. (B) 1 mM proanthocyanidin with 300 U/g laccase. (C) 1 mM catechin with 300 U/g laccase. (D) 300 U/g laccase in distilled water.

In Fig 2A, we compare the effect of phenolic compound type on the G' of heat-set emulsion gel samples prepared according to sequence A. Here, it is apparent that the oxidation of various phenolic compounds exerts a notable enhancement on the storage modulus (G') of the emulsion gel (blue arrows). This observed effect is plausibly attributed to the trace protein content present in curdlan (average nitrogen content as quantified by the Dumas method: 1.63 ± 0.01%). Oxidised phenolic compounds had likely interacted covalently through nucleophilic attack on the amino acids present within the trace protein content present in curdlan (Adelakun et al., 2012). In contrast, oxidised phytochemicals seemed to have negligible impact on CFN owing to its lower protein content (average nitrogen content as quantified by the Dumas method: 0.56 ± 0%), as verified in a separate rheological assessment

of emulsions containing CFN as the sole fibre (data not shown). Fig. 2A suggests that an increase in the molecular weight of native phenolic compounds (accompanying which is greater density of hydroxyl groups) correlates with the promotion of curdlan networking, resulting in an increase in G' (red arrows). This increment in G' has conceivably arisen from non-covalent interaction between the hydroxyl groups in the phenolic compounds and the amino acids of the trace protein in curdlan. Considering that our curdlan exhibited the function of lowering oil-water interfacial tension (Tan & Phoon, 2023), the observed increase in G' might have stemmed from immobilised curdlan particles (in addition to CFN) at the oil-water interface, which could facilitate inter-oil drop crosslinking to encourage an active-filler phenomenon (Oliver et al., 2015). These combined effects are likely contributors to the heightened firmness observed in the emulsion gel samples containing phenolic compounds, with or without laccase-catalysed oxidation, in Figs. 2A and 2C.

In the aqueous phases corresponding to the emulsion gel samples in Fig. 2A, there is a decline in G' in the presence of phenolic compounds regardless of their oxidative state (Fig. 2B, black arrow). This could potentially be attributed to the interaction between phenolic compounds and the trace proteins originating from the activated CFN strands. This function might lead to reaggregation of the CFN strands, disrupting their initially homogenous distribution within the emulsion gel template matrix while still freshly activated, and consequently leading to a reduction in G'. Thus, any interaction between phenolic compounds and curdlan might be offset by the re-aggregation in CFN, leading to no significant difference found among aqueous phases containing native versus oxidised phenolic compounds.

For better narrative flow, we continue the discussion first with Fig. 2D, which compares the effect of process sequencing during the preparation of aqueous phase samples on their subsequent G' upon heat-setting. In Fig. 2C, FA was focused upon as an example. Here, the aqueous phase from sequence B/C shows higher G' for oxidised FA than for native FA (blue arrow). This disparity differs from sequence A, as previously elucidated, due to the prolonged reaction between the trace protein from curdlan and oxidised FA products that were progressively generated during the 16 h of enzymic reaction. This prolonged reaction time led to a notable thickening effect (Sun et al., 2007) within the aqueous phase, consequently resulting in an increase in G'. The lower G' in the aqueous phase from Sequence B/C, compared to that from Sequence A (long black arrow), might be due to hydrophobic re-aggregation of activated CFN strands driven by prolonged exposure to higher temperature (i.e., 45ºC). The effect of re-aggregation of activated CFN strands has been commented earlier on.

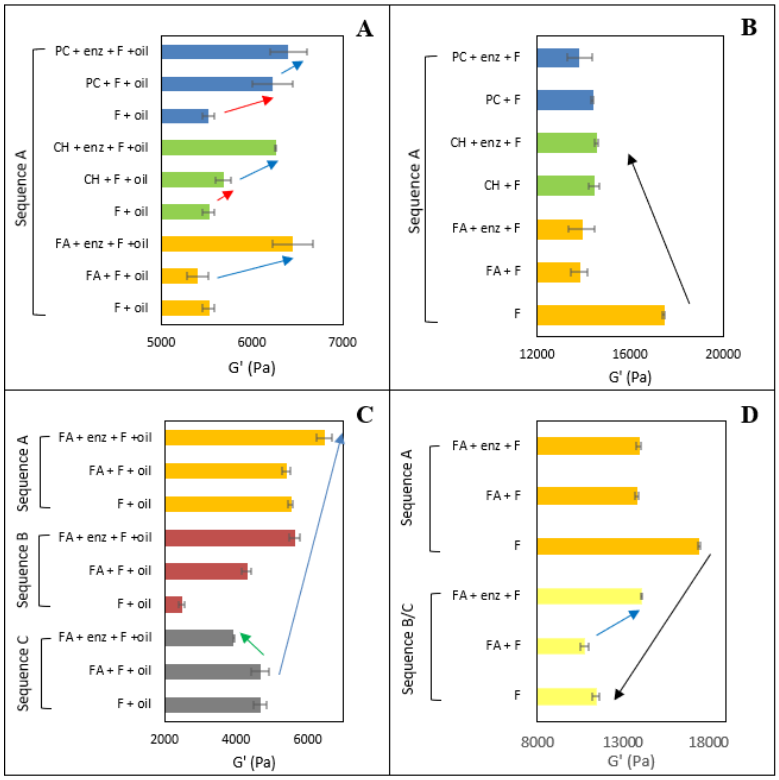

Fig. 2. Average storage modulus (G') of emulsion gel samples and their corresponding aqueous phases, with and without phenolic compounds (native/oxidised by laccase). (A) Emulsion gel samples prepared by sequence A, containing FA/CH/PC. (B) Aqueous phase samples prepared by sequence A, containing FA/CH/PC. (C) Emulsion gel samples prepared by different sequences A, B, and C, containing FA. (D) Aqueous phase samples prepared by different sequences A, B, and C, containing FA. G' measurements were from oscillatory rheometry (at 1 Hz, 0.2% strain) at 21ºC, at the end of a cooling ramp after rapid heating to 95ºC. All emulsion gel samples had 50% (w/w) sunflower oil load. F: CFN and curdlan fibres; enz: laccase; FA: ferulic acid; CH: catechin; PC: proanthocyanidin.

With the emulsion gel samples corresponding to the aqueous phase samples in Fig. 2D, the main pattern observed is that in terms of the magnitude of G', it is best attained with using oxidised FA, followed by using FA, and lastly not using any FA (Fig. 2C, long blue arrow). In the presence of oil, there would be partial removal of CFN strands from the aqueous phase owing to CFN adsorption at the oil-water interface, which enhances the accessibility of both native and oxidised FA to the aqueous curdlan particles to interact non-covalently, thereby boosting curdlan networking. Oxidised FA brings the added advantage of promoting inter-drop cross-linking involving curdlan particles adsorbed at the oil-water interface. An exception to the main pattern observed is found in sequence C samples: the use of FA did not increase G' but could even decrease G' with FA oxidation (green arrow). Here in sequence C, the prolonged reaction between the trace protein from curdlan and oxidised FA products, progressively generated during the 16 h of enzymic reaction, could pose the risk of substantial clustering of oil drops and micro-phase separation. Such clustering could stem from the formation of inter-oil drop bridges, facilitated by the same cross-linking of partially absorbed curdlan particles.

In summary, Fig.2 reveals a complex interdependence among the presence of fibres, oil, and the timing of enzymic oxidation of phenolic compounds. The observations lay the foundation for a more comprehensive understanding of the interactions involving phenolic compounds, laccase-mediated reactions, fibres, and oil, and how they collectively impact the rheological attributes of the emulsion gel.

CONCLUSIONS

Our findings illustrate the feasibility of enhancing the G' in emulsion gels using of phenolic compounds oxidised by laccase. However, it is important to note the process sequence of preparing the emulsion gel templates matters. We see further room in optimising the process sequence and we will follow up with more in-depth investigation, toward modulating the thermal and textural attributes of these fibre-based emulsion gels. These endeavours are expected to yield valuable insights in diversifying their potential applications for industrial uptake.

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PICKERING EMULSIONS STABILIZED BY OSA-YAM STARCH: EFFECTS OF STARCH MODIFICATION AND HEAT TREATMENT ON EMULSIFYING CAPACITY

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Abstract: Native yam starch has its limitations in food applications, therefore starch modification is essential in improving its properties. Yam starch granules can be modified with octenyl succinic anhydride (OSA) and serve as an emulsifier in stabilizing Pickering emulsion. Hence, this study is designed to chemically modify yam starch with OSA and its emulsifying capacities such as droplet size, microstructure, emulsification index, accelerated stability test, and its effect on heat treatment were investigated. OSA yam starch showed low gelatinisation temperature (68.5°C) compare to its native form (74.7°C). OSA yam starch Pickering emulsions exhibited smaller droplet sizes (27.66±5.97µm) thus improving the stability of Pickering emulsions with high emulsification index (EI) and least affected after accelerated stability test compared to native yam starch Pickering emulsions. The heat-treated emulsions have smaller droplet sizes than non-heat-treated emulsions with 100% EI. Modification of yam starch granules using OSA and their performance in stabilizing Pickering emulsions could give an understanding to the food industry in finding an optimum and efficient formula that suits their requirements.
Keywords: yam starch, OSA, starch modification, Pickering emulsions, emulsifying capacity

INTRODUCTION

An emulsion is defined as a mixture of two immiscible liquids stabilized by emulsifiers to form droplets. Emulsions are commonly applied in industries such as cosmetics, food, and pharmaceutical products. Those emulsions can be stabilized by a variety of emulsifiers such as surfactants, proteins, biopolymers, and particles (Zanini et al., 2017). The choice of emulsifier depends on the type of emulsion. For example, in the case of particles that are used as emulsifiers, the emulsion is called Pickering emulsion. In this study, the technique of oil-in-water emulsion stabilized by solid particles or known as Pickering emulsion is applied.

Yams, also known as *Colocasia esculenta*, is a herbaceous plant that grows in Malaysia. However, other native yams that are planted in tropical Asia were categorized in the genus of Dioscorea. Native yam starch has its limitation in food application due to its limitation in hydrophobicity. Native starch granules are inefficient in stabilizing emulsion due to low hydrophobicity. Octenyl Succinic Anhydride (OSA) modified starch is widely used in the food industry as a stabilizer and emulsifier. A previous study has reported that modified breadfruit starch by OSA can stabilize the emulsion of algae oil and fish oil. Yam starch can be a suitable emulsifier in Pickering emulsions due to starch granules have less sensitivity towards pH, and isoelectric point, compared to synthetic surfactant- or protein-based emulsifiers(Yan et al., 2022). The hydrophobicity of starch is important in Pickering emulsion because it can affect the stability of the emulsion. In Pickering emulsions, the particles create a physical barrier which adsorbing at the oil-water interface that can prevent droplet contact and interface interaction through volume exclusion. These particles are also thought to be irreversibly adsorbing at the interface (Timgren, Rayner, Sjöö, & Dejmek, 2011).

Therefore, this study is designed to determine the ability of modified yam starch by OSA to stabilize the Pickering emulsions. Emulsions' stability was determined by monitoring the droplet size using microscopy and its emulsification index. In addition, the characterization of particle size of modified yam starch will also be studied using Scanning Electron Microscopy (SEM). Apart from that, the comparison of heated and non-heated Pickering emulsions

can be beneficial information to the processing industries that have an emulsion system in heat processing foods. The aim of this study is to characterise native and modified yam starch granules, and its emulsifying capacity in stabilizing oil-in-water Pickering emulsions.

MATERIALS AND METHODS

Materials

Yams (*Colocasia esculenta*) will be bought from the Mydin grocery store located at Gong Badak, Kuala Terengganu, Terengganu. After the isolation of starch, the native starch is in powder form prior to use. Octenyl Succinic Anhydride (OSA) (CAS Number: 42482-06-4) was obtained from Sigma-Aldrich (M) Sdn. Bhd. Palm oil was bought from grocery store in Kuala Terengganu, Terengganu. The chemicals used in this study were analytically grade and obtained from Merck, Germany.

Isolation of Yam Starch

The isolation of yam starch was done by using method of Marefati, Wiege, Haase, Matos, and Rayner (2017) with a slight modification. The yams were cut into small pieces and homogenized using a blender. Then, the starch paste was centrifuged and the supernatant were removed. The washing steps were repeated three times to remove the impurities. The starch was dried in an oven at 55°C for 24 hours and was sieved to get an even size.

OSA Modification of Yam Starch

The method is modified from the research of Marefati et al. (2017). Native yam starch was dissolved in distilled water with the pH was adjusted to 8.5 using sodium hydroxide (NaOH) solution. At a maintained pH, the OSA was added slowly with continuous stirring for three hours until pH decrease to 6.5. The esterified starch suspension was washed and centrifuged 3 times with distilled water and once with acetone. The esterified starch paste was dried at 50 °C for 24 hours. The dried OSA yam starch was crushed into a powder and the degree of substitution was determined using a method from Zięba, Kapelko, and Szumny (2013).

Preparation of Native and OSA Yam Starch Granules Stabilized Pickering Emulsions

The method of preparing Pickering emulsions stabilized by native and OSA yam starch was adopted from Abdul Hadi, Marefati, Matos, Wiege, and Rayner (2020) with slight modification. An oil-in-water Pickering emulsion with a total volume of 7mL and 10% v/v oil fraction was prepared using native and OSA yam starch granules as emulsifiers with starch concentration was 200mg of starch per mL of oil. The mixture was homogenized at 20000 rpm for 60 seconds. For heated Pickering emulsions, the freshly prepared native and OSA yam starch Pickering emulsions were heat treated by heating the emulsion until 80 °C and hold for two minutes.

Morphology of Starch Granules

Starch granule morphology was observed by method from Shao et al. (2020) with slight modification using a scanning electron microscope (JEOL JSM 6360LA, JEOL Ltd., Japan). The starch were coated with vacuum spray gold. The samples were examined and photographed using SEM at an accelerating voltage of 10.0kV and their particle size was estimated.

Differential Scanning Calorimetry (DSC)

The method is adopted from research conducted by Zhu et al. (2017) with a slight modification. The starch was placed in 20 µL capacity aluminium pans and deionized water was added using a microliter syringe in a ratio of 1:8. The pans were scanned with a scanning rate of 10 °C/min from 30 °C to 120 °C. An empty aluminium pan was used as a reference. The onset temperature of gelatinization (To), peak gelatinization temperature (Tp), conclusion temperature of gelatinization (Tc), and enthalpy of gelatinization (ΔH) was recorded.

Microscopy

The prepared Pickering emulsion was diluted by using distilled water at a ratio of (1:2) and one drop of the mixture was placed on a glass slide. The emulsion droplet size was observed at a magnification of 10x using a compound microscope with a camera (PRIMOSTAR, CarlZeiss, Germany). The images of each sample were taken in three different areas to observe the homogeneity of emulsion droplets. The images obtained were analyzed using Image J v1.52a software (National Institutes for Health, Bethesda, Maryland, USA).

Emulsification Index

Approximately 7mL of native and OSA yam starch Pickering emulsions were placed in a glass vial and let stand for 24 hours. The sedimentation or creaming layer of Pickering emulsion was measured as well as the total height of Pickering emulsions. The height of the creaming layer and the volume of total emulsion were measured and recorded. The creaming index was calculated as follows equation from Timgren, Rayner, Dejmek, Marku, and Sjöö (2013):

Emulsification Index (%) = (height of sedimentation or creaming layer)/(height of total emulsion) ×100

Accelerated Stability Test

An accelerated stability test is applied to determine the Pickering emulsion stability by applying a gravitational force to the Pickering emulsion system (Restu et al., 2015). Approximately 7mL of native and OSA yam Pickering

emulsions was placed in a centrifuged tube. The emulsion was then centrifuged at 500, 1000, 2000 and 3000 rpm respectively for 10 minutes. The height of the emulsion retained was measured and recorded.

RESULTS AND DISCUSSION

Morphology of Starch Granules

Figure 1 illustrated SEM micrographs of native and OSA yam starch granules. Native yam starch granules exhibited irregular polygonal shapes with a smooth surface while OSA yam starch exhibited a rough surface and still in irregular polygonal shapes. OSA yam starch granules also have polygonal and irregular shapes but the surfaces are rough compared to native yam starch granules.

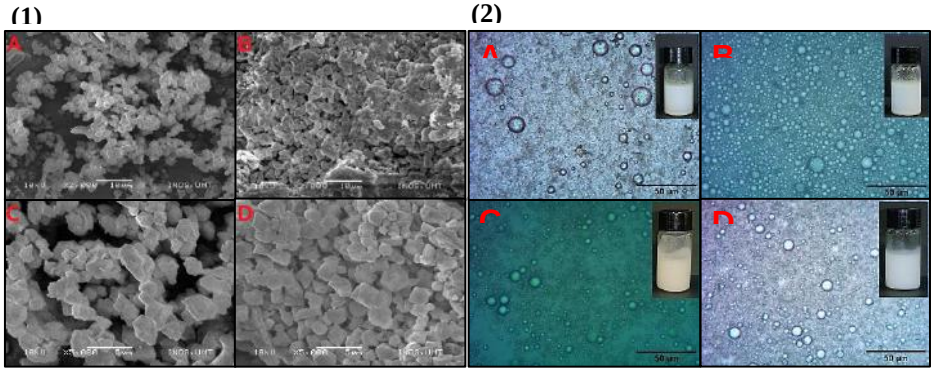

Figure 1. 1) Scanning electron micrographs of native and OSA yam starch granules: (A) native yam starch and (B) OSA yam starch at 2000x, (C) native yam starch, and (D) OSA yam starch at a magnification of 5000x. 2) Microscopy picture of droplet size of Pickering emulsion: (A) non-heated native yam starch Pickering emulsion and (B) non-heated OSA yam starch Pickering emulsion, (C) heated native yam starch Pickering emulsion, and (D) heated OSA yam starch Pickering emulsion.

Native and OSA yam starch has no significant difference in terms of granule size with size 1.42±0.30 µm and 1.87±0.29 µm, respectively. Hence, it is proved that modification of OSA did not alter the granule size of yam starch. The granule size of both native and OSA yam starch is almost similar but the surface of native yam starch has been altered into rough after undergoing modification using OSA.

Thermal Properties

The gelatinization temperature of native and OSA yam starch was characterized as shown in Table 2. The gelatinisation temperature of native yam starch were higher than the OSA yam starch with the T_o, T_p and T_c were 74.70 ± 0.3 °C, 79.11 ± 0.9 °C, and 88.80 ± 4.9 °C, respectively. The OSA starch showed lower gelatinisation temperature attributed by the introduction of OSA group to the starch structure (Zhu et al., 2017).

Table 1. Gelatinization temperature for native yam starch.

Yam Starch	Gelatinization parameter **			ΔH (J/g)
	T_o (°C)	T_p (°C)	T_c (°C)	
Native	74.7 ± 0.3[a]	79.1 ± 0.9[a]	88.8 ± 4.9[a]	2.74[a]
OSA	68.5 ± 0.5[b]	70.5 ± 0.2[b]	73.5 ± 1.2[b]	3.65[b]

Values are means ± SD. Values with the same letters in the same column are not significantly different ($p < 0.05$). T_o, T_p, T_c, and ΔH indicate the onset temperature, peak temperature, conclusion temperature, and crystal melting enthalpy, respectively.

Droplet Size Distribution

The result indicates that the droplet size of non-heated OSA yam starch Pickering emulsion was found to be smaller compared to non-heated native yam starch Pickering emulsion as shown in Table 2 and Figure 1. OSA yam starch provides good wettability which resulted in smaller droplet size of emulsion formed. Research conducted by Tesch, Gerhards, and Schubert (2002) shown that OSA-modified starch due to the substitution of functional groups which improved starch wettability to stabilize emulsions. The droplet size of the non-heated native yam starch Pickering emulsion obtained showed larger size than heated native yam starch Pickering emulsion with size obtained 48.55 ± 16.45 µm and 18.05 ± 4.15 µm, respectively. Research conducted by Sjöö, Emek, Hall, Rayner, and Wahlgren (2015) found that the droplet size of Pickering emulsion stabilized by starch increased when the temperature of heat treatment increased. The swelling of the starch layer after heating was the immediate source of this increase, although the interior oil drop size was unaltered (Sjöö et al., 2015).

Table 2. Droplet size of heated and non-heated native and OSA yam starch Pickering emulsion.

Yam Starch Pickering Emulsion	Droplet Size (µm)	Emulsification Index (%)	Accelerated test (Total emulsion retained (%))			
			500	1000	2000	3000
Non- Heated Native	48.55 ± 16.45^{a}	90.28 ± 2.41^{b}	95.15 ± 1.05^{cA}	96.66 ± 0.53^{bA}	96.97 ± 1.05^{bA}	97.57 ± 1.05^{aA}
Heated Native	18.05 ± 4.15^{c}	100.00 ± 0.00^{a}	92.12 ± 2.10^{bcA}	92.73 ± 1.82^{cA}	91.52 ± 1.05^{cA}	90.91 ± 1.82^{bA}
Non- Heated OSA	27.66 ± 5.97^{b}	86.96 ± 0.00^{c}	95.76 ± 1.05^{bA}	96.97 ± 1.05^{bA}	96.36 ± 0.00^{bA}	97.57 ± 1.05^{aA}
Heated OSA	26.35 ± 5.99^{b}	100.00 ± 0.00^{a}	100.00 ± 0.00^{aA}	100.00 ± 0.00^{aA}	100.00 ± 0.00^{aA}	100.00 ± 0.00^{aA}

* Values are means ± SD. Values with the same letters in the same column are not significantly different ($p< 0.05$). a-c Within each set of data means in the same column with different lowercase letters are significantly different in each total emulsion formed ($p< 0.05$). A-C Within each set of data means in the same row with different capital letters are significantly different in each total emulsion formed ($p< 0.05$).

Emulsification Index (EI)

As shown in Table 2, OSA yam starch Pickering emulsions had a lower emulsification capacity compared to native yam starch where the EI was 86.96% retained after 24 hours compared to native yam starch Pickering emulsion with EI measured was 90.28%. There was no significance difference between the emulsification index of heated native and OSA yam starch Pickering emulsions where the emulsion retained at 100.00% after 24 hours storage.

Accelerated Stability Test

Comparing the yam starch Pickering emulsions that are heat-treated, the Pickering emulsion stabilized by OSA yam starch possess a better performance compared to native yam starch. The heated OSA yam starch Pickering emulsion retained 100% EI after high speed of centrifugation gravity which indicates the emulsion were truly in a stable state.

CONCLUSIONS

OSA was substituted into native yam starch with degree of substitution of 0.0109 showed no significant difference in granule sizes. Native yam starch starts to gelatinize at a temperature of 74.40 °C higher than the OSA yam starch. The droplet size of both heat-treated native and OSA yam starch emulsion were proved to be smaller than the non-heated emulsion. The emulsification index of heated native and OSA yam starch emulsion did not show a significant difference where both of the heat-treated emulsions retained 100% of the emulsion after 24 hours of storage. This study would provide insight in utilising yam starch as plant-based emulsifier for industrial purposes.

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EFFECTS OF THE ENZYMATIC TREATMENT FACTORS ON THE EMULSIFYING STABILITY OF RICE BRAN NANOFIBERS PRODUCED THROUGH LACCASE TREATMENT

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Abstract: One of the alternatives in recycling an underutilized agricultural waste like rice bran is by isolating the nanocellulose from this biomass for its purpose as an emulsifier to stabilize Pickering emulsion. In this study, factors affecting the enzymatic treatment like enzyme concentrations and substrate loading were studied to determine their effects on the emulsifying properties of the rice bran nanofibers (RBN) produced. The defatted rice bran (DRB) was subjected to 5% (w/v) potassium hydroxide (KOH) followed by ultrasonication step for 15 min. The laccase treatment was conducted by varying the process parameters using single factor optimization at a constant time (24h) and temperature (50°C): Enzyme concentrations (0, 1, 3, 5, 7 and 9 % (w/w)) and substrates loading (0.3, 0.5, 1, 2 and 2.5 % (w/v)). The percentage of delignification, visual inspection, emulsion index (%) and emulsion stability (%) were determined. Results of the study revealed that the laccase treatment was suitable to be conducted under the following conditions: 3% (w/w) enzyme concentrations and 1% (w/v) substrate loading to achieve an optimum emulsion stability. Our results enhance understanding about the relationship between the process conditions of laccase treatment and their effects on the ability of RBN produced as an emulsifier.

Keywords: Food engineering, Rice bran, Cellulose nanofiber, Laccase, Pickering emulsion

INTRODUCTION

Over the past few decades, the number of consumers whom are aware of a healthier lifestyle is consistently increasing. Consequently, the use of natural ingredients in food industry has increased with the aim of developing safer and healthier products. Emulsions stabilized by surfactant has limited application and practicality (Costa et al., 2021) due to their high cost and short-term stability. Therefore, the use of solid particles to stabilize an emulsion which is also known as "Pickering emulsion" are of great interest nowadays. Pickering emulsion are formed when solid particles were used as emulsifier that accumulate at the oil-water interface and led to a steric barrier which can avoid coalescence and Ostwald ripening (Pickering, 1907). Particles like silica (SiO_2) (Zhang et al., 2022) and titanium dioxide (TiO_2) (Wang et al., 2021) that had been used as emulsifier had risen consumer awareness in term of their suitability to be use in food-grade emulsion. Hence, sustainable food-grade particles like starch granules and cellulose draw much attention in recent studies.

Cellulose is the main component of plant cell walls which offers great potential to be used as one of the ingredients in food industry. Previous studies have reported the ability of cellulose fibres isolated from various agricultural sources to stabilize oil-in-water emulsions without the aid of surfactants (Huang et al., 2020). Rice bran is one of the potential agricultural wastes that can be used to isolate the cellulosic materials. It is an abundant and underutilized by-product of rice processing. Considering its environmental-friendly properties that are biodegradable, non-toxic and sustainable, several research had been conducted previously to isolate cellulose in the nanosize form called nanocellulose from rice bran (Angkuratipakorn et al., 2017; Arun et al., 2020).

Acid hydrolysis method is the common method involved in the extraction of nanocellulose from rice bran (Ilias et al., 2021). However, the use of strong acid like sulfuric acid is harmful not just to the consumers but also to the environment as well. Therefore, the use of enzymatic treatment which is an environmental-friendly method as an alternative to the acid hydrolysis process is able to eliminate the usage of solvents and/or chemical reagents in the nanocellulose extraction and increase its feasibility to be used as food-grade emulsifier. Though several studies have

reported on the use of laccase in the production of nanocellulose (Poddar et al., 2015; Pula et al., 2021), limited study has been carried out on the use of this enzyme in the production of nanocellulose to serve its function as an emulsifier.

Inspired by our previous work (Ilias et al., 2023), the effect of varying the process parameters of enzymatic treatment like enzyme concentrations and substrates loading on the emulsifying properties of nanocellulose was clarified. The amount of lignin reduction, emulsifying index, accelerated stability test and visual appearance of the emulsion produced were investigated and the results obtained were useful in evaluating the emulsion stability of the nanocellulose-stabilized Pickering emulsion.

MATERIALS AND METHODS

Rice bran from MR 219 paddy variety was collected from a local seed company in Pendang, Kedah, Malaysia. Defatted rice bran (DRB) was prepared by using Soxhlet extraction following the method by Abdul Khalil et al. (2016). Potassium hydroxide (KOH) was obtained from R&M Chemicals (Malaysia). Laccase from *Trametes versicolor* (EC No.: 420-150-4, ≥ 0.5 U/g), sodium acetate ($C_2H_3NaO_2$), acetic acid (CH_3COOH) and 1-hydroxybenzotriazole (HBT) were procured from Sigma Aldrich (Darmstadt, Germany).

The DRB produced after Soxhlet extraction was subjected to the pulping procedure by using 5% (w/v) potassium hydroxide (KOH) following the ultrasonication (90% amplitude, 0.3s pulse cycle) for 15 min. The laccase treatment was carried out by varying the process parameters using the following conditions: Enzyme concentrations in the range of 0, 1, 3, 5, 7 and 9 % (w/w) and 0.3, 0.5, 1, 2 and 2.5 % (w/v) of substrates loading (DRB concentrations). The time and temperature of the enzymatic treatment were set at a constant time of 24 hours and 50°C respectively according to Moniruzzaman and Ono (2013). The effect of varying the parameters on the percentage of lignin removal were determined (TAPPI T222 om-11, 2011). Further influence of parameter variations were also investigated on the nanocellulose ability to perform oil-in-water Pickering emulsion and its emulsion stability was evaluated through accelerated stability test (Restu et al., 2015), its emulsifying index and also visual appearance (Luo et al. (2021). The optimum process conditions were selected based on the level of parameters that give the highest amount of percentage lignin removal, emulsion index and emulsion stability.

RESULTS AND DISCUSSIONS

The effect of varying the laccase enzyme concentrations on the percentage of lignin content were studied using the enzyme concentrations of 0, 1, 3, 5, 7, and 9 % (w/w) from the total defatted rice bran (DRB) as shown in Figure 1. The increase in enzyme concentration led to a rapid increment in the rate of reaction which increased proportionately until a saturation point was reached. At this point, further increment in the enzyme concentration contributes to a small or no effect on the reaction rate. Enzyme saturation is most often caused by a lack of available substrate sites for the enzyme to bind to, or it could be caused by product accumulation. Figure 1 reveals a steady increase in the delignification until the enzyme saturation concentration of 3% was reached with the lowest percentage of lignin content observed at 16.97%. The untreated DRB contains 24.72% of Klason lignin (Ilias et al., 2023), hence, from this original amount of lignin, the use of 3% of enzyme concentrations contributed to a 31.37% of lignin reduction which is the highest of all concentrations. It is also observed that further increment in the enzyme concentrations from 5% to 9% (w/w) from the weight of DRB had no appreciable influence on the rate of enzymatic treatment. This is also supported by insignificant reduction in lignin content as the enzyme concentrations increased until 9% (w/w). A similar finding was observed in the study done by Ishmael et al. (2016) which reported a consistent weight loss as the enzyme concentration was increased more than 20 IU/g. The influence of varying the enzyme concentrations were also evaluated on the ability of the nanocellulose produced to emulsify oil-in-water Pickering emulsion. Results obtained from the emulsifying index and visual appearance also revealed similar findings whereby 3% (w/w) of enzyme concentrations showed the highest emulsion stability.

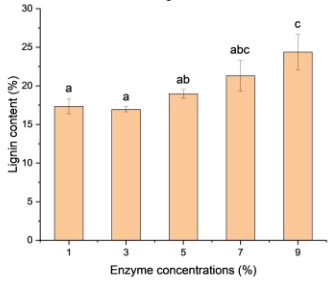

Figure 1. Effect of varying the enzyme concentrations on the percentage of lignin content. All values are expressed as mean value (n=3) ± SD, and different alphabets denote significant differences (P<0.05).

Results of the effect of substrates loading on the percentage of lignin reduction showed an optimum value at 1% (w/v) of substrate concentration with the highest lignin removal of 50.20% as tabulated in Table 1.

Table 1. Effect of varying the substrates loading on the lignin content. All values are expressed as mean value (n=3) ± SD, and different alphabets denote significant differences (P<0.05).

Substrates loading (%)	Lignin content (%)	Lignin reduction (%)
0.3	15.73 ± 0.14	36.37
0.5	16.58 ± 0.37	32.93
1.0	12.31 ± 0.14	50.20
2.0	12.91 ± 0.79	47.78
2.5	13.33 ± 0.54	46.08

As the substrate concentration increased after 1% (w/v), a slight decrement can be observed in the lignin reduction, verifying that increasing the substrate concentration of the enzymatic treatment leads to a reduction in the delignification activity of laccase due to the reduction of surface contact between enzyme and substrate (Shah et al., 2016). The accelerated stability test which was carried out to evaluate the emulsion stability of the nanocellulose-stabilized Pickering emulsion at different substrates loading also revealed a correspond finding as the highest emulsion stability was also recorded when 1% (w/v) of DRB was used as the substrate for the laccase treatment as shown in Figure 2.

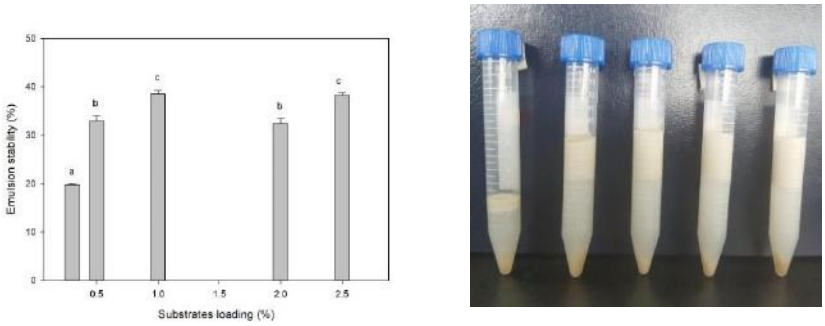

Figure 2. Effect of varying the substrates loading on the emulsion stability. All values are expressed as mean value (n=3) ± SD, and different alphabets denote significant differences (P<0.05).

Slight decrement in the emulsion stability can be observed as the substrate concentrations were increased, which also might be contributed by the higher lignin content compared to 1% DRB. This result explains the importance of lignin removal in the lignocellulosic biomass which can maximize the cellulose exploitation and increase the amount of nanocellulose to stabilize the emulsion (Song et al., 2019).

CONCLUSIONS

The process conditions of enzymatic treatment by using laccase enzyme were optimum at the enzyme concentration of 3% (w/v) and 1% (w/v) of substrate loading. The low concentration of the optimum enzyme and substrate used have contributed to the economic benefits of the enzymatic treatment which can act as an alternative to replace the traditional preparation of cellulose.

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MAPPING OF FOOD ACCESSIBILITY AND AFFORDABILITY CONDITIONS AT SMALL SCALE FARMERS HOUSEHOLD
(CASE STUDY IN OGAN ILIR REGENCY SOUTH SUMATRA-INDONESIA)

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Abstract: Indonesia faces food security issues, including availability, affordability and staple food price. The condition of food security affects the ability of households to access and achieve food fulfillment. The study aims to map the availability and affordability of staple food in Ogan Ilir District, South Sumatra-Indonesia. The staple food accessibility conditions map and Staple food affordability map were carried out through scoring analysis using the class interval method. The result of this study shows that domestic food capacity was in the unfavorable criteria. Food reserves are in the less good criteria. This condition indicates that households do not have staple food reserves at home. The score for supplying food from local sources is in the not good criteria. The food aid condition is not good because there is no food aid, received by the communities. The food logistics system is not in good criteria because the majority of households in the village do not have refrigerators, the people's purchasing power for staple food is good, but other additional food is still unable to buy. The price of staple food shows a price increase that is quite frequent in rural then households have difficulties fulfilling staple food continuously. The household income level show that when income decreases, they will reduce the amount of food purchased.
***Keywords*:** Food Mapping, Food Security, Accessibility, Affordability, Food Price Level.

INTRODUCTION
Indonesia faces food security issues, including availability, accessibility, and stability. Food utilization is crucial in efforts, with challenges in agriculture, transportation, and restriction regulation. Opportunities exist (Rozaki, 2021). Regulations govern the responsibilities and powers of Indonesian food control agencies, overseen by central, provincial, and local governments. These regulations cover fresh and processed food types in Indonesia (Barinda & Ayuningtyas, 2022). Premium prices hinder sustainable food consumption, creating affordability for customers by treating liberal spending on alternative foods as prudent while conventional foods are considered imprudent (Bååth, 2022). Agripreneurship techniques can improve food security by ensuring availability, accessibility, and affordability. Policymakers should focus on supply-side and demand-side factors. Agripreneurship, in collaboration with donor communities and development partners, can transform the agriculture sector, promoting food security, poverty reduction, and socio-economic transformation (Kazungu & Kumburu, 2023).
The effects of agricultural export incentives on domestic food security show that agro-export promotion negatively impacts urban areas and national levels due to increased food prices. Rural households benefit more, with stronger effects from international market volatility and declines in domestic productivity can further deteriorate access to food (Aragie et al., 2023). Low-income and food-insecure households face challenges in sustaining a nutritionally adequate diet, particularly in childhood, highlighting the importance of addressing household-level factors.(Eicher-Miller et al, 2023). The conditions of availability and affordability of food in an area describe the food security conditions, where households can meet their basic food needs under proper conditions, and to fulfill this reason, research needed that explores food security and food availability in Ogan Ilir Regency to obtain an overview of the food security through mapping carried out based on the current situation from the opinions of respondents who represent the population of farming households in food insecure locations. The purpose of this research

MATERIALS AND METHODS
The study uses a purposive sampling method to represent the population of Ogan Ilir Regency and gather opinions on studies. The sample includes 90 respondents within 30 per study area, focusing on households with low income and experiencing food insecurity conditions. Primers and seconds data are the kinds of data used in this study. Data

analysis for staple food accessibility and affordability condition performance was conducted using the interval class method, with ordinal scales based on categorical values.

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RESULTS AND DISCUSSION

A. Conditions of Staple Food Availability in Ogan Ilir Regency

Food availability refers to the total food production, including ports and buffer stocks in government granaries. It involves production and distribution at household, community, state, and international levels. Self-production is the primary means for the majority of the hunger. Distribution involves food and products to humanitarian and retail outlets, with well-functioning market systems determining availability (Kumar et al., 2021). Food security, originating in the 1970s, assesses a nation's food availability by estimating kilocalories of domestic and imported foodstuffs (Kolog et al., 2023). The condition of food availability in Ogan Ilir Regency is seen from several indicators, namely the food production capacity, food reserves, provision of food from local resources and food aid. The condition mapping result can be seen in Figure 1.

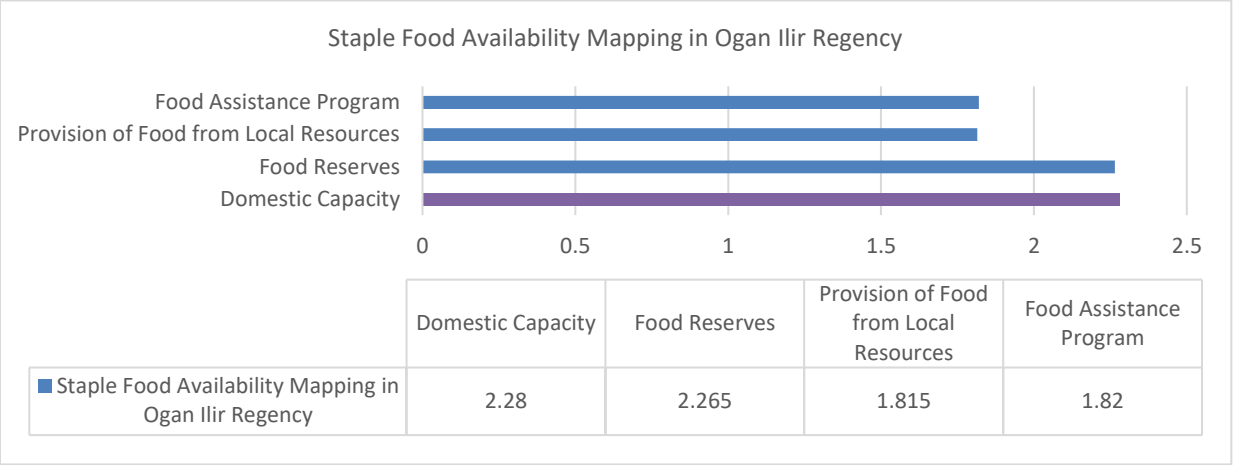

Figure 1. Conditions of Staple Food Availability Mapping

Food Production Capacity

The growing global population demands food, with healthy nutrition a major issue. Agriculture faces challenges like land competition, water shortages, and climate change while contributing significantly to environmental impacts (Buchholz dkk., 2023). Food production capacity measures the quantity of food produced in Soak Bato Village and Lebak Pering Village, affecting food availability in Ogan Ilir District. The data shows that the total domestic food capacity in Soak Bato Village meets the unfavorable criteria with a score of 2.19. This condition can be seen from the statement of respondents that the amount of rice production currently does not meet the needs of consumption at home. Households in this village also do not have ponds or livestock that can be used to meet daily consumption.

Food Reserves

Available food reserves determine household food availability, ensuring sufficient stock to meet long-term needs and the proper availability of staple foods as main foods.. The data result that food reserves in Soak Bato Village meet the unfavorable criteria with a score of 2.21. This condition indicates that households in Soak Bato Village do not meet staple food reserves at home. The only food available was rice, while other staple foods were unavailable at home in the form of stock or reserves.

Provision of Food from Local Resources

Provision of food from local resources is one indicator of household food availability, if a household can meet food needs by obtaining its own crops or livestock, food availability will be better. Lebak Pering and Soak Bato villages struggle to provide food from local resources, with crops and livestock insufficient for household needs. Households lack vegetables, fish ponds, and livestock, limiting self-cultivation opportunities.

Food Assistance Program

Improving local food access and assistance services is crucial for recovery from public health emergencies, especially for those with high food insecurity and disproportionately high prevalence among people (Larson et al., 2021). Food asisitance program is one of the factors that can assist households in providing staple food at home. Mapping the condition of food aid in the study area aims to determine the contribution of food aid in assisting the availability of staple food in farmer households.. The data shows that the condition of food assistance in the two villages was not good, with a score of 1.66 in Soak Bato Village and 1.98 in Lebak Pering Village. These two conditions were due to the absence of food assistance received by the people in these two villages. The community feels that there is no food assistance provided by the government, especially food other than rice. For Soak Bato Village, there was absolutely no food aid, while in Lebak Pering Village, food aid arrived but was still very little.

B. Conditions of Staple Food Affordability in Ogan Ilir Regency

Global population growth demands affordable, nutritious food, requiring agricultural industry improvement (Schulman et al., 2023).The explores how suppliers, intermediaries, and customers in a market-based alternative food network assess the economic value of alternative foods while constructing them as affordable. Alternative food refers to local, small-scale, and organic produce, contrasting supermarkets and industrial production methods. These foods are generally more expensive than conventional, requiring steeper prices to challenge the current system (Bååth, 2022). The condition of food affordability in Ogan Ilir Regency is seen from several indicators, namely the food logistics system, staple food purchasing power, staple food prices, and total household income.

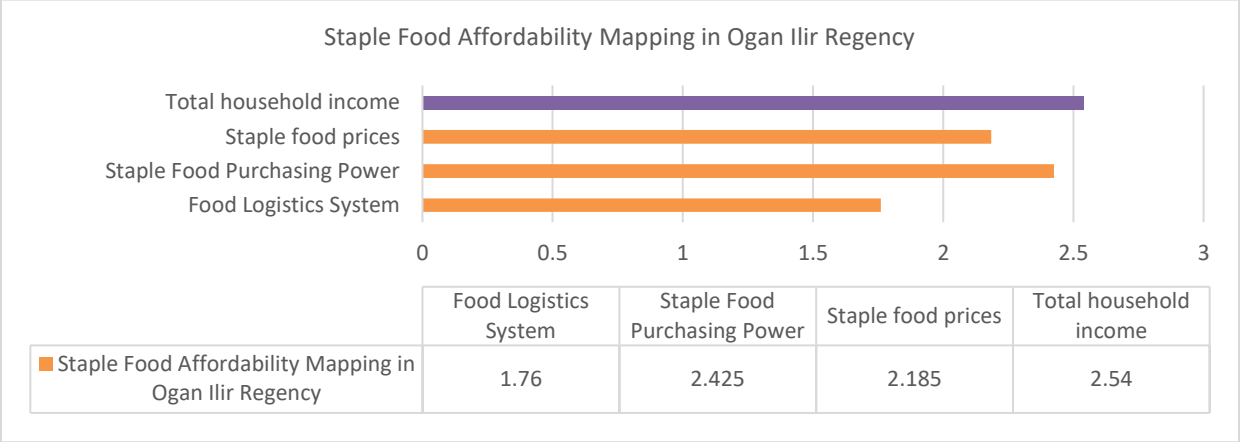

Figure 2. Conditions of Staple Food Affordabilit Mapping

Food Logistics System

The logistics system describes the affordability of food for households. The better the logistics or food storage system for the household, the better the affordability of food for the household. In rural farming households, the average staple food logistics system is still manual or traditional so food preservation is also limited in time. On average, households in these two villages have poor food logistics. This is because the majority of households do not have refrigerators, so food is purchased only for the same day. There is no logistics system for farming households, so this results in potential food insecurity or hunger.

Staple Food Purchasing Power

The purchasing power of staple foods for households shows that households have access to staple foods that are needed daily. The higher the purchasing power of food, the better the affordability of staple food for the households. Purchasing power is an indicators that the quality of staple foods consumed is better. The results of the study in the two villages in Ogan Ilir Regency show that the purchasing power of the people in this area for staple food is good, the average farmer household is able to buy all types of staple food, but for food other than staple food such as additional types of food such as cakes, snacks, snacks and various drinks such as milk, soda, and others, respectively, are not good.

Staple food prices

The price of staple foods affects the affordability of food for households. The more stable food prices, of course, the higher the ability of households to reach staple foods in the market. Food security can be fulfilled if staple food prices

are stable and do not experience large increases, so that households are able to continuously provide a daily supply of staple food for their family members. The results of an analysis of staple food prices in Ogan Ilir district show that prices increase quite frequently in rural areas, so households have difficulties fulfilling staple food continuously. Increases in staple food prices that often occur in rural areas are due to a lack of stability in the supply of staple foods to remote areas. Unstable supply causes very frequent price fluctuations, so people have difficulty buying staple foods.

Total household income

Total household income is an indicator of food affordability; the higher the level of household income received, the greater the household's ability to reach staple food to meet the daily needs of the family. The results of the analysis of household income levels in Ogan Ilir District show that the amount of food purchased will follow the income earned by the household. When income decreases, households will reduce the amount of food purchased, and when income increases, households will try to improve the quality of food consumed. This condition shows that the affordability of staple foods in rural households is highly dependent on the income received by farmers.

CONCLUSIONS

The conclusion of this study shows that domestic food capacity was in the unfavorable criteria. Food reserves are in the less good criteria. This condition indicates that households do not have staple food reserves at home. The score for supplying food from local sources is in the not good criteria. The food aid condition is not good because there is no food aid, received by the communities. The food logistics system is not in good criteria because the majority of households in the village do not have refrigerators, the people's purchasing power for staple food is good, but other additional food is still unable to buy. The price of staple food shows a price increase causes households have difficulties fulfilling staple food.

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EFFECTS OF CASEIN HYDROLYSATE PRODUCED USING SEQUENTIAL ENZYMATIC HYDROLYSIS ON CALCIUM UPTAKE BY CACO-2 CELL

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Abstract: Calcium is important in strengthening bones and teeth, it also plays crucial roles in many physiological processes. While dietary inhibitors of absorption such as phytate form complexes with calcium in the intestine, bioactive peptides could potentially enhance calcium absorption. This study aimed to investigate the effects of peptides derived from casein on calcium uptake by Caco-2 cell. High-performance liquid chromatography analysis showed that glutamine/glutamic acid (21.66% of total amino acid) was the major nonessential amino acid (NEAA) in casein, whereas the two most abundant essential amino acids (EAA) were leucine and lysine (15.88%). Casein was subjected to sequential hydrolysis at 50-55 °C, pH 8.0-8.5 by Alcalase (0.5 g/100 g protein) for 30 min and then Flavourzyme (0.25 g/100 g protein) for 16 h. Casein hydrolysate achieved 52.90% degree of hydrolysis, as determined by the o-phthaldialdehyde method. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis revealed that casein hydrolysate contained peptides with molecular weight less than 10 kDa. Preliminary results demonstrated that the effects of casein hydrolysate (1-5 mg/mL) on intracellular free calcium concentration was concentration dependent (2-6 mM calcium) in Caco-2 cell population model. These results provide insight into the nutraceutical potential of casein hydrolysate as an enhancer of calcium absorption.

***Keywords*:** Bioactive peptides, Casein hydrolysate, Calcium absorption, Caco-2 Cell, Alcalase and Flavourzyme

INTRODUCTION

Short-chain protein molecules with less than 20 amino acid residues and exhibit biological activities are known as bioactive peptides. These amino acid sequences do not show any activity as part of the parent protein, however, they become biologically active when they are freed through enzymatic or chemical hydrolysis. Bioactive peptides are found in enzymatic protein hydrolysates and fermented dairy products; they are also generated during gastrointestinal digestion of proteins by digestive enzymes.

The conversion of protein into hydrolysate using enzyme offers many benefits besides producing bioactive peptides (Novozymes, 2019a). Protein hydrolysate is usually soluble over a broad pH range and hence has a wide range of applications. In addition, hydrolysate with a targeted degree of hydrolysis (DH) and molecular weight profile can be produced using combinations of exo- and endopeptidases. Hydrolysate containing peptides is also absorbed better in

the body than the parent protein. In terms of production, enzymatic reaction is carried out under mild temperature and pH that requires only relatively low-cost technology, therefore it is green to the environment and sustainable.

In addition to its function in building and maintaining bones and teeth, calcium is also involved in formation of blood clot, regulation of muscle contraction, transmission of nerve impulses, secretion of hormones, and activation of some enzyme reactions in the human body (Gallagher, 2008). Calcium adequacy depends on the quantity consumed as well as its absorption rate. Phytate, oxalate, and tannic acid are dietary components that can decrease calcium absorption. On the other hand, bioactive peptides could potentially enhance calcium absorption. Demands for nutraceuticals and functional food that provide health benefits beyond basic nutrition are increasing, driven by increases in life expectancy, health consciousness, and personal disposable income.

Multiple mechanisms of calcium absorption mediated by peptides have been proposed. One of which is peptides function as mineral carriers, where calcium would chelate with peptides and the chelated calcium is absorbed by intestinal cells. Hydrolysis of casein using trypsin produced phosphopeptides that chelated calcium and formed stable soluble peptide-calcium complexes (Cross et al, 2005). Calcium-binding peptides were also found in soybean protein hydrolysates produced using Neutrase, Flavourzyme, protease M, and pepsin by Bao et al. (2008), the highest calcium binding effect observed was 66.9 mg calcium/g protein. Furthermore, desalted duck egg white peptides produced using Protamex had effectively enhanced calcium absorption through the interaction with transient receptor potential vanilloid type 6 (TRPV6) calcium channel of intestinal cells (Hou et al., 2015). TRPV6 calcium channel plays an important role in the transcellular absorption of calcium, particularly when calcium intake is low.

The effect of peptides derived from casein by sequential hydrolysis of Alcalase and Flavourzyme on intestinal calcium absorption is not well known. This study aims to produce casein hydrolysate using the two enzymes at their respective optimal pH, temperature, and enzyme:substrate ratio. The hydrolysate was then characterised in terms of the degree of hydrolysis achieved and the molecular weight profile of the peptides produced. Caco-2 cells were used to investigate the effect on intestinal calcium absorption as mediated by casein hydrolysate. Caco-2 cells express transporter proteins, efflux proteins, and Phase II conjugation enzymes to resemble a variety of transcellular pathways and are often used as a model of human intestinal absorption of drugs and other compounds.

MATERIALS AND METHODS

Alcalase® and Flavourzyme® were obtained from Novozymes Malaysia Sdn. Bhd.. Sodium caseinate was obtained from Promac Enterprises Sdn. Bhd.. The Pico-Tag method described by Heinrikson and Meredith (1984) was used to determine the amino acid content of casein. The application sheet published by Novozymes (2019a) on dairy protein hydrolysates was referred to during preparation of casein hydrolysate. The o-phthaldialdehyde (OPA) method described by Nielsen et al. (2001) was used to determine the degree of hydrolysis (DH) of casein hydrolysate. The sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) protocol outlined by Merck (2023) was used to separate proteins in casein hydrolysate based on size. The simulated gastrointestinal digestion method of Hernández-Ledesma et al. (2007) was used to determine the stability of casein hydrolysate towards hydrolysis by pepsin and pancreatin. Calcium absorption study using Caco-2 cells population model was performed following the procedure described by Hou et al. (2015), with some modifications.

RESULTS AND DISCUSSION

Casein had more non-essential amino acids (63.22%) than essential amino acids (36.80%). Leucine (8.45%) and lysine (7.43%) were the two most abundant essential amino acids in casein. Phenylalanine (4.66%) and valine (5.36%) were present in moderate amounts. Histidine, isoleucine, methionine, and threonine were present at lower quantities (2.47-3.06%). On the other hand, glutamine/glutamic acid (21.66%) was the major non-essential amino acid in casein. Others that present at moderate amounts included alanine (7.91%), arginine (6.71%), and asparagine/aspartic acid (6.71%). Cysteine, glycine, proline, serine, and tyrosine were all found at concentration less than 5.60%.

Casein contained 85.41% of protein was used to produce hydrolysate. After being subjected to sequential hydrolysis by Alcalase and Flavourzyme, a casein slurry (85.41 mg protein/mL) was made into a casein hydrolysate (38.66 mg protein/ml). The recovery of casein hydrolysate was 45.26%. The casein hydrolysate had achieved a degree of hydrolysis (DH) of 52.90%. According to Novozymes (2019a), when hydrolysis was conducted at the optimum pH and temperature of the enzymes, the maximum DH is 15-25% for Alcalase and approximately 45% for Flavourzyme.

Figure 1(a) shows the electrophoretic separation of casein and its hydrolysate. The four casein subunits, the αs2-, αs1-, β-, and κ-caseins (in a decreasing order of relative molecular weight) had molecular weight between 37 and 25 kDa (lane 1). Hydrolysis with first, Alcalase and then, Flavourzyme had resulted in no residual of the casein subunits, this implies an extensive hydrolysis of casein had taken place. The four casein subunits were resolved into smaller peptides with molecular weight less than 10 kDa (lane 2). A distinctive band with peptides that had molecular weight less than 2 kDa was observed. Alcalase is endopeptidase while Flavourzyme is exopeptidase. Despite being classified as exopeptidase, Flavourzyme also displays endopeptidase activity (Novozymes, 2019b). The molecular weight profile of casein hydrolysate was found to be in good alignment with the DH achieved.

Research on bioactive peptides must take into consideration one important aspect of the compounds, that is, the peptides must show resistance to digestion by gastrointestinal enzymes. Figure 1(b) shows that casein was hydrolysed by pepsin and pancreatin into peptides with molecular weight less than 10 kDa (lane 2). The peptides in casein hydrolysate undergone a small extend of hydrolysis by pepsin and pancreatin, yielding peptides with molecular weight around and less than 5 kDa (lane 4). It is postulated that the bigger peptides in casein hydrolysate were hydrolysed into smaller peptides, meanwhile, some of the smaller peptides remained intact and some were hydrolysed. Alcalase and Flavourzyme are peptidases, pepsin and pancreatin are peptidases too. Despite that, peptidases are different based on their catalytic mechanism. Therefore, the peptides obtained via hydrolysis by the enzymes might not be the same.

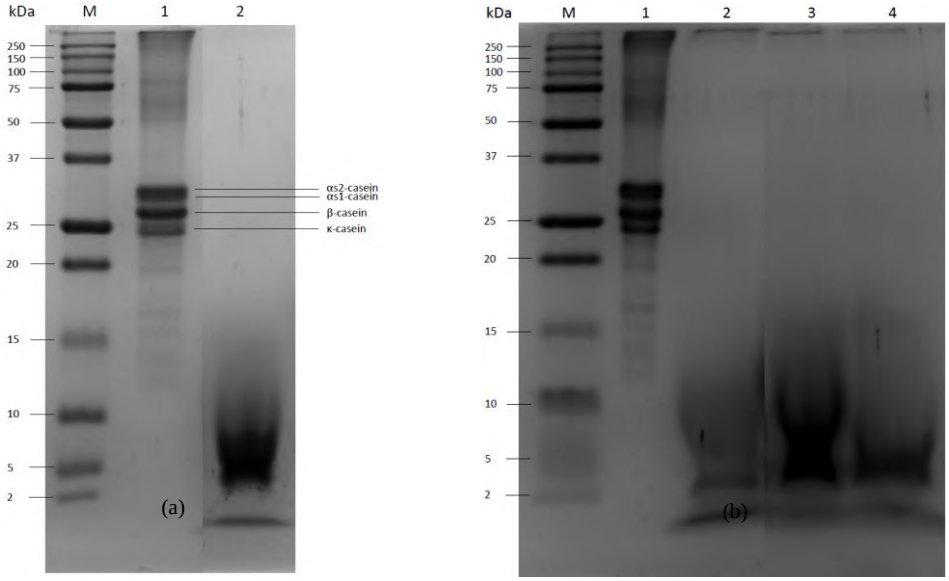
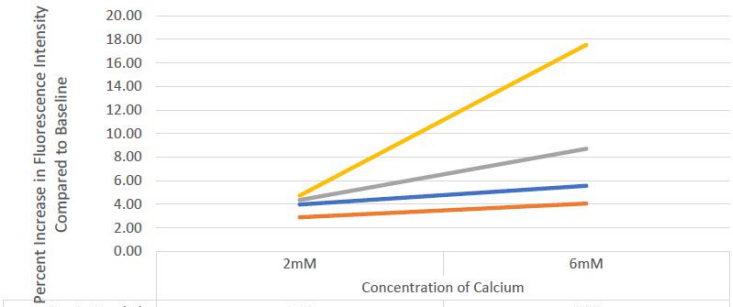


Figure 1: (a) SDS-PAGE analysis of prestained protein standards, 2-250 kDa (M), casein (lane 1), and casein hydrolysate (lane 2); (b) SDS-PAGE analysis of prestained protein standards (M), casein (lane 1), simulated digestion of casein (lane 2), casein hydrolysate (lane 3), and simulated digestion of casein hydrolysate (lane 4).

Figure 2 shows that calcium uptake by Caco-2 cells was influenced by (1) the concentration of casein hydrolysate and (2) the extracellular concentration of calcium ([Ca^{2+}]). As the concentration of casein hydrolysate increased from 1 mg protein/mL to 5 mg protein/mL, calcium uptake by Caco-2 cells increased slightly (4.37% to 4.75%) at extracellular [Ca^{2+}] of 2 mM and increased 2-fold (8.71% to 17.53%) at extracellular [Ca^{2+}] of 6 mM. This result is consistent with literature on soybean protein hydrolysate, whereby the peptides in the hydrolysate were postulated acting as calcium carriers to transport calcium into the cytosol (Bao et al., 2008). According to the researchers, the structures containing Glu, Asp, and Pro played a crucial role in binding calcium. As had mentioned earlier, casein contained 28.37% glutamine/glutamic acid and asparagine/aspartic acid in combination, the peptides generated from this parent protein bind calcium and promote the uptake of the mineral by intestinal cells as the peptides are absorbed. Meanwhile, increasing the concentration of unhydrolysed casein from 1 mg/mL to 5 mg/mL did not increase calcium uptake by Caco-2 cells.



	2mM	6mM
Casein 1mg/ml	3.99	5.57
Casein 5mg/ml	2.91	4.07
CaseinH 1mg/ml	4.37	8.71
CaseinH 5mg/ml	4.75	17.53

Figure 2: Intracellular calcium concentrations expressed as percent increase in fluorescence intensity compared to baseline (fluorescence intensity without the addition of casein or casein hydrolysate).

CONCLUSIONS

Results provide insight into the potential of peptides in casein hydrolysate as nutraceutical that promote calcium absorption by intestinal cells. Ongoing studies focus on investigating the effects of casein hydrolysate on calcium absorption by Caco-2 cells using a monolayer cells model and on TRPV6 calcium channel expression, as well as the calcium-binding properties of peptides in casein hydrolysate.

ACKNOWLEDGEMENT

This work was supported by Taylor's University through the Emerging Researchers Funding Scheme [project code TRGS/ERFS/1/2017/SBS/006].

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TEXTURISATION APPROACHES FOR 3D PRINTING OF FABA BEAN PROTEIN AS PRAWN MIMICS

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Abstract: Offering versatility and automation, 3D printing has the potential to create structures resembling meat and seafood using plant-based ingredients. Nonetheless, for printed plant-based foods to gain consumer acceptance, they need to offer the firmness of animal-derived counterparts. Therefore, suitability of curdlan gum and protein cross-linking enzyme, transglutaminase (TGase) as texturizing agents were evaluated in the context of mimicking prawn which is widely consumed in Asian cultures. Faba bean protein (FBP) was selected for its light colour and xanthan gum was added to FBP to impart post-printing structural stability. Printed curdlan-containing mimics were steamed for 9 min, while printed TGase-containing mimics were incubated at 55 °C for varying durations before steaming. Chewiness of the mimics could be strengthened to match that of prawn through adding curdlan gum (0.0625 - 0.125% w/w) or, TGase and incubating an hour. For curdlan, this was mainly through its hydrogel physically reinforcing the FBP network. Whereas TGase-catalysed isopeptide cross-linkages facilitated β-sheet formation and disulfide bonding, thereby enhancing compactness of the protein structure. Although TGase requires incubation, it appeared more effective since it conferred mimics comparable cutting strength. Overall, curdlan gum and TGase texturisation can be extended towards developing other 3D-printed meat and seafood mimics.

***Keywords*:** 3D printing, faba bean protein, transglutaminase cross-linking, protein-polysaccharide interactions, protein structure analysis.

INTRODUCTION

3D printing offers versatility in terms of ingredient selection and structures which can be printed, allowing automated fabrication according to user specifications. An emerging application of 3D printing is in creating structures resembling meat and seafood using plant-based ingredients, contributing towards the need for more sustainable alternatives of these products. Through their comparable appearance, printed mimics have huge potential to align with consumer dietary habits and, divert meat and seafood demand towards them. To actualize this, the printed mimics would need to offer the firmness of animal-derived counterparts. Curdlan gum gelation and protein cross-linking enzyme, transglutaminase (TGase), were thus evaluated as post-printing texturization agents. Since both curdlan and TGase require heating to be activated, it is hypothesized that their texturisation will only be induced post-printing, retaining printability of the ink until activation. These were evaluated in the context of mimicking prawn since prawn is widely consumed in Asian cultures, but plant-based alternatives are mainly polysaccharide-based and hence lack protein content. Resemblance of mimics treated with TGase and curdlan to prawn were compared via texture profile analysis. Underlying changes to microstructure and molecular interactions were characterised. Overall, both TGase and curdlan could strengthen the mimics to confer chewiness matching that of prawn.

MATERIALS AND METHODS

Materials

Microbial transglutaminase (TGase) blend (Activa TG-SR-MH) was purchased from Ajinomoto (Singapore). Curdlan gum was purchased from Opal Biotech (Zhengzhou, China). Faba bean and citric acid were purchased from the local supermarket. All other chemicals were of analytical grade and purchased from Sigma Aldrich Inc. (St Louis, USA).

Preparation and 3D printing of Faba bean protein (FBP)-based food inks

Faba bean protein (FBP) was extracted by milling faba bean into flour which was dispersed into deionized water at 10% w/v. The dispersion was adjusted to pH 9 ± 0.1 to solubilize proteins. Proteins in the supernatant were precipitated at pH 4.5 ± 0.1 then redispersed at pH 8.0 ± 0.1. Protein content in the extract was determined by measuring nitrogen content via a modified Dumas method (FlashSmart CHNS Elemental Analyser, ThermoFisher Scientific) and using a nitrogen-to-protein conversion factor of 5.4. The control was prepared by diluting the FBP extract and emulsifying with coconut oil to achieve comparable macronutrient composition to prawn, 19.8% w/w protein and 0.7% w/w lipid. Variants consisting of 0.8% w/w TGase with xanthan gum at 1% w/w (1% XG (TGase)) and 2% (2% XG (TGase)); or xanthan gum at 1% with curdlan gum at 0.0625% (1% XG (0.0625% CG)), 0.125% (1% XG (0.125% CG)) and 0.25% w/w (1% XG (0.25% CG)) were prepared. FBP-based inks were 3D printed into prawn structure via a Foodini printer (Natural Machines) using a 1.5 mm printer nozzle. The mimics were then coated with astaxanthin and after steamed for 9 min, in the same manner as prawn. Mimics measured at 0 h were steamed thirty min after emulsification. For TGase-containing mimics measured at 1 h and 2 h mark, thirty min after emulsification, these were incubated at 55 °C accordingly before steaming.

Texture Profile Analysis and Cutting Strength

A TA-XT2i texture analyzer with 5.0 kg load cell coupled to Exponent software (Stable Micro Systems) was used. For cutting strength, samples (40 mm × 10 mm × 10 mm) were measured using a Warner-Bratzler blade at 200 mm/min cut speed. For texture profile analysis, samples (10 mm × 10 mm × 10 mm) were measured using the P25 probe under 4.0 mm penetration depth. Speeds were 1 mm/s for pre-test and test; 5 mm/s for post-test.

Scanning electron microscope (SEM)

Freeze-dried samples were platinum sputter coated. Micrographs were taken at 15 kV accelerating voltage under 300× magnification using a JSM6701F SEM (Jeol Ltd).

Structural changes

To determine intermolecular forces, samples were homogenised into denaturing solvents S1 – S5 and centrifuged, before measuring protein content in each supernatant via Bradford assay as modified from Chen et al. (2015). S1 was deionised water, S2 was 0.6 M sodium chloride with 0.1 M Tris buffer (pH 8.0), S3 was composed of S2 and 1.5 M urea, S4 was S2 and 8 M urea, while S5 was S4 and 2.5% (w/w) βME. Differences in protein solubilised (mg/mL) between S1 and S2 indicate ionic interactions, S2 and S3 indicate hydrophobic interactions, S3 and S4 indicate hydrogen bonds and, S4 and S5 indicate disulfide bonds.

Fourier-transform infrared spectroscopy (FTIR) spectra were collected on freeze-dried samples using Alpha-E Platinum FTIR spectrometer and OPUS version 7.2 (Bruker) then baselined. Baselined spectra were Fourier self-deconvoluted and second derivatized. Peaks in the amide I region (1700 – 1600 cm^{-1}) were then calculated as a proportion of total peak area of this region and assigned to secondary structures following Martínez et al. (2017).

Statistical analysis

Biological triplicates were measured, averaged from technical duplicates. Results were expressed as mean ± standard deviation. Statistical analyses were performed using Prism 9 (GraphPad Software). Significant statistical differences ($p < 0.05$) were evaluated using one-way analysis of variance (ANOVA) with Tukey's post-hoc test or unpaired t-tests.

RESULTS AND DISCUSSION

Adding curdlan gum and incubation with TGase increased hardness and hence chewiness of mimics ($p < 0.05$) **(Table 1)**. Based on chewiness, mimics with the closest texture to prawn had 1% xanthan gum, with curdlan gum added at 0.0625% (1% XG (0.0625% CG)) and 0.125% (1% XG (0.125% CG)); or contained TGase (1% XG (TGase) and 2% XG (TGase)) and incubated for an hour. However, cutting strength was only strengthened during incubation of TGase-containing mimics, with those incubated for an hour matching that of prawn. Hardness and chewiness being non-penetrative are associated with initial perception before biting, while cutting strength being penetrative is associated with perception on biting. This suggested the texture of one hour incubated TGase-containing mimics were closer to prawn than mimics containing 0.0625% and 0.125% curdlan gum. Although xanthan gum had to be added for post-printing structural stability i.e., inhibit flowing of the ink, raising xanthan gum content as per 2% XG (TGase) mimic relative to 1% XG (TGase) weakened gel strengthening during the second hour of incubation. This was reflected by hardness and thus chewiness ($p < 0.05$), as well as cutting strength. Nonetheless, this did not affect textural resemblance of TGase-containing mimics to prawn since they already achieved comparability in the first hour of incubation.

Table 1. Textural analysis of control and mimics relative to prawn.

	Hardness (g)	Chewiness (g)	Cutting strength (kN mm^{-1})
Prawn	1256.4	956.9	1353.3
0 h			
Control	479.2 ± 20.9^{C}	441.6 ± 18.2^{D}	690.0 ± 70.0^{A}
1% XG (0.0625% CG)	718.5 ± 17.3^{B}	818.2 ± 109.4^{BC}	740 ± 78.1^{A}
1% XG (0.125% CG)	797.8 ± 42.4^{AB}	940.6 ± 130.6^{B}	770 ± 91.7^{A}
1% XG (0.25% CG)	869.9 ± 15.4^{A}	1508.4 ± 15.2^{A}	783.3 ± 170.4^{A}
1% XG (TGase)	521.6 ± 25.8^{Cc}	624.0 ± 51.7^{Cc}	673.3 ± 110.2^{Ac}
2% XG (TGase)	447.1 ± 54.3^{Cc}	502.7 ± 7.07^{CDc}	786.7 ± 125.0^{Ab}
1 h			
1% XG (TGase)	619.7 ± 21.5^{Ab}	927.2 ± 71.3^{Ab}	1330.0 ± 52.9^{Ab}
2% XG (TGase)	618.9 ± 24.4^{Ab}	937.7 ± 32.9^{Ab}	1313.3 ± 40.4^{Aa}
2 h			
1% XG (TGase)	1017.6 ± 7.3^{Aa}	1958.1 ± 19.6^{Aa}	1563.33 ± 85.0^{Aa}
2% XG (TGase)	822.7 ± 14.7^{Ba}	1425.7 ± 66.2^{Ba}	1396.7 ± 110.2^{Aa}

In each column, significant differences ($p < 0.05$) are represented by different capital letters amongst means of mimics within the same incubation time and by different lowercase letters across incubation durations for each mimic.

To interpret how TGase, curdlan and xanthan influence gel strength, gel structures were analysed via SEM **(Figure 1).**

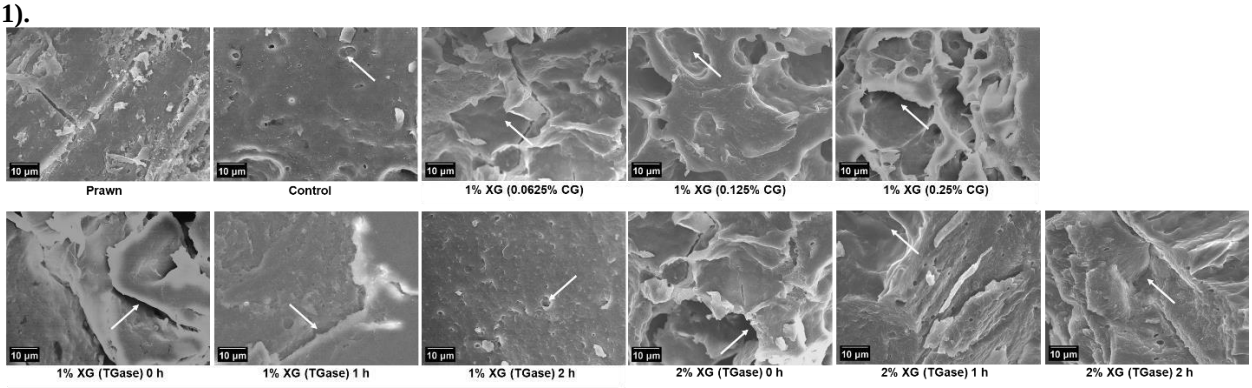

Figure 1. SEM micrographs of the control and prawn mimics relative to real prawn. White arrows indicate pores.

In both TGase-containing mimics, SEM network connectivity was enhanced with incubation. This is attributable to TGase promoting isopeptide cross-linkages (Romano et al., 2016). Fragmented microstructures of mimics containing curdlan gum or additional 1% xanthan gum are typical of protein-polysaccharide gels under SEM (Lin et al., 2023). Greater fragmentation of 2% XG (TGase) than 1% XG (TGase) mimic suggested more extensive xanthan gum penetration and hence obstruction to TGase-catalysed cross-linking of FBP in 2% XG (TGase). This may explain its weaker gel strengthening. While xanthan is merely a thickener, curdlan forms hydrogel complexes which could serve as fillers to strengthen the FBP network.

Hydrogen bonding was significantly reduced and disulfide bonding increased in TGase-containing mimics after the second hour of incubation ($p < 0.05$) **(Table 2)**. Disulfide bonding was also promoted in the mimic with the highest curdlan content relative to counterparts with lower curdlan gum contents ($p < 0.05$). Promotion of disulfide bonding through adding curdlan gum and incubating with TGase respectively could have strengthened the gels (Chen et al., 2015), thereby raising chewiness. Additionally, β-sheet gradually increased ($p < 0.05$), at the expense of α-helix, throughout the 2 h incubation in both TGase-containing mimics **(Table 2)**. β-sheet formation indicates aggregation of FBP which is attributable to TGase-catalysed isopeptide bonds. This may have further strengthened the TGase-containing mimics during incubation (Martínez et al., 2017).

Table 2. Key intermolecular interactions and secondary structure components altered by TGase and curdlan.

	Intermolecular interactions		Secondary structures (%)	
	Hydrogen bonding	**Disulfide bonding**	**α-helix**	**β-sheet**
Prawn	12.16	3.83	11.67	53.78
0 h				
Control	10.52 ± 0.30^{B}	1.99 ± 0.13^{D}	12.44 ± 0.15^{A}	49.74 ± 0.53^{A}
1% XG (0.0625% CG)	12.71 ± 0.36^{A}	1.48 ± 0.30	12.97 ± 0.37^{A}	49.44 ± 0.32^{A}
1% XG (0.125% CG)	12.62 ± 0.17^{A}	1.74 ± 0.09^{B}	12.74 ± 0.15^{A}	49.54 ± 0.69^{A}
1% XG (0.25% CG)	12.49 ± 0.07^{A}	2.45 ± 0.03^{A}	12 ± 170.4^{A}	47.66 ± 0.32^{B}
1% XG (TGase)	12.46 ± 0.03^{Aa}	1.53 ± 0.17^{BC}	12.65 ± 0.32^{Ac}	48.94 ± 0.70^{ABc}
2% XG (TGase)	12.76 ± 0.02^{Aa}	1.40 ± 0.12^{CDc}	12.91 ± 0.04^{Ab}	49.58 ± 0.50^{Ab}
1 h				
1% XG (TGase)	12.17 ± 0.59^{Aa}	1.79 ± 0.09^{BC}	12.40 ± 0.01^{Ab}	50.59 ± 0.34^{Ab}
2% XG (TGase)	12.37 ± 0.13^{Ab}	1.74 ± 0.01^{Ab}	12.71 ± 0.23^{Aa}	50.18 ± 0.82^{Ab}
2 h				
1% XG (TGase)	10.59 ± 0.07^{Ab}	3.27 ± 0.23^{BC}	12.35 ± 0.22^{Aa}	52.27 ± 0.65^{Aa}
2% XG (TGase)	9.39 ± 0.03^{Bc}	2.96 ± 0.10^{Ba}	11.81 ± 0.02^{Aa}	52.12 ± 0.38^{Aa}

Significant differences ($p < 0.05$) are represented by different capital letters amongst means of mimics within the same incubation time and by different lowercase letters across incubation durations for each mimic.

CONCLUSIONS

Evaluated in the context of mimicking prawns, curdlan and incubation with TGase effectively texturised 3D printed plant protein-rich inks. These can maintain printability of the ink, undergoing ink-to-solid transformation only on heat-induction to yield comparable chewiness to prawn. For curdlan, this was mainly via serving as a physical filler. For incubation with TGase, this was via catalysing isopeptide cross-linkages which also promoted β-sheet formation and disulfide bonding, jointly enhancing compactness of the protein network. Although curdlan could be more efficient since it does not require incubation, TGase appeared more suitable since it could confer comparable cutting strength to prawn. Overall, these approaches complement the capability of 3D printing in producing high resolution seafood and meat mimics with comparable protein content, by conferring them comparable textures. Through aligning with consumer dietary habits, well-texturised 3D printed mimics would have huge potential to facilitate dietary shifts.

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DEVELOPMENT OF OMELETTE MIMIC: IMPROVING TEXTURAL RESEMBLANCE OF AMARANTH PROTEIN-STABILISED EMULSION GELS VIA LECITHIN

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Abstract: To mitigate the ASEAN egg shortage situation, plant-based alternatives are needed since they can offer greater sustainability and food security. For plant-based alternatives to effectively capture egg demand, texture is key. In mimicking texture of omelettes, lecithin-protein interactions are hypothesised to be crucial. To evaluate this, amaranth protein was selected as the protein source for its neutral colour and lecithin was derived from soy. Amaranth protein-stabilised emulsions with lecithin varying within the typical range it is present in eggs (0.26 - 2.10%) were fried in the same manner as omelette. Tensile properties of the mimics were strengthened on raising lecithin content, such that mimics with 0.52% and 1.05% lecithin content achieved comparability to omelette. Confocal images suggested adsorption of lecithin in place of proteins at oil droplet interface reduced oil droplet volume and thus proportion exceeding pore size of the matrix. Overlap of protein and lecithin signals in the confocal images suggested their interactions. Indeed, raising lecithin content gradually exposed hydrophobic and sulfhydryl groups. Hence lecithin likely strengthened the mimics through reducing oil droplet size and promoting disulfide bonding. Since the mimics also have comparable protein content and can be fried as per eggs, they have potential to divert demand.
***Keywords*:** Omelette mimic; Amaranth protein; Soy lecithin; Protein conformation analysis; Confocal imaging

INTRODUCTION

The ASEAN egg shortage situation is partially attributable to unprecedented spread of the H5N1 virus and, rising feed and fuel costs. To mitigate this, plant-based alternatives are needed since they can offer greater sustainability and food security. To effectively divert demand, these alternatives need to align with consumer dietary habits in terms of protein content and textures formed on cooking. Targeting omelette, this study hypothesised that comparable textures can be achieved by adhering to the nature of omelette as a protein-lecithin emulsion network. This will be evaluated by determining the influence of varying soy lecithin content, within the typical range lecithin is present in eggs, on textural resemblance of the plant-based mimic to omelette. Amaranth protein was selected as the protein source for the mimic given its reportedly good gelation and emulsification properties, coupled with neutral colour (Gürbüz et al., 2018). Adhering to macronutrient composition of egg, amaranth protein-stabilised emulsions with varying lecithin content were prepared and fried as per omelette. Textural resemblance was assessed via tensile behaviour and underlying changes to microstructure and molecular interactions were characterised. Overall, lecithin was observed to strengthen mimics through reducing oil droplet volumes and hence the obstruction they posed to protein cross-linking. Moreover, lecithin-protein interactions exposed sulfhydryl groups which could facilitate disulfide bonding amongst proteins for gel strengthening. Lecithin content hence influenced textural resemblance of the mimics to omelette.

MATERIALS AND METHODS

Materials

Organic *Amaranthus hypochondriacus* grains originating from India were purchased from Bob's Red Mill Natural Foods (Milwaukie, Oregon, United States). Lecithin from soybean (P3644) was purchased from Sigma Aldrich Pte.

Ltd. (Singapore). Lutein extract (80% purity) was purchased from BLD Pharmatech Ltd. (Shanghai, China). Hen eggs from Malaysia (Pasar) and rice bran oil from Thailand (Ricefield) were purchased from NTUC Fairprice Co-operative Ltd (Singapore). All other chemicals were of analytical grade and purchased from Sigma Aldrich Inc. (St Louis, USA).

Preparation of omelette and amaranth protein-based mimics

A liquid egg control was prepared by diluting whole eggs 7/8 with deionized water. Amaranth grains were grounded into a flour which was defatted at a 1:10 flour-to-hexane ratio for two cycles of 10 h each. Hexane was then removed by vacuum filtration. After drying overnight, the defatted flour was dispersed into deionized water at 10% w/v. The dispersion was adjusted to pH 9.0 ± 0.1 to solubilize proteins. Proteins in the supernatant were precipitated at pH 4.0 ± 0.1 then redispersed at pH 8.5 ± 0.1. Protein content of the extract was determined by measuring nitrogen content via Dumas method (FlashSmart CHNS Elemental Analyser, Thermo Fisher Scientific) and using a nitrogen-to-protein conversion factor of 5.85. Moisture content was accounted for via an infrared moisture analyser (MOC63u, Shimadzu). To the diluted amaranth protein extract, lecithin was added for 30 min hydration, before emulsifying for 2 min at 10,000 rpm with lutein-added rice oil. This yielded emulsions with comparable contents of protein (12.4% w/w emulsion), lipid (9.74% w/w) and lecithin (0.26% (1×), 0.52% (2×), 1.05% (4×), 2.10% w/w (8×)) to egg. The emulsions were then diluted 7/8 with deionized water prior to frying in a similar manner as the liquid egg control.

Tensile behaviour

TA-XT2i texture analyzer with 5 kg static load cell coupled to Exponent software (Stable Micro Systems, Surrey, UK) was used. Omelette and its mimics (40.0 mm × 15.0 mm × 1.0 – 3.0 mm) were secured between A/MTG Mini Tensile Grips such that initial sample length was 20.0 mm. Speeds were 1 mm/s during displacement. Tensile stress (σ_t, Pa) which is the force per unit of time divided by initial contact surface area, and tensile strain (ε) which is the ratio of sample length at time t as a proportion of initial sample length (20.0 mm) were determined.

Protein conformational changes

Surface hydrophobicity S_0 of omelette and its mimics were measured using 8-Anilinonaphthalene-1-sulfonic acid according to Zhang et al. (2022) except samples were dispersed in a 0.01 M Tris buffer (pH 8.0). S_0 was determined as the slope of a linear regression of fluorescence intensity against protein concentration (Biotek Cytation 5). Free sulfhydryl (-SH) content was measured by homogenising the samples (3.0 g ± 0.2 g each) in 10 ml of 0.1 M Tris buffer (pH 8.0) solution containing 0.6 M NaCl, 0.5% SDS and 8.0 M urea at 10 000 rpm for 1 min (T 25 digital Ultra-Turrax®, IKA). Supernatants were diluted to 1.25 mg protein/mL based on protein content measured via Bradford assay (Epoch 2, Biotek). To 300 µL aliquots, DTNB (10 µL, 10 mM in 0.1 M Tris-HCl buffer at pH 8) was added and absorbance read at 412 nm (Epoch 2, Biotek) **(Eq. 1)** (Ellman, 1959).

$$\textbf{Free -SH content (µmol/g)} = \frac{A_{412} \times 73.53}{1.25} \qquad \textbf{(1)}$$

where A_{412} is the absorbance at 412 nm, 1.25 was the protein concentration in mg ml^{-1}, 73.53 was the unit conversion factor for M to µM divided by molar extinction coefficient of $1.36 \times 10^4\ M^{-1}\ cm^{-1}$ (Ellman, 1959).

Confocal laser scanning microscopy (CLSM) imaging

Samples were soaked for 15 min in an aqueous solution containing 160× diluted CellMask™ Deep Red (lecithin dye), then 10 min in ethanol containing 29 ppm FITC (protein dye) and 171 ppm Nile Red (oil dye). Subsequently, samples were rinsed in a 50% v/v ethanol solution. Z-stacks were imaged at 100× magnification using a CLSM microscope (LSM 710, Carl Zeiss) and ZEN 2011 software (Black edition, Carl Zeiss). Excitation/emission wavelength were 488/500 - 534 for FITC, 543/558 – 616 nm for Nile red and 633/644 - 758 nm for CellMask™ Deep Red. Imaris software (Bitplane Company) was used to construct 3D models from the Z-stacks to measure oil droplet volumes.

Statistical analysis

Biological triplicates were measured, averaged from technical duplicates. Results were expressed as mean ± standard deviation. Statistical analyses were performed using Prism 9 (GraphPad Software). Significant statistical differences ($p < 0.05$) were evaluated using one-way analysis of variance (ANOVA) with Tukey's post-hoc test or unpaired t-tests.

RESULTS AND DISCUSSION

From tensile stress-strain graph, several parameters were determined. Young's modulus, gradient of the initial linear portion of the graph, was significantly enhanced on raising lecithin content in the mimics to 2× ($p < 0.05$) **(Table 1)**. Ultimate strain (maximum strain before breaking) was significantly higher in the mimic with 8× lecithin content ($p < 0.05$). Raising lecithin content also gradually enhanced ultimate strength (maximum stress before breaking) and toughness (area under curve) ($p < 0.05$). Mimics with 2× and 4× lecithin contents were similar to omelette across all tensile parameters ($p > 0.05$).

Table 1. Parameters determined from tensile stress-strain curves.

	Young's modulus (kPa)	Ultimate strain (%)	Ultimate strength (kPa)	Toughness (kPa)
0×	95.8 ± 4.6^{a}	11.72 ± 1.08^{a}	9.96 ± 0.63^{d}	1.34 ± 0.17^{d}
1×	153.7 ± 45.8^{ab}	13.56 ± 4.08^{a}	15.62 ± 1.44^{c}	2.41 ± 0.09^{cd}
2×	195.6 ± 29.1^{a}	13.87 ± 1.54^{a}	22.15 ± 1.17^{b}	3.81 ± 0.54^{bc}
4×	202.0 ± 33.1^{a}	14.22 ± 1.10^{a}	25.93 ± 0.46^{b}	3.96 ± 0.81^{b}
8×	216.1 ± 39.0^{a}	20.6 ± 0.90^{b}	34.46 ± 0.92^{a}	6.50 ± 0.76^{a}
Omelette	143.2 ± 12.7^{ab}	16.89 ± 0.68^{ab}	21.87 ± 3.21^{b}	2.69 ± 0.25^{bcd}

Significant differences ($p < 0.05$) amongst the sample means for each column are represented by different letters.

Lecithin enhanced Young's modulus of the mimic which indicates stiffness; ultimate tensile strain which indicates increasing elastic range; ultimate strength and toughness which indicates more energy can be withstood prior to rupturing. Consequently, mimics with 2× and 4× lecithin contents resembled omelette. Since mimics rely on amaranth proteins for gelation, their textures may be closer to omelette relative to many commercial plant-based eggs which rely on polysaccharides. This is because protein networks contain disulfide bonds conferring tensile behaviour (Bailey et al., 2002), unlike polysaccharide gels which thus tend to lack elasticity.

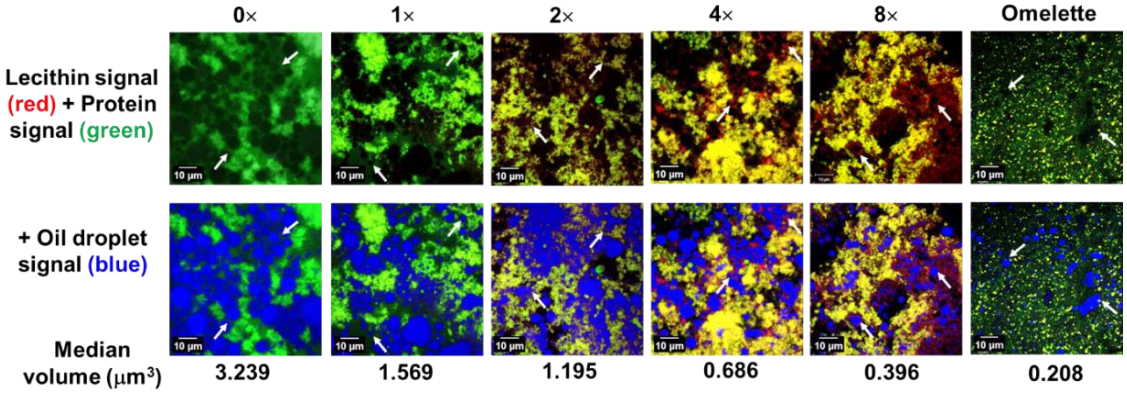

Figure 1. Confocal laser scanning microscopy (CLSM) images of mimics relative to omelette. White arrows show oil droplet interfaces and median oil droplet volumes (μm^3) are indicated below.

Corresponding to extensive coalescence in the CLSM image of the mimic without lecithin, raising lecithin content gradually reduced median oil droplet volume from 3.239 μm^3 to 0.396 μm^3 **(Figure 1)**. Lecithin signals at the oil droplet interface were also gradually intensified on raising lecithin content. Thus, the reduction in oil droplet volume on raising lecithin content was likely due to adsorption of lecithin to the oil droplet interface in place of proteins gradually reducing surface tension (Zhou et al., 2016). This likely strengthened the mimics since oil droplets exceeding pore size of a protein network would weaken it (Torres et al., 2016). Moreover, since raising lecithin content leads to its signals (red) increasingly overlapping with those of the protein network (green), this suggests lecithin-protein interactions which will subsequently be evaluated.

Surface hydrophobicity S_0 was distinctly lowered in the mimic containing 1× lecithin relative to that without lecithin (0×). likely due to lecithin interacting with proteins, thereby enfolding hydrophobic residues within the complexes (Zhu et al., 2019) **(Table 1)**. Raising lecithin content up to 4× ($p < 0.05$) gradually increased S_0 with weak increase from 4× to 8×. This was likely due to hydrophobic sites exposed through lecithin-induced protein conformation changes increasingly exceeding those enfolded (Zhang et al., 2022). Lecithin-induced protein conformational changes also exposed buried -SH groups as reflected by the significant increase in free sulfhydryl (-SH) content on raising lecithin content in the mimics ($p < 0.05$) **(Table 1)**. Exposed -SH groups could facilitate disulfide bonding when exposed to heat during the frying process, thereby strengthening the mimics (Zhang et al., 2022).

Table 1. Effect of lecithin on protein conformational changes in mimics.

	Surface hydrophobicity S_0	Free -SH content (μmol/g)
0×	83.11 ± 3.33^{a}	0.249 ± 0.005^{c}
1×	13.50 ± 1.59^{d}	0.287 ± 0.002^{b}
2×	20.13 ± 1.61^{c}	0.309 ± 0.014^{ab}
4×	56.51 ± 0.82^{b}	0.307 ± 0.011^{ab}
8×	60.38 ± 2.06^{b}	0.311 ± 0.008^{a}

Significant differences ($p < 0.05$) amongst sample means in each column are represented by different letters.

CONCLUSION

Mimics containing 2× and 4× lecithin contents achieved textural resemblance to omelette. This substantiated the hypothesis that plant-based mimics adhering to the structure of omelette as a protein-lecithin emulsion network would achieve textural resemblance to it. Lecithin-induced strengthening was through lecithin reducing oil droplet size and hence obstruction posed by them. Moreover, lecithin-induced protein conformational changes which exposed sulfhydryl groups. This could have promoted disulfide bonding amongst the proteins. Besides texture, the mimics have similar frying behaviour to omelette which would enable consumers to add ingredients according to their tastes. These alongside comparable protein content to egg confer these mimics promising potential to divert egg demand.

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TEXTURED TEMPE (FERMENTED SOY) PROTEIN PRODUCED BY COOKING SINGLE SCREW EXTRUSION: PHYSICOCHEMICAL PROPERTIES AND SENSORY EVALUATION

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Abstract: This study aimed to evaluate the impact of screw speed (SS) on the physicochemical and sensory evaluation of textured tempeh protein (TTP). The extrusion conditions were set at a fixed input moisture level of 55%. The barrel temperature was divided into three distinct zones, namely zone I, zone II, and zone III, with temperatures of 50 °C, 80 °C, and 120 °C, respectively. The variable screw speed (SS) was manipulated at five different levels: 20, 30, 40, 50, and 60 rpm. Subsequently, an analysis was conducted on the TTP to evaluate its proximate composition, water absorption capacity (WAC), oil absorption capacity (OAC), and sensory attributes. The findings of the study revealed that the levels of moisture, protein, fat, ash, and carbohydrate in the samples varied within the ranges of 41.2-45.3%, 29.37-32.21%, 11.76-13.13%, 0.66-0.94%, and 11.54-13.89%, respectively. The colour values (L*, a*, b*) range from 45.61-64.43, 6.68-7.21, and 40.67-47.15, respectively. Notably, TTP with SS 40 rpm demonstrated the most elevated level of acceptability, as indicated by its highest overall rating of 4.04. Tempeh is anticipated to supplant soy protein in the manufacturing of analogues meat due to its cost-effectiveness, favourable taste profile, high nutritional value, and widespread consumption the global population.
Keywords: Extrusion, Sensory, Sustainable, Tempe, TVP

INTRODUCTION

There is a growing focus among scientists, food producers, and consumers on the topics of environmental degradation, animal welfare, and health implications linked to the eating of meat (Lin et al., 2022). On the contrary, there has been a global growth in the population of individuals adhering to vegan, vegetarian, and flexitarian diets, mostly driven by factors such as health concerns, religious beliefs, ethical considerations, and various other motivations. The elevated lipid content present in meat can potentially contribute to the elevation of cholesterol levels within the bloodstream. Cholesterol represents a significant risk factor for the development of coronary heart diseases (CHD). Consequently, those who possess predisposing health conditions such as diabetes, obesity, and cardiovascular disease are advised to refrain from consuming meat and instead choose for vegetarian alternatives (Larsson & Wolk, 2006; Mehta et al., 2015). Tempeh, a legume-based fermented product, has been produced in Java, Indonesia for several centuries. Numerous competitive benefits associated with tempeh have been recognised, including its notable content of crude protein, isoflavone, vitamin B12, folate, fat, and carbohydrate. The present study aimed to produce a new high moisture textured vegetable protein by partially substituting soy protein isolate with tempeh at a ratio of 50% using a single screw extrusion cooking process. This study aimed to evaluate the impact of screw speed (SS) on the physicochemical attributes and sensory evaluation of textured tempeh protein (TTP).

MATERIALS AND METHODS

1. Materials

The materials utilised in the production of TTP include soybean (*Glycine max*) sourced from Wang Lai Store in Pingtung, Taiwan. The tempeh starter culture (*Rhizopus spp*.) was kindly provided by INDEX Store in Pingtung, Taiwan and soy protein isolate (SPI) were acquired from Archer Daniels Midland (ADM) Company, USA.

2. Extrusion Process

The extrusion process was carried out using a single screw extruder manufactured by Taiyu Industrial Co., Ltd., located in Taipei, Taiwan. The production of TTP involves the combination of TF and SPI in a 50:50 (w/w)

proportion. The experimental parameters for extrusion were established using a feed moisture content of 55%. The barrel temperature was partitioned into three distinct zones: zone I maintained at 50°C, zone II at 100°C, and zone III at 130°C. The variable screw speed (SS) encompasses a range of 20, 30, 40, 50, and 60 rpm.

3. Proximate Composition

Using a method developed by the AOAC (2005), the proximate analysis of textured tempeh protein was performed on the moisture, ash, crude fat, crude protein, and carbohydrate.

4. Water Absorption Capacity (WAC) and Oil Absorption Capacity (OAC)

The water absorption index and oil absorption capacity (WAC and OAC) measurements were conducted with modifications to method Hong et al. (2022).

5. Sensory Evaluation

15 non-trained students from the National Pingtung University of Science and Technology conducted a sensory evaluation using the hedonic test.

6. Data Statistical Analysis

SPSS (Statistical Package for the Social Sciences, Version 22.0, SPSS Inc., Chicago, IL, USA) was used to conduct an analysis of variance (ANOVA) on the study's data. The Duncan's multiple range test (DMRT) produced significantly different data with a 95% confidence interval ($P < 0.05$).

RESULTS AND DISCUSSION

1. Proximate Composition

Based on the findings, it was observed that the moisture content of TTP exhibited a statistically significant difference ($P < 0.05$). Elevating the rotational velocity of the screw during the extrusion procedure leads to an augmentation in the water content of the resultant product. The moisture content of TTP exhibits a range of variability, with values ranging from 41.20 to 45.30 percent, as seen in Table 1. An increase in barrel temperature and a drop in SS velocity lead to a greater amount of water vaporising within the barrel, thereby causing a reduction in moisture content. The moisture content was influenced by factors such as the nature of the material, the temperature during extrusion, and the subsequent drying process (Patria et al., 2021).

The observed variations in TTP's ash content did not demonstrate statistical significance ($P > 0.05$). The ash content of TTP exhibits a range of 0.71 to 0.88 percent, as indicated in Table1. A reduction in screw speed leads to an extended residence time of the material in the barrel, hence causing a fall in ash content. This decrease can be linked to a reduction in water content, which in turn results in a decrease in the concentration of minerals that are carried away by evaporating water during the extrusion process. According to Puteri et al. (2018), the ash concentration of tempeh is higher compared to that of SPI due to the solubility of ash in water, resulting in significant degradation during the extraction process.

Table 1. Chemical component of textured tempeh protein and TSP (wet basis)

Samples	Moisture Content (%)	Ash Content (%)	Protein (%)	Lipid (%)	Carbohydrate (%)
SS20	41.20 ± 0.2^{a}	0.71 ± 0.3^{b}	29.37 ± 0.5^{a}	14.11 ± 0.5^{f}	14.61 ± 0.2^{f}
SS30	42.40 ± 0.1^{b}	0.75 ± 0.1^{bc}	30.03 ± 0.3^{b}	13.25 ± 0.3^{e}	13.57 ± 0.1^{e}
SS40	43.20 ± 0.2^{c}	0.80 ± 0.4^{cd}	30.73 ± 0.2^{c}	12.50 ± 0.1^{d}	12.78 ± 0.5^{d}
SS50	44.10 ± 0.4^{e}	0.84 ± 0.2^{de}	31.36 ± 0.3^{d}	11.74 ± 0.3^{c}	11.96 ± 0.2^{c}
SS60	45.30 ± 0.2^{f}	0.88 ± 0.2^{ef}	32.21 ± 0.1^{e}	10.94 ± 0.4^{b}	10.68 ± 0.3^{b}

According to the DMRT test, there are statistically significant differences ($P < 0.05$) between the notations on the same column.

The protein content of all TTP samples was tested and found to range from 29.37 to 32.21 percent, as shown in Table 1. Based on the findings, the samples exhibit a statistically significant difference ($P < 0.05$) in terms of protein content. As the screw speed slows, there is a decrease in the protein content. The duration of material heating in the barrel is directly proportional to the decrease in screw speed. The observed reduction in protein content can be attributed to protein denaturation resulting from the significant heat energy to which the feed was subjected during the process of extrusion. According to Omohimi et al. (2013), he process of extrusion induces thermo-mechanical effects, leading to the gelatinization of starch, protein denaturation, and the inactivation of enzymes, microbes, and anti-nutritional agents.

There were observed statistically significant differences ($P < 0.05$) in the lipid content of TTP. Based on the data presented in Table 2, the lipid content in TTP exhibits a range of 10.94% to 14.1%. As the rotational velocity of the screw dropped, there was an observed increase in the concentration of lipids inside a specific segment of the TTP.

The concentrations of carbohydrates in the TTP samples ranged from 10.68% to 14.61 % (Table 1). The screw speed treatment had a substantial impact ($P < 0.05$) on the amount of carbohydrates. Significant modifications occur in carbohydrates when extrusion conditions are modified. Starch, a form of carbohydrate, is included in a diverse range of extrusion products and serves as a crucial constituent for structural integrity. The study discovered a correlation between the increase in barrel temperature and the deterioration of starch-lipid complexes. Additionally, the creation of these complexes was found to be significantly influenced by the screw speed (De Pilli et al., 2012).

2. Water Absorption Capacity (WAC) and Oil Absorption Capacity (OAC)

The observed WAC values exhibited significant variation ($P < 0.05$), as seen by the range of measured WAC values for all TTP samples, which ranged from 1.46 to 1.80 g/g (Table 2). This resulted in an extended cooking time for the material, leading to the gelatinization of its starch component. That is relate with statement Wang et al. (2017) the process of gelatinization of starch induces expansion in extruded products and enhances their ability to retain water.

There was a substantial variation ($P < 0.05$) in the observed OAC, as indicated by the measured OAC values of all TTP samples, which ranged from 1.94 to 2.36 g/g (Table 2). The potential benefits of enhanced oil absorption in extrudates are evident when considering the application of the product in the creation of broths or sauces. Osen et al. (2014) reported that The application of extrusion heat treatment resulted in an increase in the oil absorption capacity (OAC) of isolated pea protein, likely due to the enhanced exposure of hydrophobic regions.

Table 2. WAC and OAC of textured tempeh protein and TSP

Samples	WAC (g/g)	OAC (g/g)
SS20	1.83 ± 0.1^{f}	2.36 ± 0.2^{f}
SS30	1.75 ± 0.4^{e}	2.21 ± 0.1^{e}
SS40	1.67 ± 0.3^{d}	2.13 ± 0.2^{d}
SS50	1.51 ± 0.2^{c}	2.08 ± 0.5^{c}
SS60	1.46 ± 0.2^{b}	1.94 ± 0.3^{b}

According to the DMRT test, there are statistically significant differences ($P < 0.05$) between the notations on the same column.

3. Sensory Evaluation

TSensory evaluation is employed as a means of assessing culinary products in order to guarantee the provision of a superior, experiential product to the consumer. The highest TTP SS40 scores for sensory texture, aroma, and taste are 4.20, 4.00, and 4.13, respectively. Nevertheless, it is worth noting that the TTP SS 40 sample has the highest overall acceptance score, measuring 4.04.

CONCLUSION

According to our research findings, it is proposed that the manufacture of TTP can be improved by adjusting the tempeh to SPI ratio, with the aim of partially or fully substituting SPI. The physicochemical characterization and sensory evaluation of TTP indicate that an increase in screw speed during TTP manufacturing can lead to a reduction in chemical composition levels, except for protein content. The augmentation of screw speed is inversely correlated with the levels of water absorption capacity (WAC) and oil absorption capacity (OAC). The TTP was achieved with a screw speed of 40 rpm (SS40), which was found to have the highest overall acceptance score of 4.04 in the sensory evaluation. There is an expectation that tempeh would replace soy protein in the manufacturing of analogues/vegetable meat owing to its comparatively lower cost, favourable taste, and high nutritional value.

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EFFECT OF TEMPERATURE ON SIMULTANEOUS OIL AND DEFATTED PROTEIN MEAL FRACTIONATION FROM BLACK SOLDIER FLY LARVAE (BSFL)

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Abstract: Black soldier fly larvae (BSFL) decompose various organic wastes into valuable oil and protein for promising biofuel, cosmetic, human food and animal feed applications. BSFL oil is a potential palm oil alternative due to their similar properties as well as the lauric, palmitic and oleic acids composition. Temperature is a key parameter affecting the extraction performance, especially the quality of both BSFL oil and protein meal. High temperature usually improves the oil yield, but this might cause protein denaturation. In this project, temperature-controlled mechanical pressing (70, 90, 110 °C) was employed to fractionate BSFL oil and defatted protein meal simultaneously. Analytical tests were then performed to investigate the effect of temperature on both fractions. The extracted oil was characterized by iodine value (IV), saponification value (SV), peroxide value (PV), acid value (AV) and free fatty acid (FFA) content, whereas the defatted protein meal was characterized by protein dispersibility index (PDI) and protein content via Total Kjeldahl Nitrogen (TKN) analysis. Results revealed that higher extraction temperature obtained higher oil yield, lower protein yield and lower total losses. BSFL oil extracted at 110 °C demonstrated the highest yield (20.44%), relatively high SV (209.33 mg KOH/g), as well as the lowest IV (53.53 g I_2/100 g), PV (7.89 meq/kg) and FFA content (1.17%). Moreover, the defatted protein meal obtained from 110 °C also exhibited the highest PDI (20.15%). Hence, the optimum fractionation temperature was found at 110 °C which produced lower oil rancidity, higher stability and quality of both oil and protein meal. Further studies on the use of BSFL oil and protein in human food are recommended. By integrating the waste-fed and sustainably-farmed BSFL in food supply chain, the high-quality oil and protein will help to improve waste management, environmental sustainability, food security and nutrition.

Keywords: Black solider fly larvae, Fractionation temperature, Mechanical pressing, Oil extraction, Protein meal

INTRODUCTION

Black soldier fly (*Hermetia illucens*) is originated in America and later spread to tropical and subtropical areas worldwide (Rahman et al., 2021). Owing to its powerful digestive enzymes and mouth parts, BSFL is able to decompose and convert various organic wastes into high-value oils and proteins. The most common substrates used to feed BSFL include but not limited to substantial portion of restaurant wastes, cereals, fruits, vegetables, manures, poultry feeds, brewery and winery byproducts, as well as marine-based seaweeds, mussels, seafoods and fish offal. Rearing of this insect requires lower cost with shorter growth cycle than the conventional way of oil crops production or other animal sources. This is associated with its lower consumption of electricity and water, land-efficiency, higher feed bioconversion efficiency, and lower greenhouse gas emissions (Kim et al., 2021; Rodrigues et al., 2022). It is an edible insect with high nutritional value that contains around 40% protein and 30% fat. To date, BSFL oil and meal are mainly employed for livestock feeds (sheep diet, aquafeed, swine and poultry feed) and biofuel production (Barragan-Fonseca et al., 2017; Rahman et al., 2021).

Several methods have been used to extract oil and/or protein from BSFL including mechanical pressing, wet mode fractionation (WMF), solvent extraction, microwave-assisted and enzyme-assisted solvent extraction. Despite solvent extraction could achieve high yield of lipid, this approach is costly, hazardous, environmentally damaging, time-consuming, and requires extensive solvent recovery. Microwave-assisted and enzyme-assisted extraction are developed in recent years with respective pros and cons. The former is superior in terms of short processing time and high oil yield, while the latter could extract oil without destroying the protein structure. However, the heating effect of microwave-assisted technique on the protein structure is still lacking of information, and it has a high environmental impact. Utilization of enzymes is not economically feasible and less efficient (Amarni & Kadi, 2010; Caligiani et al., 2018). Due to the absence of hazardous solvent and relatively low energy consumption, both WMF and mechanical

pressing are more eco-friendly. The main difference between WMF and mechanical pressing is the use of juicer and oil presser to process BSFL with high moisture and low moisture, respectively. Washing and steam blanching are involved in WMF, where additional step is required to purify the product (Ravi et al., 2021). Thus, WMF is more time-consuming and costly than the conventional mechanical pressing.

In this project, temperature-controlled mechanical pressing was used to fractionate BSFL oil and protein simultaneously due to its simple operation, efficiency, greenness, low cost, and industrial feasibility. Different extraction parameters such as temperature, moisture content and pressing time will influence the product quality in different ways. Temperature is one of the most significant parameters that affecting the extraction yield, structure, quality, properties and final application of the valuable oil and protein. The effect of temperature on BSFL oil extraction had been researched, where oxidation and hydrolysis took place at high temperature causing low oil quality. Nevertheless, studies of temperature effects on the defatted protein meal are still absent. It is known that excessive heating would denature and degrade the protein structure, but low temperature would reduce the extraction efficiency of oil significantly. This is due to the fact that BSFL oil has a high saturation level and it will solidify easily at low temperature. Hence, careful control of temperature during fractionation process is crucial to yield high recovery of oil and protein, at the same time to prevent degradation of both product quality. This research aimed to investigate the effect of temperature on the fractionation of oil and defatted protein meal from BSFL. The extraction performances were gauged based on the yield and quality of products such as IV, SV, PV, AV, FFA content, and PDI. These properties are the important indicators which commonly used to evaluate the oxidative stability, rancidity, saturation level and physical form of oil, as well as the solubility of heat-sensitive protein to estimate the extent of processing and denaturation (da Silva Oliveira et al., 2019; Palić et al., 2011).

MATERIALS AND METHODS

Raw dried BSFL were obtained from BioLoop Sdn. Bhd. (Teluk Intan, Perak, Malaysia), and they were stored at room temperature before use. Dried BSFL were mechanically pressed at different temperatures (70, 90, 110 °C) to extract the oil and defatted protein meal simultaneously. After pressing, the defatted meal was collected and weighed to calculate the protein yield. Meanwhile, the pressed oil was filtered and centrifuged at 8000 rpm for 5 min. The supernatant was then collected and weighed to calculate the oil yield.

Defatted BSFL meal was subjected to TKN analysis via digestion, steam distillation and titration to determine the protein content in both solid and liquid samples for calculation of PDI (Matthäus et al., 2019). While for the characterization of BSFL oil, IV was measured using Wijs method through the reaction of iodine monohalide with the double bonds in oil sample, followed by titration with sodium thiosulphate in the presence of starch indicator to estimate the amount of unreacted halogen. PV was measured as the amount of free iodine produced by the reaction of potassium iodide with the peroxides in oil sample to determine the extent of primary oxidation. Sodium thiosulphate and starch indicator were also employed for titration. Besides, SV was measured under reflux at 100 °C for an hour, followed by titration with hydrochloric acid in the presence of phenolphthalein indicator to determine the amount of unreacted potassium hydroxide. AV was measured through the titration of neutralized ethanol containing oil sample with sodium hydroxide using phenolphthalein indicator (da Silva Oliveira et al., 2019).

RESULTS AND DISCUSSION

As shown in Table 1, the oil yield increased at higher temperature due to rupturing of oil cells and a faster mass transfer rate was promoted during the mechanical pressing process. At elevated temperature, the oil became less viscous and more fluidized, thereby facilitated the oil extraction from BSFL as well as reduced the total mass losses. During low temperature extraction process, higher oil viscosity caused some of the BSFL materials remained in the presser and larger sediments were observed after centrifugation, which resulted in greater mass losses. Nevertheless, further increase of pressing temperature in an effort to improve the overall yield was not recommended as heat could induce adverse effects on the quality of both oil and protein. Since larger amount of oil was extracted at higher temperature, the yield of defatted protein meal was lower with lesser residual oil.

Table 1. Yield of BSFL oil and defatted protein meal at varying temperatures.

Mass yield (%)	Pressing temperature		
	70 °C	90 °C	110 °C
Oil	16.82 ± 0.80	19.80 ± 0.97	20.44 ± 0.73
Defatted protein meal	73.07 ± 0.75	70.95 ± 1.52	70.37 ± 0.40
Loss	10.10 ± 0.44	9.25 ± 0.56	9.20 ± 0.91

After TKN analysis, it was found that the raw BSFL had lower protein content (34.09%) as compared to the defatted meal after pressing (39.6% to 41.8%). As oil had been extracted, the protein in defatted meal was higher in purity. The protein content of defatted meal extracted at 90 and 110 °C were comparable. The lowest protein content of 70 °C meal was due to the aforementioned high viscosity that led to greater mass losses. Table 2 exhibits the PDI of defatted BSFL meal was around 13.5% to 20.2%. In fact, heat treatment tends to cause protein denaturation and coagulation which eventually reduce the solubility and digestibility of protein. Greater extent of processing or heating will decrease the protein stability and PDI value (Palić et al., 2011). However, the findings revealed that PDI of the protein meal increased with pressing temperature. This was attributed to the stability of BSFL protein up to 110 °C for short duration of pressing and the breakdown of larger proteins into smaller sizes which enhanced the protein dispersibility. Moreover, lower residual hydrophobic oil left in the protein meal extracted at higher temperature also increased the PDI.

Table 2. Properties of BSFL oil and defatted protein meal at varying temperatures.

Parameter	Pressing temperature		
	70 °C	90 °C	110 °C
Protein content of defatted meal (%)	39.67 ± 0.20	41.67 ± 1.34	41.75 ± 0.32
Protein dispersibility index (%)	13.52 ± 0.11	18.78 ± 0.11	20.15 ± 0.70
Iodine value (g I_2/100 g)	55.25 ± 1.15	56.03 ± 1.07	53.53 ± 1.33
Saponification value (mg KOH/g)	214.63 ± 5.01	205.84 ± 14.80	209.33 ± 13.81
Peroxide value (meq/kg)	9.08 ± 0.27	9.57 ± 0.15	7.89 ± 0.28
Acid value (g/100 g)	2.76 ± 0.19	2.59 ± 0.21	2.34 ± 0.30
Free fatty acid (%)	1.39 ± 0.09	1.30 ± 0.10	1.17 ± 0.15

IV can be used to predict the stability and physical form of an oil sample. Higher IV represents lower saturation level and the presence of more double bonds in the oil, which is prone to fast degradation reactions including autoxidation or polymerization (Tiefenbacher, 2017). Besides, lower SV indicates higher mean molecular weight or chain length of fatty acids. As shown in Table 2, the IV of extracted BSFL oil ranged from 53 to 56 g I_2/100 g, while the SV of BSFL oil was approximately 205 to 215 mg KOH/g, which were similar to palm oil (Mba et al., 2015). The oil sample at 70 °C had the highest SV and smallest chain length, which was desired for better absorption and digestibility. When the pressing temperature increased from 70 °C to 90 °C, a relatively higher amount of unsaturated fatty acids with longer chain length were fluidized and extracted at higher oil yield, leading to higher IV and lower SV. It was found that further increase of temperature to 110 °C obtained the lowest IV and relatively higher SV of oil sample. Higher temperature provided sufficient energy to fluidize the saturated fatty acids with higher melting point. Therefore, greater amount of saturated fatty acids with shorter chain length such as palmitic and lauric acids were extracted at 110 °C.

Low oil quality is usually attributed to oxidation and hydrolysis processes. These processes cause rancidity, off-flavour and low oxidative stability which eventually affect the shelf life of oil and fat products. PV can be used to measure the extent of primary oxidation of oil where the hydroperoxides are formed. Based on Table 2, the PV of BSFL oil displays similar trend as IV, confirming the presence of higher unsaturation level would accelerate the rate of lipid oxidation. The PV of BSFL oil was around 7.8 to 9.6 meq/kg, in which 110 °C produced the lowest PV of oil. They were considered fresh with satisfactory oxidative stability as the PV well below 10 meq/kg. Since the raw BSFL was fed with substrates derived from oil palm biomass, the extracted oil was intense orange in color. Hence, it was deduced that the presence of natural antioxidants such as tocotrienols and carotenoids also played a vital role to reduce the degree of oil oxidation. Upon hydrolysis, the ester bonds in triglycerides are broken where the FFA are released. Table 2 shows the AV of BSFL oil ranged from 2.3 to 2.8 g/100 g, whereas the calculated FFA content was approximately 1.1% to 1.4%. When the pressing temperature increased especially from 90 °C to 110 °C, the AV and FFA content of BSFL oil decreased significantly. This was due to the evaporation of moisture at elevated temperature which reduced the extent of lipid hydrolysis process, leading to lower AV. Although the FFA content was desirable for animal feed, it was necessary to refine the crude pressed BSFL oil for human consumption. Oil refining such as neutralization and deodorization processes can be conducted to further reduce the FFA content below 0.05% to meet industry standards for the edible oils (Matthäus et al., 2019).

CONCLUSIONS

BSFL are promising sources of fat and protein for diverse applications. This insect was mechanically pressed at controlled temperature for simultaneous oil and protein fractionation. Higher temperature had obtained higher oil

yield, lower protein yield and lower total losses. The optimum pressing temperature was found at 110 °C which showed the best overall performance in this work. BSFL oil extracted at 110 °C demonstrated the highest yield, saturation level and oxidative stability, relatively smaller chain length, as well as the lowest FFA content. Meanwhile, PDI of the defatted meal also increased with temperature, suggesting better protein quality obtained from 110 °C. Furthermore, it was found that BSFL oil closely resembled the properties of palm oil, making it a highly potential palm oil alternative.

Different properties and qualities of BSFL oil and protein will lead to different applications. Though the applications of BSFL oil and meal are greatly focused on animal feed and biofuel, it was reported that BSFL contains essential amino acids that human needs. However, the use of BSFL in human food is an ongoing challenge due to the food safety concerns and consumer acceptance. Therefore, in-depth studies are recommended to investigate the suitability of BSFL oil and protein for human consumption, based on the guidelines defined by FAO/WHO and international standards such as Codex Alimentarius. The presence of any detrimental component to human health should be explored, while the fatty acid and amino acid profiling of BSFL can be conducted to better analyze the composition. Additionally, it is also suggested to refine or purify the BSFL oil to further improve its shelf life and quality for human food applications. Ultimately, this novel food resource can help to address the global issue of food-fuel competition, as well as alleviate the stress of traditional agricultural production in the limited arable lands. Maximum utilization of the waste-fed and sustainably-farmed BSFL to generate high quality oil and protein will help to tackle climate change, achieve circular economy, improve waste management, enhance food security and nutrition.

ACKNOWLEDGEMENTS

This work was supported by Xiamen University Malaysia Research Fund (XMUMRF) [XMUMRF/2019-C3/IENG/0010], BioLoop Sdn. Bhd. [EENG/0041], and World Wildlife Fund (WWF) Education for Nature Program [EF16984].

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THE EVALUATION OF PHYSICOCHEMICAL PROPERTIES OF BANANAS (*Musa acuminate*) COATED WITH DIFFERENT POLYSACCHARIDE-BASED COATING

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Abstract: Bananas are among the most important fruits in global trade. However, bananas have a short shelf life which results in financial losses for farmers and can lead to food waste. Therefore, developing an edible coating for bananas may help to extend their shelf life and reduce food waste. There are various studies on formulating the edible coating solution including polysaccharide-based coating that was proven to extend the shelf life of foods. **Problem statement**: However, limited research tested the effect of the physicochemical properties of the coated fruit, especially bananas. **Objective**: Thus, an edible coating with the combination of polysaccharide-based (alginate, carrageenan and pectin) and ZnONPs for bananas was developed. **Method**: The bananas were dipped in the formulations produced; AlZnONPs, CZnONPs, PZnONPs and R (uncoated). The coated bananas were stored at room temperature for 15 days to observe the physicochemical changes (weight loss, TSS, TA, pH, reducing sugar and firmness) that happened. **Findings**: The physicochemical properties of the coated bananas were significantly different ($P < 0.05$) between the coated and uncoated bananas with the increased storage time. **Conclusion**: Coated bananas were found to have significant changes in their physicochemical properties and these findings offer excellent potential in post-harvest technologies that could increase income for farmers, reduce food waste and also food waste management. **Future recommendation**: This polysaccharide-based coating should be tested on other types of food that could benefit local farmers.
***Keywords*:** edible coating, polysaccharide, zinc oxide nanoparticles, shelf-life, banana

INTRODUCTION

Bananas, scientifically referred to as Musa spp., have a prominent global trade position due to their extensive consumption, economic value, and contribution to livelihoods (Mahmud et al., 2022). They rank as the fourth most crucial crop worldwide (Pongprasert et al., 2021). This crop's substantial export value significantly benefits economies and offers livelihoods to farmers and labourers across the supply chain (Qin et al., 2023). Bananas, rich in nutrients like potassium and fibre, are staple foods globally, ensuring steady demand (Munia et al., 2019). With its favourable climate, Malaysia also participates in the global banana trade. It cultivates diverse banana varieties for local and international markets, contributing to its economy and food industry. However, bananas have a short shelf life which results in financial losses for farmers and can lead to food waste (Tkáč et al., 2022). Therefore, developing an edible coating for bananas may help to extend their shelf life (Panariello et al., 2022). There are various studies on formulating the edible coating solution including a polysaccharide-based coating that was proven to extend the shelf life of foods (Kong et al., 2022). However, limited research tested the effect of the physicochemical properties of the coated fruit, especially bananas.

Thus, an edible coating with the combination of polysaccharide-based (alginate, carrageenan and pectin) and ZnONPs for bananas was developed. The utilization of polysaccharides as biopolymers in creating edible film coatings is driven by their effective oxygen barrier properties due to strong hydrogen bonds leading to organized structures (Song et al., 2021). Polysaccharides such as cellulose, pectin, carrageenan, alginate, starch, chitin, and chitosan replace traditional packaging materials, aligning with sustainability trends. These coatings, often transparent and oil-free (de Oliveira et al., 2021), prolong fruit shelf life by preventing microbial growth while maintaining aerobic conditions (Ilyas et al., 2022). To address the hydrophilic nature of polysaccharides and enhance their moisture barrier, they can be combined with hydrophobic materials (Ng et al., 2022). Alginate, derived from marine brown algae, serves as a stabilizer and thickener in the food industry. It is applied as a coating to slow fruit ripening, involving immersion in alginate solutions containing glycerol and calcium chloride (Bibi et al., 2023). Carrageenan, from red algae, forms coatings that alter fruit atmospheres, extending shelf life. Kappa-carrageenan, approved by the FDA, is used in edible coatings to reduce respiration rates, maintain firmness, delay ripening, and improve fruit quality (Smola-Dmochowska et al., 2023). Pectin, derived from plant cells, is a soluble fibre rich in galactose, arabinose, and rhamnose. Sourced from fruits like apples and citrus, pectin is used as an edible coating that effectively protects fruits by offering barriers against oxygen and carbon dioxide (Menezes & Athmaselvi, 2016).

Zinc oxide nanoparticles were added to the formulation due to the properties of antibacterial, antimicrobial, and UV-blocking properties (Jiang et al., 2018). In an investigation by La et al. (2021), it was determined that the optimal concentration for creating a uniform layer on banana peels within edible coatings was 0.5% ZnONPs. These nanoparticles are considered safe inorganic oxides suitable for human consumption, holding approval from the FDA (Lian et al., 2021). In this study, the bananas were dipped in the formulations produced; AlZnONPs, CZnONPs, PZnONPs and R (uncoated). The coated bananas were stored at room temperature for 15 days to observe the

physicochemical changes (weight loss, TSS, TA, pH, reducing sugar and firmness) that happened. Coated bananas were found to have significant changes in their physicochemical properties and these findings offer excellent potential in post-harvest technologies that could increase income for farmers and reduce food waste and food waste management.

MATERIALS AND METHODS

Preparation of polysaccharide-based coating solution

Briefly, two solutions are prepared namely solution A and solution B. For solution A, 1g (0.5%) of zinc oxide into 80mL of distilled water. The solution was stirred until dissolved by constant stirring using a water bath sonicator for 10 minutes. For solution B, the alginate was weighted to produce 1.0%, then the alginate was mixed with 80mL of distilled water and stirred at 50°C for about 30 minutes using a magnetic stirrer. After 30 minutes, 1g (0.5%) of glycerol is added into solution B to support binding with zinc oxide solution. Finally, solutions A and B are mixed with the addition of 40mL of distilled water and stirred for 2 hours to produce an AlZnONPs coating solution (Bahrami et al., 2019; Eldib et al., 2020). The solution was allowed to be cooled at 28°C or room temperature before applying banana coating. The procedure was repeated by replacing the alginate with carrageenan (CZnONPs) and pectin respectively (PZnONPs).

Physicochemical analysis of coated banana

Physicochemical analysis of coated bananas was observed through six analyses which are weight loss (Mooktida Saekow et al., 2019), firmness (Mooktida Saekow et al., 2019), TSS (Wani et al., 2021), TA (Nguyen et al., 2021), pH (Wani et al., 2021) and reducing sugar (Sahu et al., 2020).

RESULTS AND DISCUSSION

Weight loss (%)

Weight loss is an important aspect to consider when evaluating the effectiveness of the coating in prolonging the shelf life and quality of the fruit. In this study, the graph illustrates the trend of percentage weight loss over a period of 15 days for coated (AlZnONPs, CZnONPs, PZnONPs) and uncoated bananas (R). All treatments exhibit a general increasing trend over time. Specifically, the R shows a steady and consistent increase in values throughout the period. The CZnONPs experienced a slight increase in values initially until day 6, followed by a significant jump at day 12, and then a slight increase again towards the end of the period. The AlZnONPs exhibit a steady increase in values over the days, with a slight acceleration in the increase around day 9. Lastly, the PZnONPs treatment shows a steady increase in values over the days, but the rate of increase slows down towards the end of the period.

Edible coatings act as an extra layer that coats the stomata and guard cells, forming a film on the surface of the fruit (Ceccarelli et al., 2021). This additional barrier created by the coating serves to reduce respiration and transpiration in the fruit, ultimately resulting in reduced moisture loss and gaseous exchange from the bananas (Zhao et al., 2021). Various studies have shown that alginate coatings have a positive effect on preventing weight loss in plum, sweet cherry, and apple fresh cuts (Korbecka-Glinka et al., 2022), indicating their potential efficacy in preserving the quality of bananas as well. One study specifically focused on the effect of alginate coatings on weight loss in bananas, using a combination of sodium alginate, ascorbic acid, olive oil, and citric acid as the coating materials (Solís-Contreras et al., 2021). This study found that the treatment of 3% calcium alginate proved to be the most efficient in reducing weight loss in bananas, followed by a treatment of 3% sodium alginate.

The use of carrageenan-based coatings on bananas has been found to have a significant effect on reducing weight loss (Dwivany et al., 2020). The use of carrageenan-based coatings on bananas has been found to have a significant effect on reducing weight loss (Dwivany et al., 2020). Dwivany reported that weight loss reduction in fresh-cut banana fruits was achieved by combining calcium chloride with carrageenan coating. Previous research on coated mangoes demonstrated that pectin coatings can significantly decrease weight loss compared to untreated fruits after 6 days of storage at room temperature (Khalil et al., 2022). One study by Placido et al. (2018) demonstrated that coating mangaba fruits with pectin biofilms increased their shelf life. Overall, the use of polysaccharide-based coatings for banana preservation has been studied and shown promising results in extending the shelf life and maintaining the quality of bananas.

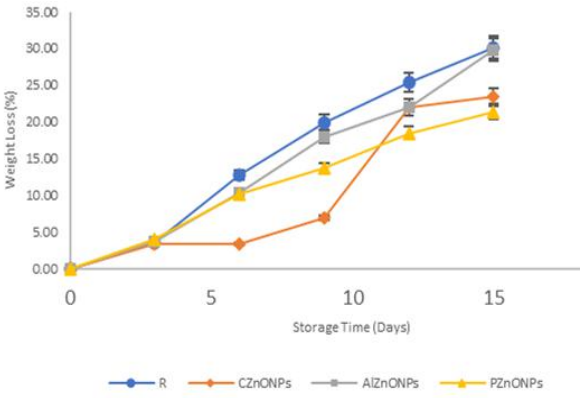

Figure 1. The weight loss of uncoated and coated bananas for 15 days of storage at room temperature

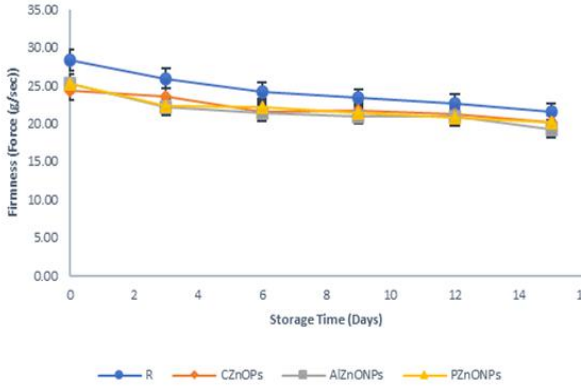

Figure 2. The firmness of uncoated and coated bananas for 15 days of storage at room temperature

Firmness

Banana firmness is an important quality attribute that greatly affects consumer preference and post-harvest handling techniques (Yang et al., 2022). In this study, the graph illustrates the trend of firmness over a period of 15 days for coated (AlZnONPs, CZnONPs, PZnONPs)

and uncoated bananas (R). All treatments exhibit a general decreasing trend in firmness over time, indicating that the substances become less firm as the storage days increase. Specifically, the R shows a steady decrease in firmness throughout the period. The CZnONPs experience an initial decrease in firmness until day 6, followed by a slight increase at day 9, and then a continued decrease until the end of the period. Similarly, the AlZnONPs exhibit a steady decrease in firmness over the days, with a minor increase at day 9. Lastly, the PZnONPs show an initial decrease in firmness until day 6, followed by a slight increase at day 9, and then a continued decrease until the end of the period.

Alginate-based coatings have been shown to extend or enhance the firmness of fruits by creating a protective barrier that inhibits moisture loss and minimises the degradation of cell walls (Mahmoud et al., 2022). Additionally, the protopectin in the peels and pulp of bananas can be transformed into soluble pectin due to the presence of an enzyme called protopectinase, which leads to a decrease in firmness during storage (Ma et al., 2019). Therefore, utilizing an alginate-based coating on bananas may also inhibit the activity of protopectinase and prevent the conversion of protopectin into soluble pectin, thereby maintaining the firmness of the fruit. In addition to the alginate-based coating, other factors such as the presence of calcium chloride and the use of chemical dips have also been shown to contribute to maintaining the firmness of coated bananas (Baite et al., 2022).

Research has shown that carrageenan-based coatings can modify the internal gas composition of fruits, specifically reducing oxygen concentrations and increasing carbon dioxide concentrations (Dwivany et al., 2020). This modification of the atmosphere surrounding the fruit can slow down textural changes and help maintain firmness (Hassan et al., 2022). The study found that the firmness of strawberries decreased over time during storage, as expected. However, the strawberries coated with carrageenan-based coatings exhibited higher levels of firmness compared to the control samples (Solís-Contreras et al., 2021). The ability of carrageenan to form a barrier between the fruit and the surrounding gas has been proven effective in reducing respiration and minimising discolouration in fresh-cut fruit (Warsiki & Manan, 2022). Additionally, carrageenan films can provide antibacterial protection and maintain fruit texture during storage (Smola-Dmochowska et al., 2023).

The pectin-based coating helped to slow down the decrease in firmness of the bananas over time (Felicia et al., 2022). This can be attributed to the fact that the pectin-based coating acts as a barrier, preventing the degradation of the cell wall and the transformation of starch, polysaccharides, hemicelluloses, and pectin into sugars during the maturation process (Sun et al., 2022). In addition to the protective function of the pectin-based coating, other compounds such as nanocellulose and sucrose ester fatty acid were also found to contribute to the firmness retention of coated bananas (Cárdenas-Barboza et al., 2021). Overall, the findings from various studies suggest that coating bananas with polysaccharide-based coatings can positively impact their firmness and postharvest quality (Soleh et al., 2022).

CONCLUSIONS

In the present work, an edible coating with the combination of polysaccharide-based (alginate, carrageenan, and pectin) and ZnONPs for bananas was developed to examine the physicochemical changes that happened to the coated bananas. The physicochemical analysis shows the weight loss (significantly lower than uncoated), TA (significantly higher than uncoated), pH (significantly lower than uncoated), TSS (significantly lower than uncoated) reducing sugar (significantly increased than uncoated) and firmness (significantly higher than uncoated) of the coated bananas within a period. The application of coating on bananas resulted in notable alterations in their physicochemical attributes. These discoveries hold promising implications for post-harvest advancements, potentially enhancing farmers' earnings and decreasing both food waste and its management. Exploring the implementation of this polysaccharide-derived coating on various food varieties could offer advantages to local agricultural producers.

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FOOD SPOILAGE-CAUSING BACTERIA INHIBITION: SYNBIOTIC EFFECT OF *BIFIDOBACTERIUM* SPECIES AND PILI (*Canarium Ovatum* ENGL.) POMACE AS POTENTIAL BIOPRESERVATIVES

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Abstract: Supplementation of food waste, Pili (*Canarium ovatum* Engl.) Pomace Powder (PPP), a prebiotic to the probiotic *Bifidobacterium* species, was studied to assess the inhibitory potential against common food spoilage-causing bacteria via in vitro analysis. Fermentation was observed by significantly lowering the pH values of the synbiotic mixture (3.67±0.58) vs. Bifidobacterium consortium alone (5.00± 0.00). Population density and viability of synbiotic mixtures (OD = 1.09 ±0.10; 9.37 ±0.02 log CFU/ml) were also significantly greater than Bifidobacterium consortium alone (OD = 0.12±0.03; 9.18±0.04 log CFU/ml). The inhibitory capacity of the food spoilage-causing bacteria vs. synbiotic mixtures grown in MRS broth with 1% PPP, shown as % survival rates [*Staphylococcus* spp. (35.34%) vs. synbiotic mixture (77.37%); *Enterobacter* spp. (44.85%) vs. synbiotic mixture (91.87%); and *Staphylococcus* spp. (56.68%) vs. synbiotic mixture (85.81%)] were all observed to be lower than those of the synbiotic mixtures, which survived after incubation. The antimicrobial substance responsible was found to be organic acids as the by-product of bifidobacterial fermentation. These results showed that the synbiotic could inhibit food spoilage-causing bacteria and suggest that this synbiotic mixture may have promising applications in food biopreservation.
***Keywords*:** *Bifidobacterium*, probiotic, prebiotic, synbiotic, *Canarium ovatum* Engl.

INTRODUCTION

Shelf-life improvement of food products through microorganisms and/or antimicrobials is a young branch of science. Metabolites produced by fermentation yield numbers of beneficial microorganisms and by-products that aid in lowering the causes of food spoilage and leave food free from pathogenic microbes. (Singh, 2018; Ganguly, 2013). The general objective of this study is to determine the synbiotic effect of Bifidobacterium species and Philippine Pili (*Canarium ovatum* Engl.) Pomace on the growth inhibition of selected spoilage-causing bacteria.

Probiotics, prebiotics, or a combination of both are synbiotics and inhibit food spoilage. This synergistic effect of a living and a non-living component in application to food products has been recently established in biopreservation. *Bifidobacterium* spp. is considered to be a potent probiotic. Adding a locally grown nut and utilizing its waste material as a prebiotic to make a synbiotic formulation is a significant addition to probiotic bacteria growth and viability. It has also been a noticeable trend recently that consumers are more aware of their food choices. **Thus, this study can be a good source of biopreservatives, thereby a potential alternative against food chemical preservatives.**

MATERIALS AND METHODS

Sample Collection

Pure cultures of *Bifidobacterium* spp. (*B. bifidum* ATCC 29521 ®, *B. breve* ATCC 15700 ®, *B. infantis* ATCC 15697 ®, *B. adolescentis* ATCC 15703 ®, and *B. longum* ATCC 15707 ®) were purchased from the Japan Collection of Microorganisms.

Philippine Pili (*Canarium ovatum* Engl.) Pomace Powder was obtained from the Department of Food Technology, College of Education, University of Santo Tomas.

Spoilage-causing microorganisms were taken from spoiled and rotten fruits. Bacterial samples were analyzed morphologically and phenotypically.

Analysis of physiological characteristics of Bifidobacterium consortium

Three physiological characteristics of Bifidobacterium spp. were studied in increasing levels of pH (2, 3, 4, 5, 6, and 7), salt (1%, 2%, 3%, 4%, 5%, and 6%), and temperature (18°C, 37°C, and 45°C) following the methods of Prabhurajeshwar & Chandrakanth (2019) and Mahmoudi et al., (2013).

Prebiotic effect of Philippine Pili (*Canarium ovatum* Engl.) Pomace (PPP) on growth of *Bifidobacterium* consortium

The fermenting capacity of *Bifidobacterium* consortium in PPP was determined and expressed in pH, optical densities, and % survival rates according to the methods of Kimoto-Nira et al., (2019) & Onal-Darilmaz et al., (2019) with modifications.

Effect of the synbiotic mixture on selected food spoilage-causing bacteria

The inhibitory capacity of the synbiotic mixture (*Bifidobacterium* consortium + 1% PPP) was tested against the individually selected food spoilage-causing microorganisms. The final pH, optical densities, total plate count, and % survival rates were observed and recorded according to the methods of Onal-Darilmaz et al., (2019) with modifications.

Characterization of antimicrobial substances

Antimicrobial substances produced by the synbiotics was examined for the production of antimicrobial substances according to the methods of Prabhurajeshwar & Chandrakanth(2019) with modifications. These are bacteriocins, organic acids, and hydrogen peroxide using agar well-diffusion technique.

RESULTS AND DISCUSSIONS

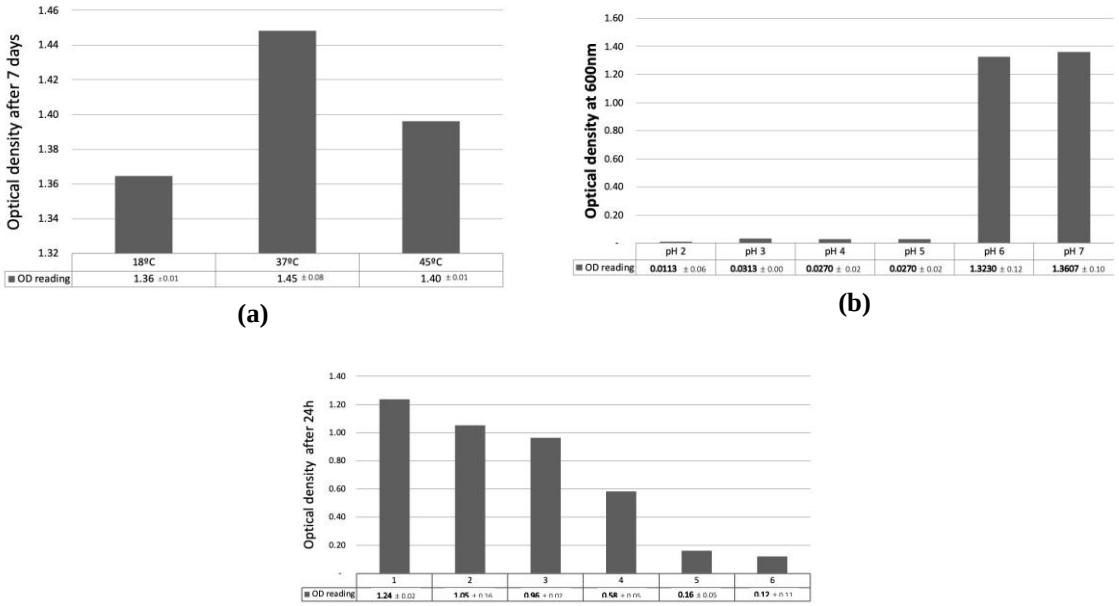

Figure 1. Analysis of physiological characteristics of *Bifidobacterium* consortium; (a) effect of temperature; (b) effect of pH; (c) effect of salt concentration

The most favorable temperature for growth of *Bifidobacterium* consortium is 37ºC (Fig. 1). In the study of Matejčeková et al., (2019) and Shah (2011) the optimum temperature for bifidobacterial growth is between the range of 36ºC to 38ºC in anaerobic conditions.

In Fig. 1, pH 6 and 7 are favorable for bifidobacterial growth. Studies by Shah (2011), Meena et al., (2014), and Sánchez et al., (2007) concluded that the optimum viability for *Bifidobacterium* spp. is between pH 6.5 to 7.0 and has a very low culturable bacteria in pH 2.5 to 6.4

Increasing percent concentrations of salt for Bifidobacterium consortium resulted to a decrease in bacterial viability (Fig. 1). Gandhi and Shah (2016) stated that bacterial membrane is damaged by salt stress on *Bifidobacterium* spp., especially in salt concentrations greater than 3.5%.

Adding 1% PPP to MRS broth significantly increased the bacterial growth of synbiotic mixtures, depicted by lower pH values, cell density, and viability. These results not only confirm the positive effect of PPP on the present study but can also suggest the availability of a synergistic effect among the *Bifidobacterium* spp (Table 1).

Table 1. pH, OD, and viability (log CFU/ml) of selected food spoilage microorganisms in control media vs. synbiotic media

Staphylococcus spp.	**Control media** (MRS w/o PPP)	**Synbiotic media** (MRS with 1% PPP)	**% Survival**	
			Bifidobacterium consortium	Staphylococcus spp.
pH	5.17[a] +/- 0.06	4.8[a] +/- 0.27	77.37%	35.34%
OD at 600nm	0.36[a] +/- 0.05	1.588[b] +/- 0.04		
Log CFU/ml	8.67[a] +/- 0.05	8.74[b] +/- 0.08		
Enterobacter spp.	**Control media** (MRS w/o PPP)	**Synbiotic media** (MRS with 1% PPP)	**% Survival**	
			Bifidobacterium consortium	Enterobacter spp.
pH	4.37 [a] +/- 0.23	2.87 [a] +/- 0.06	91.87%	44.85%
OD at 600nm	1.04 [a] +/- 0.03	2.07 [b] +/- 0.14		
Log CFU/ml	9.26 [a] +/- 0.01	9.41 [b] +/- 0.02		
Streptococcus spp.	**Control media** (MRS w/o PPP)	**Synbiotic media** (MRS with 1% PPP)	**% Survival**	
			Bifidobacterium consortium	Staphylococcus spp.
pH	4.8 [a] +/- 0.20	4.7 [a] +/- 0.20	85.81%	56.68%
OD at 600nm	0.39[a] +/- 0.03	1.70[b] +/- 0.42		
Log CFU/ml	9.21[a] +/- 0.02	9.29 [b] +/- 0.01		

Values are means +/- standard deviations from the experiments done in triplicates. The correlations between final culture pH and viability of strains synbiotics are indicated by mean scores followed by different letters are significantly different at P< 0.05.

The inhibitory capacity of the food spoilage-causing bacteria vs. synbiotic mixtures grown in MRS broth with 1% PPP, shown as % survival rates [*Staphylococcu*s spp. (35.34%) vs. synbiotic mixture (77.37%); *Enterobacter spp*. (44.85%) vs. synbiotic mixture (91.87%); and *Staphylococcus spp.* (56.68%) vs synbiotic mixture (85.81%)] were all observed to be lower than those of the synbiotic mixtures, which survived after a period of incubation (Table 1). This indicates that synbiotic cultures can inhibit spoilage-causing bacteria.

Table 2. Comparison of bacteriocin, organic acid, and hydrogen peroxide assays for antimicrobial substances characterization

Food spoilage-causing bacteria	***Zone of inhibition (mm)***		
	Bacteriocin assay	***Organic acid assay***	***Hydrogen peroxide assay***
Staphylococcus spp.	*8.00 +/- 1.53*	*No zone*	*16.00 +/- 2.08*
Enterobacter spp.	*2.00 +/- 0.58*	*No zone*	*13.00 +/- 2.65*
Streptococcus spp.	*11.00 +/- 2.58*	*No zone*	*14.00 +/- 2.08*

The results presented in Table 2, shows that the culture supernatants of the synbiotic mixture treated with pronase did not affect the inhibitory capacity against food spoilage-causing bacteria. This indicates that the inhibitory effect of the synbiotic mixture was not due to bacteriocin production. Moreover, culture supernatants treated with catalase also did not affect its inhibitory capacity. This indicates that inhibition by the synbiotic mixture was not due to hydrogen peroxide production. However, neutralized synbiotic supernatant (pH 6.5) did not exhibit inhibitory activity and is attributed to the organic acid production. Hence, this analysis concludes that the synbiotic mixture is responsible for organic acid production.

CONCLUSIONS

This study concluded that the synbiotic mixtures of Bifidobacterium consortium and Philippine Pili (*Canarium ovatum* Engl.) Pomace inhibited food spoilage-causing bacteria: *Staphylococcus* spp., *Enterobacter* spp., and *Streptococcus* spp. These findings suggest that this synbiotic mixture may have promising applications in food biopreservation.

It is recommended that future studies focus on varying concentrations of PPP to standardize the best prebiotic concentration. Second, is to study it compared to chemical preservatives used in food products. Lastly, there is an application of the synbiotic mixtures in actual food products to establish actual biopreservation capacity.

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BRIDGING SCIENCE AND HALAL: CREATING A COMMUNICATION PLAN AND MATERİALS FOR DOST-ITDI'S HALAL COMPLIANT FOOD PRODUCTS

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Abstract: Halal Science involves the systematic acquisition of knowledge through observation, experimentation, and practice to describe and explain natural phenomena involving halal practices. However, communicating this knowledge to the public through traditional scientific manuscripts can sometimes lead to confusion and misinterpretation, particularly among non-experts. To address this, a contextual model of disseminating information was employed to effectively convey the true meaning of halalness to stakeholders involved in handling and manufacturing halal food. In this study, a science communication plan was prepared to raise awareness among food manufacturing industries in the Philippines (where almost 90% of the population are non-Muslims), on halal and cultural sensitivity, and to encourage them to become halal compliant. Various science communication materials, including rundown sheets for broadcast media, opinion pieces, editorial calendars, brochures, infographics, and elevator pitches, were developed specifically for halal compliant food products developed by DOST-ITDI. These materials facilitated stakeholders' understanding of the importance of halal compliance and provided a comprehensive view of the Halal Assurance System. Overall, the initiatives taken in this study enhanced the amount of information that can be communicated to stakeholders, resulting in a better understanding of halal compliance and its importance in food manufacturing.
***Keywords*:** Halal, Science communication, Muslim, Compliant, Food Products

INTRODUCTION

This study aims to develop a science communication plan and materials for the halal compliant food products developed by the Industrial Technology Development Institute of the Department of Science and Technology (DOST-ITDI), particularly the dehydrated foods fruits, vegetables, and root crops. These communication plan and materials seek to provide halal awareness to non-Muslim and comprehensive view of the Halal Assurance System in every step of the production of the aforementioned dehydrated products. Food materials like those coming from fruits (mango, coconut and pineapple), vegetables (carrots and malunggay), and root crops (cassava, sweet potato and purple yam "ube") are considered halal (Alzeer *et al.*, 2018); however, once these materials are processed, in order to be considered Halal, the ingredients, processing methods, packaging materials, and equipment used must be halal certified and/or halal compliant (Made & Prima, 2022).

The Philippines is considered as a Muslim minority country (Ilahan-Bakil, 2021). Due to this circumstance, the majority of industry collaborators at the DOST-ITDI are non-Muslim small and medium-scale manufacturers who primarily serve the non-Halal market. Consequently, there is a limited awareness of halal guidelines. Despite of that, a lot of companies are interested to venture in halal products because of the growing demand of halal food industry in the world (Nurrachmi, 2017).

For the Muslims, Halal is a way of life. It is not just a list of do's and don'ts, but a holistic practice of living in the lawful way according to the Qur'an (Rahman *et al.*, 2020). The approach towards Halal certification centers mostly to direct identification of haram (unlawful) materials. This approach can lead to several inaccurate interpretations as different Islam sects/branches may have different guidelines for halalness. For manufacturers to understand the importance of halal and to develop a mindset for ensuring halalness, it is also important to communicate the Halal Assurance System in an empathic sense - that is, developing a culture of halal in the food chain as if the industry players were halal-practicing individuals themselves. Aside from developing the capacity to test food materials for halalness, cultural and religious sensitivity towards Islamic halal guidelines is hoped to be developed through the science communication activities. As more manufacturers enter the halal market, the country will progress toward inclusive development by incorporating the needs of our Muslim citizens into the nation's priorities.

MATERIALS AND METHODS

The development of science communication plan and materials in the DOST-ITDI's halal compliant dehydrated food products were conducted during the Science Communication Fellowship Program jointly organized by the Philippine Council for Industry, Energy and Emerging Technology Research and Development (PCIEERD) and University of the Philippines Diliman in 2022. The science communication activities were designed based on the lessons learned during the said fellowship.

The science communication activity of this study started by preparing a science communication plan. Objectives, intended stakeholders, communication materials, and communication activities were identified in the communication plan. The ultimate goal of the activity was established based on the knowledge gaps of the target audience (Borowiec, 2023). A contextual model was used for disseminating information considering the levels of knowledge, awareness, needs, and attitudes of key players in the halal dehydrated food value chain. This approach allowed for the adjustment of communication materials to make them more comprehensible and engaging for the target audience (Lewenstein, 2003). Based on the prepared science communication plan, several communication materials were prepared for the intended stakeholders such as infographics, brochures, Halal Assurance Management System (HAS) Manual, short videos, training materials, and more. The effectiveness of the communicated information was assessed through evaluations by key players.

RESULTS AND DISCUSSION

The science communication plan designed for DOST-ITDI's halal-compliant dehydrated foods is shown in Table 1. It has been given the title 'Step by Step Towards Halal Science: The Halal Way of Food Dehydration,' with the main objective of communicating the halal concepts to stakeholders who will be involved in handling and manufacturing halal dehydrated food, especially non-Muslims.

Table 1. Science communication plan

Project Title: Step by Step Towards Halal Science: The Halal Way of Food Dehydration			
Main objective: To communicate halal concepts to stakeholders that will be involved in handling and manufacturing halal dehydrated food (especially to Non-Muslim).			
Science communication objectives	**Intended stakeholders**	**Communication materials**	**Communication activities**
To raise awareness on halal and cultural sensitivity	Main: Food SMEs Farmers, Traders, Toll Packers, Consumers	Information materials	Informal written and visual communications (e.*g.*, infographics, short videos) posted on online media platforms
To inform and convince food manufacturers to be halal compliant	SMEs from Food Dehydration Sector	Brochures	Knowledge sharing activity
To guide project collaborators on halal compliance of process, product, and facility and introduce the Halal Assurance Manual	Interested collaborators from Food Dehydration Sector	HAS Manual Audit guidelines infographics	Focus group discussions Site Visit
To provide technical assistance in making customized Halal Assurance Manual in preparation for certification	Interested collaborators from Food Dehydration Sector	Information materials HAS Manual	Customized training

On June 29 2021, the project team responsible for developing DOST-ITDI's halal-compliant dehydrated foods conducted an online seminar on Halal Awareness. The seminar attracted 989 participants from various regions in the Philippines, primarily representing private companies, government entities, students, and academia. During the webinar, participants were asked to suggest topics for in-depth discussions. The results of the seminar evaluation revealed that attendees still had knowledge gaps regarding Halal certification guidelines, the requirements for dehydrated foods to achieve halal compliance, and the Halal Assurance Management System (HAS). Following the development of the science communication plan, informational materials such as infographics and videos were created. Infographic materials, as shown in Figure 1, and videos were posted on media platforms to further raise awareness of halal practices and cultural sensitivity.

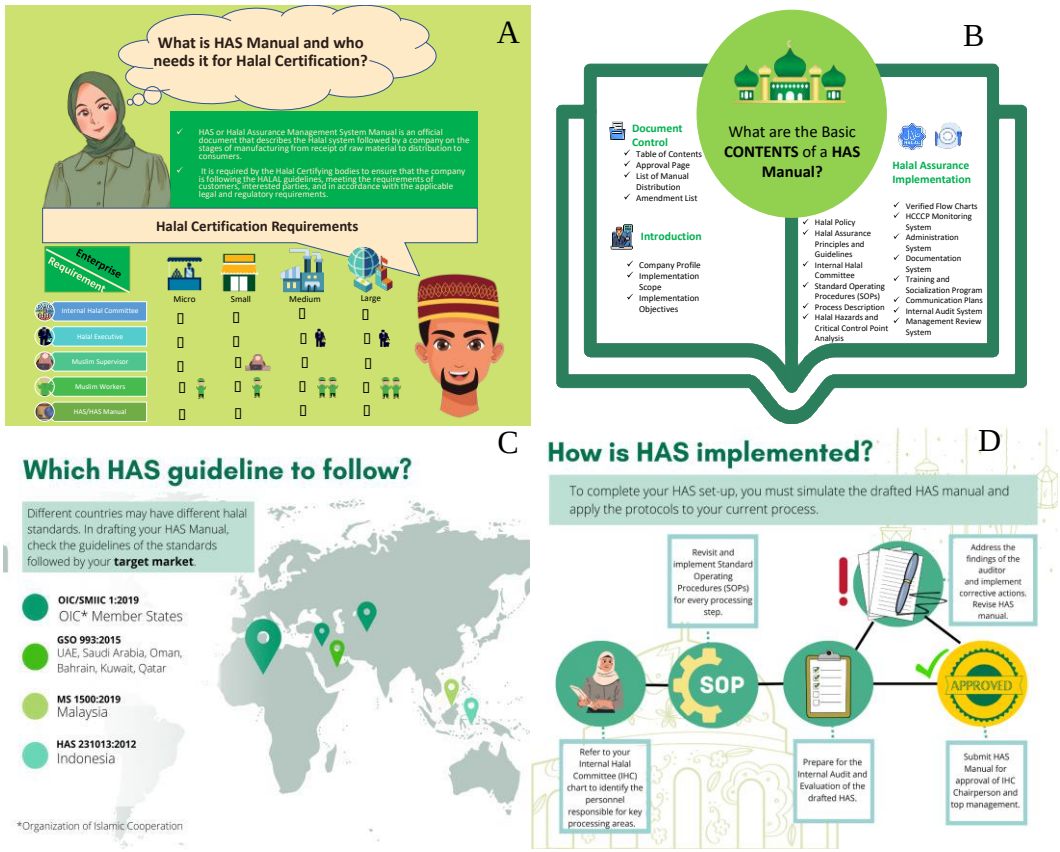

Figure 2. (a) Comparison (b) List (c) Geographic (d) Road Map Infographics

A rundownsheet was prepared for a webcasted program envisioned for halal dehydrated food products developed by DOST-ITDI. The program features the halal concepts and practices from the perspective of halal experts/researchers and halal-practicing individuals. This platform can serve as an avenue to inform the public of the accomplishments of the aforementioned Halal Project and to convince food manufacturers to become halal compliant. Furthermore, this program can be used to strategically promote ITDI-developed Halal products, Halal certified R&D facilities, and Halal-related technical services.

A brochure informing and convincing the Food Manufacturers, particularly those involve in food dehydration, on how to become Halal Compliant through the help of DOST-ITDI was made available in printed copy and in web-based version posted in social media. The target audience of this technical material are employers, entrepreneurs, managers, and/or technical staff from food industries aging from 22 to 39 years old. Brochure was chosen as the communication channel for the intended audience because it is versatile as it can be distributed at trade shows, can be put inside the visitor's lounge area of the Food Processing Division of DOST-ITDI, can be sent to the target audience via direct mail or e-mail, and can be published in DOST website and social media.

To guide project collaborators on halal compliance of processes, products, and facilities and introduce the Halal Assurance Manual, the project team for halal dehydrated food products conducted a seminar titled "How to HAS? Application of Halal Assurance Management System in Food Industries." The topics covered included the Requirements of Halal Certifying Body, Requirements of Halal Auditor, Principles of HAS, and Implementation of HAS. The seminar took place on June 29, 2022, and was attended by 393 participants from all over the Philippines. Seventy percent of the attendees were employers or employees from private companies aged between 22 and 39 years old. The majority of these participants were non-Muslims from the National Capital Region (NCR) and Region 4A. The results of the training evaluation showed that a significant number of attendees rated the appropriateness of the training methodology used as outstanding.

A site visit was also conducted by the project team and collaborators from the Food Dehydration Sector to a halal-certified restaurant in Mindanao and halal facility in Metro Mamila. This visit provided them with an actual experience and immersion in a facility that is halal certified. Additionally, the halal R&D facility at DOST-ITDI was made available for a site visit to provide interested collaborators with a walkthrough and an opportunity to gain firsthand experience.

Lastly, a HAS Manual Writing Workshop was organized for the Certified Halal Lead Auditors from the DOST system. This workshop aims to provide technical assistance in creating customized Halal Assurance Manuals, making it easier to prepare for Halal certification for interested food companies. Feedback from the workshop attendees indicated that the activity's objective was met very satisfactorily.

CONCLUSIONS

In conclusion, science communication proves that scientific ideas and result of research can be properly and effectively communicated to the target audience, provided that appropriate media, messages, and activities were properly planned and executed. The contextual model is the appropriate approach to use for Halal science, as it involves concepts and philosophy aside from the technical, scientific or empirical aspects. Other communication materials that were not presented in the paper will be showcased during the oral presentation. It is recommended to make use of these additional communication materials as well.

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EFFECTS OF BANANA PEEL FLOUR (*MUSA PARADISIACA* AAB (SUB-GROUP PISANG NANGKA) SUBSTITUTION ON PHYSICOCHEMICAL AND SENSORY PROPERTIES OF BUN

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Abstract Banana is among the most widely cultivated fruit compared to the other fruits. The consumption of banana pulp contributed to waste generation, particularly banana peels, which are valuable resources for waste valorization. Banana peel flour (BPF) was produced as an alternative to substituting wheat flour in bun production. The banana peel flour was prepared by using a drying process, while the bun was prepared by incorporating banana peel flour from 5 to 10% as wheat flour substitution. This study aims 1) to compare the physicochemical properties of banana peel flour against wheat flour, and 2) to compare the physicochemical properties of 5% and 10% banana peel flour bun with bun from wheat. Physicochemical analyses were conducted on both the flour and bun, while sensory evaluation was specifically performed on the bun. The findings showed that banana peel flour and banana peel flour bun demonstrated the highest ash, mineral (magnesium and calcium) and fibre content. Banana peel flour had lower moisture content and water activity than wheat flour. Besides, 5% banana peel flour bun attained the highest overall sensory panelist acceptability score of 3.53± 0.82 compared to 10% banana peel flour bun with a value of 2.53± 0.94. Future research could explore BPF's antioxidant and antimicrobial properties for innovative agricultural byproduct use.

***Keywords*:** banana, banana peel, banana peel flour, banana peel bun, waste valorisation.

INTRODUCTION

This paper studied the physicochemical properties of banana peel flour (BPF) in comparison to wheat flour. The banana peel flour was incorporated into baked product such as bun in 5 and 10% as a wheat flour substitution. The physicochemical and sensorial properties of the bun was investigated and compared to bun made with no BPF substitution. Bananas are one of the most produced fruit globally including in Malaysia (Taib *et al*., 2021) and this lead to banana peel as agriculture waste generation (Zaini *et al*., 2020). Banana peel accounts for 40% from banana weight which would causing both environmental pollution and economic losses if discarded (Zaini *et al*., 2020). Thus, the incorporation of banana peel into food product such as baked product may reduce those problems and enhance the nutritional profile in bun as BP has high crude fiber and other minerals (Amini Khoozani *et al.*, 2019). Physicochemical analyses were conducted on the flour and bun while sensory evaluation conducted on the bun. The banana peel was processed to get the BPF by drying process. The BPF recorded higher nutrients compared to wheat flour. The physicochemical properties of bun made from BPF also parallel to BPF results and 5% BPF substituted bun had higher acceptability compared to 10% BPF substituted bun.

MATERIALS AND METHODS

1. Banana peel flour production

The banana peel was dipped in 0.5% (w/v) citric acid solution and then dried using a drying cabinet for 48 hr at 65°C and ground into powder. The powder was then sieved using 50 mesh screens and stored in airtight plastic packs at 5±2°C (Zaini *et al*., 2020; Deb *et al.*, 2022).

2. Bun production

There were three types of bun prepared: control (wheat bun), 5% and 10% banana peel flour bun.

3. Flour analyses:

3.1 Moisture content and water activity

Moisture content was determined following the standard AOAC protocol used by Qadir and Wani (2022).

Water activity of flour was measured by a water activity meter (Series 4TE, Aqua Lab) at 25°C (Padhi and Dwivedi, 2022).

3.2 Ash content

Ash content was determined as describe by Qadir and Wani (2022).

3.3 Colour

The colour of the flour was determined using a colourimeter (Chroma Meter CR 400, Konica Minolta), with the results expressed as lightness (L*), redness (a*) and yellowness (b*) (Al-Sahlany and Al-musafer, 2020).

3.4 Total soluble solids

The total soluble solids was determined using a bench refractometer (REICHERT) (Mutshinyani *et al*., 2020).

3.5 Mineral

Mineral content was determined by method described by Bhinder *et al.* (2021) using Atomic Spectrophotometer (AAS) (Analyst 400, Perkin elmer).

3.6 Crude fibre

The crude fibre content was determined as describe by Das *et al.* (2022).

4. Bun analyses:

4.1 Colour

The colour analysis was carried out on the bun crumbs and crust using colourimeter (Chroma Meter CR 400, Konica Minolta) (Nasir *et a*l., 2020).

4.2 Texture profile analyses

Texture profile analyses was carried out using a texture analyser (TA-XT plus Texture Analyser (Corrado *et al*., 2023).

4.3 Mineral content

The mineral (Mg and Ca) was analysed using Atomic Absorption Spectrophotometer (AAS) (Bhinder *et al*., 2021).

4.2 Crude fibre

The crude fibre was analysed according to the method described in Tanweer and Imran (2018).

4.5 Sensory evaluation

Thirty (30) untrained panelists from Universiti Teknologi MARA (UiTM) Kampus Kuala Pilah, Negeri Sembilan were involved. The subjects indicated their degree of acceptance through the 5-point Hedonic (García-Gómez *et al*., 2022).

5. Statistical analysis

The experimental data were evaluated using independent sample T-test for flour analyses and One-way ANOVA with Turkey's test (α=0.05) for bun analyses.

RESULTS AND DISCUSSION

In Table 1, the moisture content in banana peel flour was insignificantly lower ($p>0.05$) than wheat flour. According to Alam *et a*l. (2020), the moisture content of flour needs to be lower than 14% to avoid mould growth. Water activity in banana peel flour was significantly lower ($p<0.05$) than in wheat flour. The result showed the ash content in banana peel flour is significantly higher ($p<0.05$) than in wheat flour. The L* colour of banana peel flour was significantly darker ($p<0.05$) than wheat flour. The dark colour of banana peel flour is caused by enzymatic browning due to the polyphenol oxidase enzyme in banana peel (Umairah and Hasmadi, 2023). On the other hand, the total soluble solid of banana peel flour was significantly higher than wheat flour. The mineral content in banana peel flour is significantly higher ($p<0.05$) than wheat flour as the ash content represents minerals in foods. In contrast, crude fibre cannot be detected in wheat flour due to the removal of the bran layer during the milling process (Ma et al., 2022).

Table 1. Physical analysis of banana peel flour and wheat flour

Parameter	Banana peel flour (BPF)	Wheat flour (WF)
Moisture (% wb)	6.55 ± 1.17^{a}	9.28 ± 1.70^{a}
Water activity (%)	0.27 ± 0.01^{b}	0.44 ± 0.02^{a}
Ash (% wb)	8.37 ± 0.27^{a}	2.41 ± 0.53^{b}
L*	49.07 ± 0.04^{b}	92.17 ± 0.08^{a}
a*	2.44 ± 0.03^{a}	-0.57 ± 0.01^{b}
b*	15.01 ± 0.09^{a}	9.7 ± 0.06^{b}
Total soluble solid (^{o}Brix)	3.20 ± 0.20^{a}	0.17 ± 0.06^{b}
Calcium (mg/100 g)	101.5±0.82a	41±0.89b
Magnesium (mg/100 g)	114.92±0.83a	25.58±1.89b
Crude fiber (%)	9.36±0.93	-

Values are mean ± SD (n=3). Values with different superscripts within the same row are statistically significant different ($p<0.05$).
-: not detected

2. Bun analyses

Table 2. Bun analyses

Parameter	Control (100% wheat flour)	Formulation 1 (5% banana peel flour)	Formulation 2 (10% banana peel flour)
Colour			
Crumb			
L*	64.25±0.90^{a}	27.63±1.40^{b}	23.02±1.22^{c}
a*	0.33±0.32^{b}	3.53±0.07^{a}	3.23±0.10^{a}
b*	23.23±1.12^{a}	12.23±0.83^{b}	9.08±0.61^{c}
Crust			
L*	74.94±1.25^{a}	44.48±0.75^{b}	35.27±0.37^{c}
a*	4.45±1.42^{a}	6.58±0.19^{a}	6.13±0.52^{a}
b*	35.04±1.59^{a}	22.15±0.74^{b}	15.81±0.33^{c}
Texture			
Hardness (g)	378.39±43.40^{b}	883.95±31.83^{a}	1127.30±345.66^{a}
Springiness (mm)	0.95±0.01^{a}	0.90±0.03^{ab}	0.88±0.03^{b}
Cohesiveness	0.77±0.02^{a}	0.78±0.06^{a}	0.73±0.06^{a}
Gumminess (mm)	292.03±39.84^{b}	686.28±79.52^{a}	814.95±97.72^{a}
Chewiness (N)	278.42±39.07^{b}	619.58±87.06^{a}	714.96±73.47^{a}
Resilience	0.36±0.01^{a}	0.37±0.03^{a}	0.34±0.02^{a}
Chemical analyses			
Calcium (mg/100 g)	24.5±0.50^{c}	27.25±0.90^{b}	31.5±1.25^{a}
Magnesium (mg/100 g)	30.33±1.28^{b}	34.08±2.96^{b}	38.83±0.38^{a}
Crude fiber (%)	-	1.51±0.15^{a}	2.29±0.65^{a}
Sensory analysis			
Texture	4.37 ± 0.809^{a}	3.83 ± 0.747^{a}	2.67 ± 1.06^{b}
Appearance	4.43 ± 0.679^{a}	3.37 ± 0.850^{b}	2.57 ± 0.858^{c}
Colour	4.27 ± 0.868^{a}	2.90 ± 0.712^{b}	2.53 ± 0.937^{b}
Taste	4.20 ± 0.718^{a}	3.50 ± 1.14^{b}	2.60 ± 1.00^{c}
Overall acceptance	4.37 ± 0.718^{a}	3.53 ± 0.819^{b}	2.53 ± 0.937^{c}

Values are mean ± SD (n=3) except for sensory (n=30). Values with different superscripts within the same row are statistically significant different ($p<0.05$).
-: not detected

The result in Table 2 shows that the banana peel flour bun contained higher amount of mineral content (Mg and Ca) and crude fibre than wheat flour. The amount of mineral and crude fibre content increased gradually with increasing substitution of banana peel flour (BPF) in bun formulations. The crude fibre in control was not detected due to the removal of carbohydrates and proteins during the acid and base solution treatment. The colour of bun substituted with BPF was significantly darker with yellow chromaticity ($p<0.05$) than control. Hardness, gumminess, chewiness, and resilience values increased with increasing substitution of BPF. According to Alkurd *et al.* (2020), hardness increase in banana peel flour bun was due to the fibre content in banana peel flour. In addition, the values of springiness and cohesiveness decreased proportionally with the increasing amount of BPF in bun. The increasing fibre content affected the elasticity due to limitation development of the gluten network. There is significant difference ($p<0.05$) between 5% and 10% BPF substituted bun in all sensorial attributes except colour, with more panelist preferred the former between these two formulations.

CONCLUSIONS

In this study, it shows that banana peel flour has higher nutrition profile than wheat flour and this is reflected in bun substituted with banana peel flour which also has higher nutrition profile than bun made with no banana peel flour substitution. The inferiority of the substitution is that higher than 5% substitution would influence panelist preference on color attribute during sensory evaluation. The banana peel flour substitution possibly reducing banana peel wastage and higher nutrition profile bakery product such as bun may be developed. All of the objectives were achieved and

further studies on banana peel properties may encourage its incorporation not only in foods but also in other practicalities as well.

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COMPARATIVE ANTIOXIDANT CAPACITIES OF EDIBLE BIRD'S NEST (EBN): CUP AND CORNER HYDROLYSATES

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Abstract: Edible Bird's Nest (EBN) is recognised for its health-enhancing properties, including antioxidants. However, limited research has focused on the antioxidant capacities of different EBN parts. This study investigated the antioxidant capacities of EBN hydrolysates from the cup and corner components of EBN, with varying enzymatic hydrolysis durations (1, 2, and 3 hours). The antioxidant capacities of Alcalase-treated EBN hydrolysates were evaluated using *in-vitro* chemical assays (DPPH, FRAP, and ABTS). In terms of DPPH radical scavenging activity and FRAP assay, the corner EBN hydrolysate consistently exhibited higher values compared to the cup EBN hydrolysate at all hydrolysis times. Regarding ABTS radical scavenging activity, the corner EBN hydrolysate consistently displayed higher values compared to the cup EBN hydrolysate, although the differences were not always statistically significant (p>0.05). It is noteworthy that the ABTS assay exhibited greater sensitivity in identifying antioxidant activity in EBN hydrolysate compared to DPPH and FRAP. In conclusion, the corner EBN hydrolysate exhibited higher antioxidant capacities when treated with Alcalase compared to the cup hydrolysate. These findings highlight the potential differences in antioxidant properties between the cup and corner components of EBN and provide valuable insights for understanding their health-enhancing effects. However, incomplete hydrolysis of hygrogel properties of EBN necessitates additional research to determine the maximum antioxidant capacities for different parts of EBN.

***Keywords*:** Edible bird's nest, antioxidant, hydrolysate, Alcalase

INTRODUCTION

Edible Bird's Nest (EBN) also known as "Caviar of the East", is a dried glutinous secretion from the salivary glands of certain species of swiftlets such as *Aerodramus maximus* (Black-nest swiftlet), and *Aerodramus fuciphagus* (White-nest swiftlet) (Dai et al., 2020). These small birds are notable for their ability to build up the EBN three times a year in a deep and dark caves or cave-like environment (bird's house) (Fan et al., 2022). These habitats mainly can be found throughout Southeast Asia and the South Pacific (Looi & Omar, 2016; Thorburn, 2015). Within the Southeast Asia region, Malaysia stands as the world's second-largest producer of EBN, following Indonesia (Yeo et al., 2021). EBN is well-known worldwide due to its richness of nutrients.

Several studies have found that EBN is abundant in nutrients such as carbohydrates, glycoproteins as well as calcium, sodium, amino acids, and potassium that promote beneficial effects for the human body (Gawade, 2021). Several research reports have shown that EBN's glycoprotein and its hydrolysates exhibit antioxidant properties (Ali et al., 2019; Dai et al., 2020; Hun et al., 2015).The antioxidant capacities that exhibited by EBN hydrolysates, which can be determined by using *in-vitro* chemical techniques such as DPPH, FRAP, and ABTS assays, may be modified and influenced by the degree of hydrolysis (Gan et al., 2020; Sbroggio et al., 2016).

According to the previous studies, most of antioxidant-related research on EBN has been conducted using the entire nest. However, there has been a lack of investigation specifically targeting the antioxidant activities of distinct parts of EBN. In addition, the corner part of EBN requires a longer hydrolysis time, this distinction arises from the differing physicochemical and chemical characteristics of the cup and corner parts of the EBN (Mohamad Ibrahim et al., 2021). Hence, in this study, the antioxidant capacities of enzymatic hydrolysates derived from different parts of EBN, utilising *in-vitro* chemical assays (DPPH, FRAP, and ABTS) Notably, the hydrolysate from the corner part of

EBN exhibited greater antioxidant capacities when treated with enzyme in comparison to the hydrolysate from the cup part.

MATERIALS AND METHODS

Preparation of EBN sample

Raw-uncleaned EBN samples (*n*=6) (Figure 1) were cleaned by an EBN producing company (Nanyang Dreams International Trading Sdn. Bhd.). The raw-cleaned EBN samples were separated into two different parts (cup and corner).

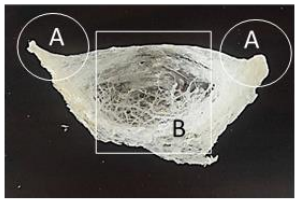

Figure 1. A) Corner part, and B) Cup part of raw-uncleaned EBN

The composite samples of both corner and cup parts were individually processed into coarse granules using a grinder (Nima®, Japan) and subsequently sieved for 5 min utilising a sieve shaker (Endercott's Minor 200-2647, England) with standard vibration mode. The samples were selected at aperture size <355 µm. The coarsely grounded EBN samples were kept in airtight containers and labelled and stored at room temperature in the dark condition for further usage.

Preparation of EBN protein hydrolysate solutions

The EBN coarse granules (corner and cup) were soaked in distilled water at a ratio of 1:20 at chilled temperature overnight respectively to soften the cement. Then, the mixture was boiled for 45 min in a 100°C water bath (LabTech LSB-015S, Daihan LabTech Co., Ltd., Korea) and cooled down to 65°C. The mixture was homogenised at 10,000rpm using Ultrasonic homogeniser (WiseTis® HG-15A, Daihan Scientific Co., Ltd., Korea). After homogenising the mixture, the sample was hydrolysed using Alcalase in the optimum pH condition suggested by the manufacturer (pH 8.6 – 8.71) at 55°C in the water bath (LabTech, Daihan LabTech Co., Ltd., Korea). The enzyme (0.5% (v/v)) was added to the homogenised mixture, and the hydrolysis was carried out for 1, 2, and 3 hours. The resulting hydrolysates were heated at the temperature of above 90°C with boiling water for 15 min to inactivate the enzymes. Then, the hydrolysates were centrifuged at 3,800rpm for 30 min using a centrifugal machine (Kubota 2420, Japan). The supernatant was separated and transferred into a 15mL centrifuge tube and then wrapped using aluminium foil prior to storage in a chiller (3°C) (Nadia et al., 2017).

DPPH, FRAP and ABTS assays

DPPH assay was conducted as described earlier (Ahmad et al., 2005; Sharma & Bhat, 2009). The samples were prepared in triplicate and the absorbance reading at wavelength, λmax=517 nm was measured spectrophotometrically (T80$^+$ UV-Vis spectrophotometer). Ascorbic acid was used as a standard and a series of standard ranging from 0 – 0.57 mM standard calibration curve was established. The difference in absorbance between test sample and control (DPPH) expressed as % radical scavenging activity.

FRAP assay was performed according to the method described before (Siti Azima et al., 2014). The readings were measured spectrophotometrically at λmax=593 nm. The linear standard calibration curve ranging from 0 – 1.0 mM Trolox was established. The final results were expressed in μM TEAC/g of hydrolysate.

ABTS assay was performed based on the previous reported method (Ali et al., 2019). The absorbance of the resultant solution was measured at 734 nm using the spectrophotometer. A standard curve was prepared by reacting 1mL of Trolox (0 – 2.0 mM) with 3.5 mL of diluted ABTS solution. The degree of ABTS radical-scavenging activity of the hydrolysates was expressed as % radical scavenging activity.

Statistical analysis

All experiments were performed in triplicate. Statistical analyses were performed using Statistical Analysis System 3.8, SAS® Studio software. The Analysis of variance (ANOVA) procedure of Duncan's Multiple Range Test at 5% level was used to compare any significant differences between mean of the antioxidant values within the hydrolysis time for each sample. T-test of Least significant difference (LSD) was calculated to compare differences

between antioxidant value for different parts of EBN sample (cup and corner). Values were expressed as mean±standard deviation.

RESULTS AND DISCUSSION

Error! Reference source not found. shows the antioxidant capacities of enzymatic hydrolysates from both the cup and corner parts of EBN at different hydrolysis durations using various antioxidant assays (DPPH, FRAP and ABTS). The data shows that at all hydrolysis times, the corner EBN hydrolysates (46.64 – 58.89%) consistently exhibit significant higher ($p < 0.05$) DPPH radical scavenging activity compared to the cup EBN hydrolysates (28.15 – 32.67%). Nonetheless, the DPPH radical scavenging activity for both hydrolysates decreased over the hydrolysis time.

Table 1: Antioxidant capacities of enzymatic hydrolysates from both the cup and corner parts of EBN at different hydrolysis durations using various antioxidant assays

Hydrolysis Time (hr)	Antioxidant capacities					
	DPPH radical scavenging activity (%)		Reducing power, TEAC (μM/g)		ABTS radical scavenging activity (%)	
	Cup	Corner	Cup	Corner	Cup	Corner
1	$32.67\pm0.00^{B*}$	$58.89\pm0.01^{A*}$	$37.29\pm4.60^{A*}$	$48.61\pm1.85^{A*}$	$98.70\pm0.00^{C*}$	$99.24\pm0.00^{A*}$
2	$38.25\pm0.00^{A*}$	$50.99\pm0.00^{B*}$	37.41 ± 2.38^{A}	42.19 ± 1.41^{B}	$99.26\pm0.00^{B*}$	$99.72\pm0.00^{B*}$
3	$28.15\pm0.00^{C*}$	$46.64\pm0.00^{C*}$	$25.61\pm1.46^{B*}$	$39.35\pm1.38^{B*}$	$99.63\pm0.00^{A*}$	$99.81\pm0.00^{B*}$

Note: Analysis of data, mean ± SD was obtained from three triplicate samples. ^{ABC} Means with different capital letter within each column were significantly different at $p < 0.05$. Asterisk (*) indicates a significant difference between cup and corner sample within each row of similar assay at $p < 0.05$.

On the other hand, FRAP assays show that the hydrolysates of EBN cup samples are between 25.61 - 37.29 μM TEAC/g while hydrolysates for corner part are between 39.35 – 48.61 μM TEAC/g. In general, the corner EBN hydrolysate showed a higher reducing power compared to cup EBN hydrolysate. However, the FRAP values for both hydrolysates have the same pattern as DPPH analysis which was decreased over the hydrolysis time. The ABTS assay indicated that both cup and corner EBN hydrolysates consistently display high ABTS radical scavenging activity, with corner EBN hydrolysates having a slightly higher activity (99.24 – 99.81%) in most cases. Based on the results, ABTS assay demonstrated the highest percentage scavenging activity compared to DPPH and FRAP assays.

Generally, the antioxidant value of EBN is known to be associated with the biopeptide, amino acid, and sialic acid content in EBN compound. The bioactive peptides act as the natural antioxidant in EBN and amino acids functions to enhance antioxidative activities (Yan et al., 2021). It is supported by another studies in which peptides plays a major role in the antioxidant action of proteins (Shahi et al., 2020). Thus, it can be assumed that corner EBN hydrolysate possesses higher in number of those compound compared to cup EBN hydrolysates, since it demonstrates the higher antioxidant capacities. On the contrary, the decrease of antioxidant values over longer hydrolysis time may be due to the proteins or peptides are broken down into inactive free amino acids, whereby these free amino acids are no longer function as an antioxidant (Samaranayaka & Li Chan, 2011). Other research also supports that further hydrolysis will lead to the formation of shorter peptides (tri- and dipeptides) and free amino acids (Sbroggio et al., 2016). Hence, the antioxidant capacity of peptides may be reduced or completely lost after conversion to free amino acids (Tironi & Añón, 2010).

CONCLUSIONS

In this study, it can be concluded that corner EBN hydrolysate possesses the significantly higher antioxidant capacities compared to cup EBN hydrolysate ($p < 0.05$). Therefore, these findings could be valuable for EBN industry in formulating functional food. However, there are few limitations during the sample preparation for both parts of EBN samples. Since EBN has hydrogel properties, the extraction of EBN hydrolysate was not fully hydrolysed even after 3 hours of hydrolysis. Thus, more work to be done to determine the maximum antioxidant capacities in EBN hydrolysates. As recommendation, EBN samples may be exposed to the longer enzymatic hydrolysis time.

ACKNOWLEDGEMENT

The authors would like to thank the Ministry of Higher Education of Malaysia (MOHE) for the financial support of this research through the Public-Private Research Network (PPRN) Grant (KPT.600-2/4/123(4)). The authors also thank Nanyang Dreams International Trading Sdn. Bhd. for providing the raw-cleaned EBN samples in this study.

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HARNESSING SARAWAK'S INDIGENOUS RESOURCES: INNOVATIONS IN PRODUCT DEVELOPMENT

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Abstract: Sarawak's tropical rainforests have a rich heritage of diverse flora and fauna, supporting some of the world's most abundant plant species. Within this biodiversity, over 100 indigenous fruits, vegetables, herbs, and spices have long been used as food sources, offering supplementary income to rural communities. These rich plant resources possess vast untapped economic potential that can be promoted for wider use, domestication, and commercialization. Among these potential indigenous resources are dabai, terung asam, and wild pepper. Through extensive research and development, MARDI Sarawak has harnessed these resources to create value-added Sarawak-based products. Our goal is to foster sustainable growth and drive economic progress in Sarawak's agri-food sector by strategically developing products and leveraging our plentiful resources for a prosperous future. This paper aims to disseminate information about the untapped potential of Sarawak's indigenous resources while showcasing MARDI's innovations and achievements in product development, including herbal drinks, condiments, and premixed powders. Developing countries are encouraged to diversify their food exports by exploring the development of indigenous crop-based products that have not been fully explored. By engaging in strategic product development, Sarawak can unlock its immense economic potential and forge a path toward a prosperous and sustainable future.
***Keywords*:**Sarawak's indigenous resources, biodiversity, innovations, product development, economic potential

INTRODUCTION

Borneo, the world's third-largest island, is renowned for its remarkable plant diversity. Situated centrally in Maritime Southeast Asia, the island is politically divided among Malaysia, Indonesia, and Brunei. Malaysia's portion of Borneo, known as 'East Malaysia' or 'Malaysian Borneo,' comprises two states, Sarawak and Sabah. Notably, Borneo has gained recognition as one of the most crucial centers of plant diversity on the planet. The botanical richness of Borneo is exemplified by an estimated 12,000 - 15,000 species of vascular plants, representing approximately 5 - 6% of the world's total. An impressive 40 - 50% of these species are unique to the island, with the majority of the endemic plants concentrated in Sarawak and Sabah. Recent research by the World Wildlife Fund (WWF) has unveiled the discovery of over 360 new species on Borneo since 1994, underscoring its ongoing significance as a botanical hotspot (Alamgir et al., 2020).

Sarawak boasts a rich legacy of diverse flora and fauna. Among these resources are more than 100 types of indigenous fruits, vegetables, herbs, and spices, which serve not only as essential food sources but also contribute to the livelihoods of rural communities. These indigenous crops have garnered attention due to their adaptability and suitability for local cultivation, setting them apart from introduced crop varieties. Several of these indigenous crops have even earned Geographical Indication (GI) status, recognized as the authentic products of Sarawak by the Intellectual Property Corporation of Malaysia (MyIPO).

Through diversifying food exports and actively promoting the domestication and commercialization of indigenous crops, the region has a significant opportunity to bolster its economic growth while simultaneously preserving its unparalleled biodiversity. Among the various botanical treasures found in this region, three principal categories of plants emerge as particularly promising: (1) edible fruits and vegetables, (2) medicinal herbs and spices, and (3) ornamental plants. This article highlights the potential of harnessing Borneo's indigenous plant resources, particularly in Sarawak, for sustainable development.

The Malaysian Agricultural Research and Development Institute (MARDI) Sarawak has played a pivotal role in advancing research on the quality enhancement and value addition of Sarawak-based plant products. Notable among these indigenous resources are dabai, terung asam, and wild pepper.

MATERIALS AND METHODS

Sample Collection &Preparation

Each indigenous fruit and vegetable sample was procured from local markets. The samples were then packed in icebox containers at a temperature of 10°C and promptly transported to the MARDI station in Kuching. Upon arrival, fruit and vegetable samples without any physical damage were selected. They were thoroughly washed under running tap water to remove impurities and then allowed to dry in the shade. The samples were subsequently stored at 4°C for further processing.

Approaches in Product Development

In order to establish a niche in the food markets, Malaysia can embark on product development grounded in indigenous knowledge encompassing aspects such as product formulation, form, and application (Aziz et al. 2005). The development of indigenous resource-based products can be structured around three innovative approaches: (i) refining the processing parameters of existing conventional or traditional products, (ii) devising new formulations by substituting local plant-based materials for the less nutritious constituents in current existing food products, and (iii) integrating with other novel processing technologies. Several illustrative examples are presented in Table 1.

Table1. Approaches and examples

Approach	**Refining the processing parameters of existing conventional or traditional products**	**Devising new formulations by substituting local plant-based materials for the less nutritious constituents in current existing food products**	**Integrating with other novel processing technologies**
Examples	Enhancement of Malaysian satay paste through the infusion of herbs with thermogenic properties to boost metabolism and promote fat burning, or the inclusion of herbs with cancer prevention attributes	Development of granola bars featuring herbal additives like stevia to substitute for sugar, along with herbal antioxidants to replace butylated hydroxytoluene (BHT)	Transformation of local fruit juices into popping boba utilizing molecular gastronomy techniques such as spherification

RESUITS AND DISCUSSION

Innovations & Product Development

Indigenous resources like dabai, terung asam and wild pepper offer significant potential for the innovation of value-added products that can benefit local economies and find success in wider markets.

Dabai

Canarium, a large fruit tree genus in the Burseraceae family, encompasses about 100 species found predominantly in Asia, Africa, and the Pacific Islands. In Malaysia, four *Canarium* species are identified, with *C. odontophyllum* being popular. Known as dabai, *C. odontophyllum* **(Figure 1)** is a seasonal fruit unique to Borneo, especially Sarawak, resembling olives but botanically distinct. Dabai's growth is upright, reaching 20 m; mature trees yield up to 300 kg of fruit per season. It has two peak seasons: May-June and December-January. Dabai, oblong (3-4 cm), with dark skin, has yellowish flesh (4-7 mm thick) around a hard seed. Quality fruit is sizeable, with nutty aroma and creamy texture.

Traditionally soaked and consumed with salt or soy sauce, dabai is rich in energy (339 kcal), protein (3.8%), fat (26.2%), minerals (potassium 810 mg, phosphorus 65 mg, calcium 200 mg, magnesium 106 mg), and vitamin E (257 ppm). Fatty acid composition resembles palm oil: palmitic acid (41.8%), linoleic acid (35.0%), linolenic acid (11.0%). Dabai shows potential for healthy oil and nutraceuticals. MARDI has conducted a study on dabai product development and storage techniques, which has resulted in the creation of innovative semi-processed products like pickled dabai and frozen dabai puree. These products aim to reduce wastage and extend dabai availability. This progress has also served as inspiration for the development of additional dabai-based products, including dabai dipping sauce, cooking paste, juice drinks, and even mayonnaise **(Figure 1)**. These developments are in alignment with Sarawak's economic projections, promising a prosperous future for the dabai industry.

Terung Asam

Terung asam *(Solanum lasiocarpum)*, commonly referred to as terung Dayak or sour eggplant, stands as one of Sarawak's prominent indigenous crops. Over time, it has gradually gained substantial commercial significance, particularly in East Malaysia (Ting & Ding, 2021).

Maintaining a healthy weight can be tedious and challenging for many of us. Given the current situation involving a rapidly growing obese population in Malaysia, MARDI has developed an innovative solution, TERUNGOLD™ beverage **(Figure 2)**, based on terung asam. This beverage efficiently helps people reduce and control their body weight. Scientific research has demonstrated the effectiveness of the product's primary constituent, hydroxycinnamic acid, in combating obesity. An analysis of the product revealed that the extract contains high concentrations of hydroxycinnamic acid group metabolites (0.42 mg/mg dried extract), including derivatives such as caffeic acid (0.15 mg/mg dried extract), 1-caffeoylquinic acid (0.09 mg/mg dried extract), along with other metabolites like gallic acid monohydrate, rutin, and quercetin acetylglucoside (Zuraida et al., 2022).

ased on a 12-week preclinical investigation involving Sprague Dawley mice, TERUNGOLD™ beverage demonstrated anti-obesity effects when compared to a control group of animals fed a high-fat diet (HFD). Similar to the effects observed with Orlistat treatment, which served as a positive control, the beverage managed to reduce the weight gain of HFD-fed mice by up to 29% **(Figure 3)**. The product's effectiveness in combating obesity, preventing weight gain, and reducing excess weight has been clearly demonstrated (Zuraida et al., 2022). The product is now ready for market distribution.

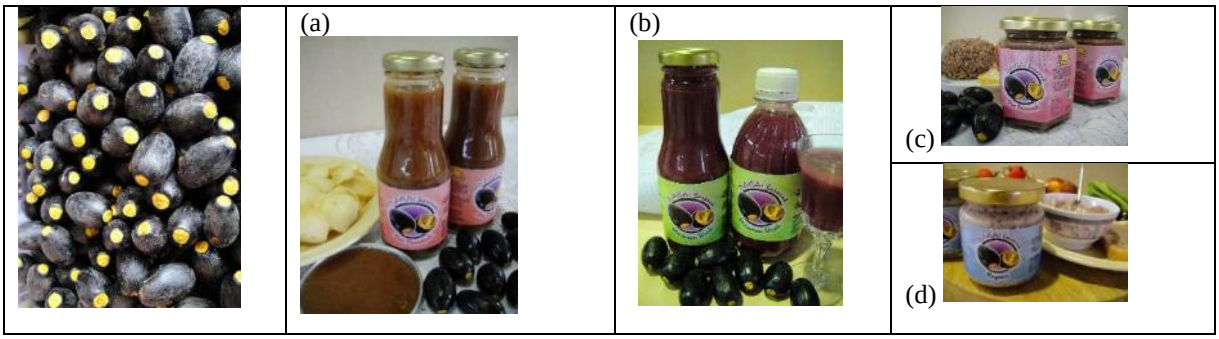


Figure 1. Dabai fruit and its value-added products: (a) dipping sauce, (b) juice drink, (c) cooking paste, and (d) mayonnaise

Figure 2. TERUNGOLD™ beverage

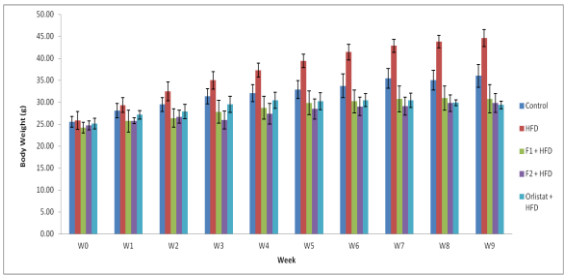


Figure 3: Weekly body weight measurement of control mouse, untreated HFD mouse, HFD mouse treated with F1, HFD mouse treated with F2 and HFD mouse treated with Orlistat for 9 weeks pre-clinical study

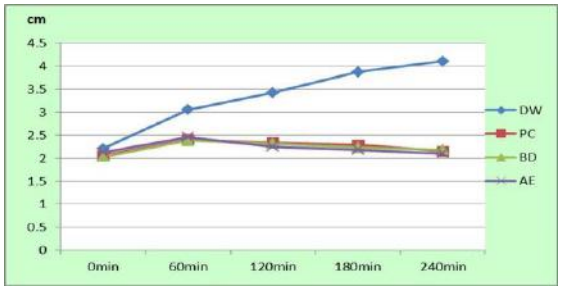
Wild Pepper

Biodiversity prospecting of Sarawak's wild Piper species is of great importance, as many have long been known to possess numerous health-enhancing properties. Wild pepper *(Piper arborescens)* is one of the most commonly distributed species. The decoction is reputed to help treat rheumatism, gout, and other inflammatory disorders among local communities. Wild pepper root extract showed no noticeable gross toxicity in all treated Sprague-Dawley mice during sub-acute toxicity evaluation. The no-observed-adverse-effect-level (NOAEL) of wild pepper root extract exceeds 5 g/kg of body weight per day.

The PIPERIA Botanical Cubes **(Figure 4)**, derived from wild pepper roots, exhibits natural anti-inflammatory activity. An anti-inflammatory study was conducted based on carrageenan-induced edema in the hind paws of Sprague-Dawley rats. The results demonstrated robust anti-inflammatory activity in the drink, comparable to that of paracetamol (normalized values of 1.08 vs. 1.05) (**Figure 5)**. PIPERIA can serve as an instant health beverage

and sweetener for a wide range of food products. Its versatility and convenience make it an ideal botanical cube for multiple applications. Furthermore, PIPERIA have the potential to be introduced as a natural alternative, reducing over-dependence on synthetic anti-inflammatory drugs.

Figure 4. Wild pepper roots (top) and PIPERIA Botanical Cubes (bottom)



AE: Carrageenan injection + aqueous extract (5 g/kg body weight)
BD: Carrageenan injection + PIPERIA drink (5 g/kg body weight)
PC: Carrageenan injection + paracetamol (20 mg/kg body weight) (Positive control)
DW: Carrageenan injection + distilled water (Negative control)
Figure 5. Figure 1. Paw carrageenan-induced edema thickness (cm) vs. time (min) (n=6)

Innovations and Impact

When considering innovations and the development of novel food products using indigenous resources, several key points come into play: ***Technology:*** This encompasses food processing and food design, with a focus on preserving and enhancing beneficial effects. This endeavor involves elements with intellectual property value or the potential to be patented, such as extraction methods, ingredients, and processing conditions. ***Novelty:*** The innovation lies in utilizing traditional knowledge to address common health issues and transforming these products into functional items. The development process can also be not only cost-effective but also simple to adopt, requiring minimal machinery. ***Utility:*** This development paradigm must offer a multitude of benefits. It enhances the utilization of indigenous plants, generates high-value products for various markets, and enriches food composition databases. From an economic perspective, it safeguards and perpetuates traditional knowledge while harnessing Sarawak's rich biodiversity. Furthermore, it fosters growth in rural areas, creating business and employment opportunities. ***Economic & Social Impact:*** This strategy brings significant economic and social benefits. It preserves and enriches traditional knowledge, strengthening cultural ties within the community. By leveraging Sarawak's unique biodiversity, it drives functional food production and boosts the local economy. Large-scale farming involves rural communities, empowering them and fostering economic growth.

CONCLUSIONS

Sarawak's status as a botanical treasure trove is emphasized by its vast plant diversity and high endemism. Its indigenous plant resources, in particular, harbor significant potential for sustainable development through the cultivation, commercialization, and export of value-added products. By harnessing these resources, Sarawak can contribute to its economic growth and the conservation of its unique biodiversity, thus ensuring a harmonious balance between human progress and the preservation of nature.

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ENHANCED STABILITY OF BUTTERFLY PEA FLOWER ANTHOCYANINS THROUGH COLLOIDAL GAS APHRONS SEPARATION METHOD USING TWEEN20 SURFACTANT

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Abstract: Butterfly pea flower (BPF) is an ornamental climber known to contain secondary metabolites including anthocyanins. However, anthocyanins are unstable and easily degraded during food processing, hence making the applications limited. This study aimed to determine the stability of anthocyanins in ethanolic extract and colloidal gas aphrons separation (CGA) which is a surfactant-based separation method. The result showed the anthocyanins recovered by CGA at volumetric ratio 20 (AV20) showed higher stability (half-life = 295d) than in ethanolic extract (EE) (half-life = 43d) and their stability increased with the concentration TWEEN20 in the CGA samples (6.07–8.58 mM). The anthocyanins loss in the CGA processed sample (AV20) (18.75%) lower than in the ethanolic extract (74.57%). The loss of anthocyanin during storage fitted to a first-order reaction model and the rate constant ranged from $0.0024d^{-1}$ – $0.0161d^{-1}$. AV20 exhibited lower values of rate constant ($0.0024d^{-1}$) compared to EE ($0.0161d^{-1}$). As a result, we draw the conclusion that CGA processed samples helped anthocyanins stabilise during storage, and volumetric ratio 20 was more stable than the ratios examined. In a summary, the inclusion of TWEEN20 aids in extending the half-life of the anthocyanins during storage, indicating that CGA might be considered as a different strategy for reducing anthocyanin degradation.
***Keywords*:** *Clitorea ternatea*, anthocyanins, surfactant, Colloidal Gas Aphrons, stability

INTRODUCTION

Butterfly pea flower (BPF) or scientifically known as *Clitorea ternatea* is one of the beautiful ornamental climbers planted in garden and found growing in the wild and is widely found in Asia(Lim, 2012). The plant is also used medicinally and plays a role in maintaining the well-being and health of population. It is also known by fact that lots of local cuisines in Thailand and Malaysia use BPF for the preparation of blue rice and pressed cakes, giving it its characteristic of vivid blue colour (Ezzudin, 2018). Numerous authors have discussed and documented the chemical constituents of BPF, particularly the polyphenols and anthocyanins. It is also well known that the anthocyanins from BPF extract have the potential to be the major source of natural pigments. However, many studies have highlighted the drawbacks of using natural anthocyanins such as degradation when exposed to heat, oxidation, and pH shift. Thus, various emerging techniques were introduced, such as the use of surfactants. The application of surfactant in a separation process has recently sparked new interest as an alternative to the organic solvents. The efficiency of extraction using surfactant as compared to water and 70% ethanol was 1.5 and 1.2 times higher, respectively (Skrypnik & Novikova, 2020).

Colloidal Gas Aphrons (CGA) is an approach of using surfactant as a medium of extraction. Defined as microbubbles generated from surfactant solutions at high-speed stirring (>8000 rpm), CGA has its own distinctive properties as compared to conventional foams, including high stability, low viscosity, and smaller bubbles size. Other than that, CGA can be easily separated from the bulk liquid; hence, no further process is needed for the recovery of compounds. Previously in our group, CGA has been used to recover polyphenols particularly anthocyanins, astaxanthin as well as proteins from various by-products (Fuda et al., 2005; MohdMaidin et al., 2018; Spigno & Jauregi, 2005). On top of that, CGA separation could also lead to stabilisation of anthocyanins during storage

(MohdMaidin et al., 2019). To the best of our knowledge, there is no study on the effect of Tween-20 on the stability of anthocyanins extracted from BPF extract. Therefore, this study is aimed at assessing the stability of anthocyanins in the CGA processed samples over time in comparison with their stability in the crude ethanolic extract (EE) (before the CGA separation). It is postulated that the CGA processed samples will have a higher stability as compared to the EE.

MATERIALS AND METHODS

Sample preparation and extraction

Dried butterfly pea flower (*C. ternatea*) (BPF) were grounded by using a grinder-mixer (Panasonic, MX-337) and sifted using a vibrating sieve shaker (Endecotts, Octagon D200 Digital) to particle size of ≤ 2mm. The extraction of polyphenols from the sifted samples was carried out by using the method proposed by MohdMaidin *et al.* (2018). Phytochemical constituents were extracted by ethanol aqueous extraction (60% v/v) in a shaking water bath (Memmert, SV1422) at 60℃ for 135 minutes. The ratio of sample to solvent was 1:8 (w/v). The extract was vacuum-filtered and collected, whereby one portion was stored at room temperature and monitored as control sample (EE) while another was further separated using CGA.

Separation of BFP ethanolic extract using CGA

The solution of 10mM Tween-20 was prepared by dissolving the concentrated Tween-20 in distilled water. CGA was generated by subjecting the surfactant solution to high-speed stirring homogenizer (IKA, T25 Digital Ultra Turrax) (≥ 8000 rpm) for 5 minutes. After the generation, the CGA was pumped into a flotation column (i.d: diameter: 4cm, height: 55cm, Volume: approximately 1000ml), readily containing BFP extract. Briefly, the BFP extract was introduced inside the column with known volume by varying the volumetric ratio with constant drainage time. The contact time between the extract and CGA was recorded. Finally, the drained aphron phase (AP) and liquid phase (LP) were collected separately for further analysis. The concentration of Tween-20 in these fractions was estimated from the knowledge of the separated volume of CGA and corresponding liquid fraction which was determined from a measurement of gas hold-up (gas volumetric ratio defined as the volume of air incorporated in each volume of CGA dispersion) of the CGA generated with this solution of Tween-20 (61.3%). The estimated concentrations were: in V4, 6.07 mM, in V8, 7.56 mM and in V16, 8.58 mM.

Storage of EE and CGA processed samples

The EE and CGA processed samples were kept in HDPE plastic bottles according to their days. The samples, ethanolic extract (EE), aphron phase with volumetric ratio 8 (AV8) liquid phase with volumetric ratio 8 (LV8), aphron phase with volumetric ratio 16 (AV16) liquid phase with volumetric ratio 16 (LV16), aphron phase with volumetric ratio 20 (AV20) and liquid phase with volumetric ratio 20 (LV20) were stored at 25℃ ± 3℃ for 85 days. Analyses were determined every day for the first four days and in the interval of 3 days afterwards up to 85 days.

Total phenolic content

Folin-ciocalteau (FC) colourimetry method was employed to determine the total phenolic content of the extract samples (Prado *et al*., 2018).

Total flavonoid content

Total flavonoid content was determined by using aluminium chloride colourimetric method according to Looi *et al*. (2020) using Quercetin as the standard and results were expressed in milligrams of quercetin equivalent per litre (mg QE/g).

Total monomeric anthocyanins

pH dilution method was employed to determine the total monomeric anthocyanins(Lee *et al.*, 2005). The absorbance values were taken at 520 nm and 700 nm within 20-50 minutes of preparation.

ABTS assay

The ABTS assay was conducted based on Tuan Putra *et al.*, (2021) with slight modification. The radical solution was prepared by mixing 5 mL of 7 mM ABTS with 80 µL of 150 mM potassium peroxodisulfate. The solution was left to stand for 12-16 hrs. For the analysis, 3 mL of ABTS+ (0.700±0.02, A734 nm) was mixed with 30 µL of sample/blank/standard. Results were expressed as % of inhibition.

RESULTS AND DISCUSSION

Table 1 shows the percentage of loss of phenolics, flavonoids, anthocyanins and antioxidant activity of CGA-processed samples along with EE sample. Among the CGA processed samples, the TPC showed the lowest losses in AV8 (11.01%), followed by EE (14.54%), AV16 (17.19%), LV8 (21.40%), LV16 (22.48%), AV20 (27.48%) and finally, LV20 (30.16%). Different trend was noted by MohdMaidin *et al*., (2019), who found that the lowest loss of TPC content from grape pomace was in V16 (4.91%) followed by V8 (5.44%), and finally V4 (6.42%). This rather contradictory result may be due to the variant phenolic compounds present in samples. Going further, the TPC values for CGA processed samples (AV8 and AV16) were comparable with EE as there was no statistical difference ($p<0.05$) between these three samples. Similar results was also reported in the recent work by MohdMaidin *et al*., (2019) which found that the extractable TPC from grape pomace by CGA (V4, V8, and V16) showed no statistical difference. The degradation of extractable phenolics compound was due to the fact that most polyphenols are highly reactive and thus unstable, and degrading into a variety of products (Kafkas *et al*., 2018).

Meanwhile, the flavonoid content of all samples reduced at the end of the 85-Day storage interval ranging from 32.88% to 70.95%. It was observed that the highest loss was in AV20 and LV20, followed by LV16, AV16, LV8, AV8 and finally EE. This showed that the presence of surfactant did not provide stability to the flavonoid compounds. The possible reasons could lie in the inability of quercetin to solubilize in Tween-20 micelles results and adsorbed onto the CGA resulting in a least stable of compound during storage. From these results too, indicates that flavonoids are less stable than phenolic compounds. The stability behavior of both compounds is in agreement with the study by Rocha-parra *et al*., (2017) whereby they reported the stability of catechin in wine are less stable than selected phenolic compounds.

Interestingly, the highest degradation of TAC was observed in the EE with 74.57%, significantly different from all the CGA processed samples. Whilst, among all CGA processed samples, the highest losses of TAC content were in LV8 (52.73%), followed by AV8 (45.63%), LV16 (45.36%), AV16 (43.70%), LV20 (26.31%) and the least loss was in AV20 (18.75%). There is little anthocyanin loss as Tween-20 concentration increases. The fact that anthocyanin acylation and glycosylation may increase anthocyanin stability may help to explain this outcome.

Table 1. Percentage of losses of phenolic, flavonoid, anthocyanin and antioxidant content of EE and CGA-processed samples during storage.

Samples	% loss of TPC	% loss of TFC	% loss of Cyanidin	% loss of Trolox
EE	14.54^{cd}	32.88^{f}	74.57^{a}	45.42^{a}
AV8	11.01^{d}	41.99^{e}	45.63^{b}	49.91^{a}
LV8	21.40^{b}	52.01^{d}	55.45^{b}	54.11^{a}
AV16	17.19^{c}	58.48^{c}	43.70^{b}	59.73^{a}
LV16	22.48^{b}	67.08^{b}	45.36^{b}	65.74^{a}
AV20	27.48^{a}	69.61^{a}	18.75^{c}	65.72^{a}
LV20	30.16^{a}	70.95^{a}	26.31^{c}	66.35^{a}

Table 2. Degradation kinetic parameters of anthocyanins during storage at room temperature.

Samples	R	K (d^{-1})	$T_{1/2}$ (d)	Losses (%)
EE	0.8668	0.0161 ± 0.00^{a}	43 ± 2.01^{c}	74.57
AV8	0.9435	0.0072 ± 0.00^{b}	97 ± 0.00^{bc}	45.63
LV8	0.9105	0.0088 ± 0.00^{b}	80 ± 14.28^{bc}	52.73
AV16	0.8922	0.0068 ± 0.00^{b}	103 ± 1.17^{bc}	43.70
LV16	0.9497	0.0071 ± 0.00^{b}	98 ± 10.89^{bc}	45.36
AV20	0.8493	0.0024 ± 0.00^{c}	295 ± 114.09^{a}	18.75
LV20	0.6853	0.0037 ± 0.00^{c}	189 ± 41.84^{ab}	26.31

Ethanolic extract (EE), aphron phase with volumetric ratio 8 (AV8) liquid phase with volumetric ratio 8 (LV8), aphron phase with volumetric ratio 16 (AV16) liquid phase with volumetric ratio 16 (LV16), aphron phase with volumetric ratio 20 (AV20) and liquid phase with volumetric ratio 20 (LV20). Results were expressed as mean ± standard deviation. Different letters show statistically significant differences between values ($p < 0.05$).

The degradation rate of EE and CGA processed samples (AV8, LV8, AV16, LV16, AV20, and LV20) was 0.0161, 0.0072, 0.0088, 0.0068, 0.0071, 0.0024, 0.0037, respectively (Table 2). Meanwhile, the respective half-life was 43, 97, 79, 103, 97, 295, and 189 days. The first order rate constant of EE was notably higher ($k = 0.0161\ d^{-1}$) as compared to all CGA processed samples. A study conducted by Wu *et al.*, (2018) deduced that the higher the k value, the faster the reaction rate and anthocyanin degradation. This implied that presence of surfactant increased anthocyanin storage stability. Among CGA processed samples, AV20 was found to have the lowest ($k = 0.0024\ d^{-1}$) first order rate constant. Significant increase in the degradation rate (k) was observed when the concentration of Tween-20 increased. The half-life ($t_{1/2}$) of anthocyanins significantly decreased when the concentration of Tween-20 increased. Therefore, it can be said that the sample with the highest stability were AV20, followed by LV20 and AV16.

CONCLUSIONS

The findings of this study gave proof that anthocyanin stability during an 85-day storage period was improved by the addition of CGA. Clearly, compared to EE, V20 (AV20 and LV20) had a decreased overall anthocyanin loss of 18.70% and 26.30%, respectively. This proved that the anthocyanins extracted by CGA were more stable and that their concentration increased with TWEEN20. The TPC and TFC losses displayed in AV20 (27.48% and 69.61%, respectively) and LV20 (30.16% and 70.95%, respectively) showed contrasting trends. The anthocyanin's stabilisation mechanisms in the CGA treated samples might still unclear, further investigation on the solubilization effect between TWEEN20 micelles and the anthocyanins might be worth to be explored.

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BIOACCESSIBILITY OF POLYPHENOLS RELEASED AND ASSOCIATED TO DIETARY FIBER IN CASHEW APPLE POMACE POWDER AS AFFECTED BY COOKIES PROCESSING

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Abstract: Cashew apples are considered waste products from cashew nut processing, while providing a relatively high polyphenols and dietary fiber. Cashew apples are not desirable to be consumed raw due to their high astringency taste. This study aims to assess the bioaccessibility of polyphenols in the cashew apple cookies (CAC) as affected by cookies processing. Cashew apple pomace powder (CAPP) was dried at 50°C and incorporated into cookies at 15% relative to the amount of the control cookies (CC). The free and bound polyphenols, as well as their bioaccessibility were evaluated. CAPP exhibited a TSP amount of 49.23 mg GAE/g dw. After incorporation into cookies, this amount reduced to 6.62 mg GAE/g, while displaying a higher value than CC. Released polyphenols demonstrated a higher value in CAC compared to CC, thereby resulting in greater polyphenols' bioaccessibility. A higher level of substitution could be considered to enhance bioaccessibility, but only if the astringency taste is reduced. Quantification of the polyphenolic compounds before and after the digestion is also recommended for further understanding.
***Keywords*:** Free polyphenols, bound polyphenols, antioxidant, food sustainability, dietary fiber.

INTRODUCTION

The cashew tree, scientifically known as *Anacardium occidentale L*. Cashew nut is the third most produced edible nut in the world. The high production of cashew nuts has led to the high production of its underutilized pseudo-fruit, which accounts for around 90% of the total weight of a cashew apple fruit. Cashew apple pomace powder (CAPP) (3.5-9.3% water) is a rich source of phenolic compounds, ranging from 49.8 to 1037.6 mg GAE/ 100 g and tannins at approximately 299 mg/ 100 g, with a significant amount of dietary fiber, accounting for around 35-79% of its carbohydrate content (44.5-77.5%) (van Walraven & Stark, 2023). This fruit is rarely eaten raw and is often discarded as waste. CAPP are added or partially substituted in various food products, with a focus on incorporating dietary fiber while reducing fat and wheat flour level. These products include burgers, chicken patties, cakes, biscuits and cookies (van Walraven & Stark, 2023). Cookies exhibited improved acceptability with a 15% substitution of cashew apple pomace powder with a higher levels of substitution led to decreased acceptability (Uchoa et al., 2009).

Bioaccessibility is defined as the amount of food constituent release from food matrix and available for absorption in the gut after digestion (Palafox-Carlos, Ayala-Zavala, & González-Aguilar, 2011). Many food processing techniques lead to phenolic compounds degradation, which often leads to low bioaccessibility. However certain techniques can also enhanced their bioaccessibility (Nayak, Liu, & Tang, 2015). Mercado-Mercado et al. (2015) reported higher bioaccessibility in decocted roselle compared to fresh roselle calyces.

Incorporating CAPP into cookies and subjected to baking temperature causes modifications in the food matrix, influencing the bioaccessibility of polyphenols in cashew apple pomace powder. In the grastrointestinal tract, phytochemicals can chemically interact with food matrix components (water, protein, lipids and fibers) and affect the release and absorption of phytochemicals during digestion (Thomas et al., 2018). Therefore, evaluating the bioaccessibility of polyphenols in cashew apple pomace powder when incorporated into cookies is of utmost importance. The total soluble polyphenols and antioxidant activities of the cashew apple cookies are also evaluated to determine the polyphenol content before being subjected to digestion for quantification of polyphenols released and associated to soluble dietary fiber. Designing a food matrix that enhances the bioaccessibility of polyphenols is crucial to ensure that polyphenol intake is not simply excreted in feces, but rather effectively absorbed in the small intestine tract.

MATERIALS AND METHODS

Materials

Cashew apples was bought from a local wet market while ingredients for cookies were from local stores.

Cashew apple pomace powder (CAPP) preparation

Cashew apples were washed, cut into smaller pieces and then subjected to a hydraulic press for juice removal. The remaining pomace was oven dried at 50°C for 48 h. The dried cashew apples were then ground into powder and sieved to get a uniform size of 250 μm. The powder was stored at -20°C for further analysis. The moisture content of the dried CAPP was 7.68 ± 0.35%.

Cookies preparation

The cookies formulation was a modification from the recipe of Abreu et al. (2019). Margarine (43%), castor sugar (10%) and brown sugar (15%) were beaten for 90 s before adding wheat flour (27%) and cocoa powder (5%). Cashew apple cookies was prepared by adding 15% CAPP into the control cookies formulation. Every 5 g of the dough was transferred into a small paper cup (3.5 cm × 5 cm × 3 cm) and baked at 150°C for 15 min.

Free polyphenols extraction

The polyphenols extraction was followed the modification method of Mercado-Mercado et al. (2015). About 500 mg sample was weighed in a capped centrifuge tube (50 ml) and 20 ml of an acidified methanol solution (composed of 50% 0.8M HCl and 50% methanol, v/v) was gradually added to the sample. After 1 h shaking at room temperature, centrifugation was performed at 4000 g for 10 min at 4°C. The supernatant was collected and 20 ml of acetone/water (70:30, v/v) was added. It was then shaken and centrifuged in a similar manner as in the first step of acidified methanol extraction. The extracts were combined and stored at -31°C until further used.

Bound polyphenols extraction

The determination of hydrolysable tannin (HT) was conducted using the published method by Hartzfeld et al. (2002) while condensed tannin (CT) was following the method described by Mercado-Mercado et al. (2015).

Total soluble polyphenols (TSP) determination

Following the modification of Folin–Ciocalteu method by Alvarez-Parrilla et al. (2011). The results were expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g dw).

Total flavonoid content (TFC) determination

The total flavonoid content was determined using the aluminium chloride colorimetric method, as described by Alvarez-Parrilla et al. (2011). The total flavonoid content was calculated from the calibration curve, and the results were expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE/g dw).

Polyphenols released by enzymatic hydrolysis (PREH) and polyphenols associated to soluble dietary fiber (PASF) determination

The assessment was conducted following the total dietary fiber method based as described by Mercado-Mercado et al. (2015). Triple enzymatic hydrolysis was carried out using α-amylase, protease and amyloglucosidase. After the *in vitro* digestion, an aliquot was taken to determine the polyphenols released from the food matrix by enzymatic hydrolysis. The samples were centrifuged, and the supernatant was subjected into dialysis tubes of the cellulose membrane (D9652-3, 0.48 m, 12,000– 14,000 Da, Sigma Aldrich) for 24 h. After dialysis, an aliquot was taken in this fraction to determine the polyphenols associated with soluble dietary fiber.

RESULTS AND DISCUSSION

Table 1 shows the quantity of both free and bound polyphenols in cookies, as affected by cashew apple pomace powder (CAPP) addition. The total soluble polyphenols (TSP) observed were higher than the commonly reported value (49.8-1037.6 mg GAE/ 100 g) (van Walraven & Stark, 2023), yet still lower than the value reported by Nguyen et al. (2023) (71 mg GAE/ g dw). The addition of 15% CAPP as an ingredient in the cookies resulted a significant increase in polyphenols of cookies compared to the control cookies (CC). However, the lower value compared to that of CAPP could be attributed to heat degradation during cookies baking process.

Table 1. Free and bound polyphenols of cookies as affected by cashew apple pomace powder incorporation

	Polyphenols	Control cookies (CC)	Cashew apple cookies (CAC)	Cashew apple pomace powder (CAPP)
Free	**Total soluble polyphenols (TSP), mg GAE/ g dw**	4.04 ± 0.04^{c}	6.62 ± 0.02^{b}	49.23 ± 0.43^{a}
	Total flavonoid content (TFC), mg QE/ g dw	9.07 ± 0.46^{c}	16.18 ± 1.32^{b}	83.56 ± 1.08^{a}
	Hydrolysable tannin (HT), mg GAE/ g dw	1.59 ± 0.12^{c}	3.21 ± 0.36^{b}	6.76 ± 0.41^{a}
Bound	**Total flavonoid content (TFC), mg QE/ g dw**	73.10 ± 0.76^{c}	77.87 ± 1.18^{b}	132.81 ± 0.92^{a}
	Condensed tannin (CT), mg CatE/ g dw	0.82 ± 0.09^{c}	2.26 ± 0.15^{b}	4.20 ± 0.24^{a}

The results in Figure 1 showed a lower amount of polyphenols released by enzymatic hydrolysis (PREH) compared to the amount obtained using solvent extraction, as shown in Table 1. This finding contrasted the results reported by Mercado-Mercado et al. (2015) and Blancas-Benitez et al. (2015), where the polyphenols released by enzymatic hydrolysis was significantly higher than that extracted by solvents. Polyphenols determination is influenced by the presence of reducing sugars, aromatic amines, sulphur dioxide, ascorbic acid, organic acids, and other compounds, often leading to overstated results (Cendrowski, Królak, & Kalisz, 2021). The digestion process for bioaccessibility assessment was conducted at high temperatures (98-100°C), which could potentially degrade the ascorbic acid, thereby contributing to the lower values reported for polyphenols after the enzymatic hydrolysis. However, the bioaccessibility of CAC was found to be higher than CC when CAPP was incorporated. The lower bioaccessibility of CAC compared to CAPP can be attributed to the complex food matrix structure in cookies, which includes starch, protein, sugar and lipids, as discussed in various studies (Shahidi & Pan, 2022).

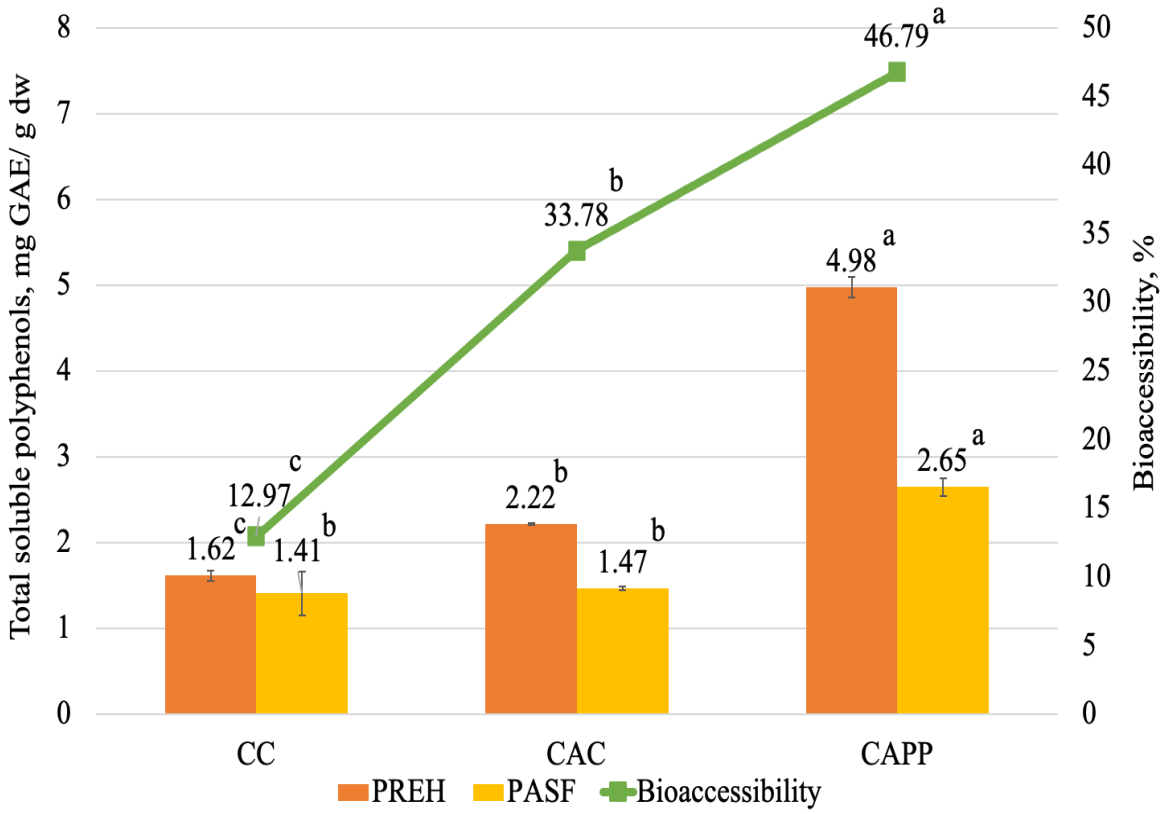

Figure 1. Bioaccessibility of polyphenols released after enzymatic hydrolysis. CC, control cookies; CAC, cashew apple cookies; CAPP, cashew apple pomace powder. Bioaccessibility (%) = $\frac{(PREH - PASF)}{PREH} \times 100$, PREH, PP released by enzymatic hydrolysis; PASF, PP associated to soluble DF.

CONCLUSIONS

Cookies processing affected the polyphenols in cashew apple pomace powder (CAPP). Heat degradation was the main factor for the lower value of polyphenols in cookies compared to CAPP. The processing of cookies also influenced the bioaccessibility of polyphenols in CAPP due to modifications in the matrix structure during cookies processing, which involved starch, protein, sugar and lipids. The baking temperature further contributed to this effect. To confirm the occurrence of polyphenol degradation as observed in this study, it is recommended to employ a different method for bioaccessibility assessment. This method should simulate real digestion conditions, utilizing gastric fluid and simulate intestinal fluid, which conducted at 37°C. High performance liquid chromatography (HPLC) technique is recommended for the quantification of polyphenolic compounds in the solvent extracts, in the extract released by enzymatic hydrolysis and associated to soluble dietary fiber. Therefore, the bioaccessibility of each compound before and after the *in vitro* digestion could be determined. A higher amount of CAPP incorporation is also suggested to enhance polyphenols bioaccessibility, or alternative delivery systems such as encapsulation techniques could also be introduced.

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MODIFICATIONS IN THE PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES OF OAT AFTER COMMERCIAL THERMAL PROCESSING

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Abstract: The main objective of this study was to compare the physicochemical and functional properties of raw (RO) and thermally-processed oat (TO). RO and TO (kilned at 115 °C for 30 min and steam-cooked at 100–104 °C for 18 min.) were obtained from a commercial processing line, rolled, and milled into flour. The chemical composition (β-glucan, crude protein, crude fat, and total starch contents), enzymatic activity (lipase and peroxidase), morphological properties, particle size distribution, thermal properties, and pasting profile were evaluated to better understand the structural and functional differences. Data have shown that TO exhibited higher β-glucan, crude protein, and crude fat contents, lower total starch content, larger component particle sizes, more aggregated starch granule clusters, higher thermal transition temperatures, lower thermal enthalpies related to starch gelatinization and melting of amylose-lipid complex, and produced a more viscous paste that displayed higher resistance to disintegration. Thermal treatment of oat was also found to be effective in lipase and peroxidase inactivation. With data gathered from this study, the thermal treatment of oat has led to the modifications of its inherent properties, which can greatly impact how it will behave in subsequent processing for a variety of food applications.
Keywords: Oat, commercial thermal processing, pasting properties, thermal properties

INTRODUCTION

Oats have high lipase activity (Zhou, Robards et al. 1999), as well as remarkable levels of lipids (3–11%) compared with other cereals. Therefore, oats are rather susceptible to hydrolytic and oxidative rancidity, which causes the production of volatile carbonyl compounds responsible for the undesirable odor and bitter taste of rancid oats (Heiniö, Lehtinen et al. 2002, Salmenkallio-Marttila, Heinio et al. 2011). To address this hurdle, different milling companies have specific processing parameters applied (e.g. time-temperature combinations, etc.) to inactivate the key enzymes that catalyze deteriorative reactions. Several thermal treatment strategies were investigated to achieve enzyme inactivation in oat in laboratory scale, including drying, hot-air roasting, infrared treatment, heat-moisture treatment, normal pressure steaming, and autoclave steaming.

Thermal treatment of oat in food industries is primarily applied to produce shelf-stable oat groat (dehulled oat), which is further processed to produce various forms of oat products like rolled oats, scotch oats, steel cut groats, oatmeal, and oat flour. Decker, Rose et al. (2014) outlined the general flow of the milling process, which included four major steps: cleaning, grading, dehulling, and kiln drying. Thermal treatment is very effective in achieving enzyme inactivation, but can affect the properties of other oat components, including starch. Starch is the predominant constituent of oat and the key driver influencing oat functionality, including thermal and pasting properties. Depending on the thermal treatment applied, the extent of modification in the physicochemical and molecular structure of starch varied when investigated using lab-scale set-up (Ziegler, Ferreira et al. 2018, Feng, Wang et al. 2019, Nguyen, Mitra et al. 2019). However, there is a lacking information on how thermal processing applied in the food industry setting has modified the physicochemical and functional properties of oats.

The main objective of this study was to compare the physicochemical and functional properties between untreated and thermally processed oat. Oat sampled from a commercial processing line with and without thermal processing step were used to better understand their structural and functional differences.

MATERIALS AND METHODS

Oat samples

Two different oat (*Avena sativa* cv. Armstrong) samples were obtained from a commercial oat processing line (Harraway and Sons, Ltd., Green Island, Dunedin, New Zealand). The first sample was obtained after milling intact raw oat groats to flour and later referred to as "raw" oat (RO). The second sample was obtained after the oat groats were kilned (115 °C for 30 min), steam-cooked (100–104 °C for 18 min), rolled, and milled to flour and later referred to as "thermally processed" oat (TO).

Determination of chemical composition

Moisture content was determined according to AACC method 44–15A. β-glucan content was determined using the mixed-linkage beta-glucan kit (K-BGLU, Megazyme, Wicklow, Ireland) according to McCleary and Codd (1991). Crude protein content was determined according to AOAC 2001.11 with minor modifications. Crude fat content was determined using the Soxhlet method. Total starch content was determined using total starch assay kit from Megazyme (K-TSTA-100A, Wicklow, Ireland).

Determination of enzyme activities

Qualitative evaluation of lipase and peroxidase enzyme activities were carried out in the Quality Control Laboratory of Harraway and Sons, Ltd. using commercial Oat-Chek 1 rapid test kit (LSB Industries through Alteca Laboratories, Kansas, USA).

Study of oat morphological properties

Scanning electron microscopy (SEM) was conducted using a benchtop scanning electron microscope (Jeol JCM-5000 Neoscope, Tokyo, Japan). SEM micrographs were obtained at 1000× and 2000× magnifications. Particle size distribution analysis was performed according to Duque et al. (2020) using a laser diffraction particle size analyser Mastersizer 2000S (Malvern Instruments, Malvern, United Kingdom).

Determination of the thermal and pasting properties

Thermal properties of oat samples were analyzed using a hot-stage microscope and TA Instruments Q2000 differential scanning calorimeter (New Castle, USA). The pasting properties were monitored according to Duque et al. (2020) using a Rapid Visco Analyser Series 4 (RVA-4, Newport Scientific, New South Wales, Australia).

Statistical analysis

Results are presented as mean ± standard error for at least 3 independent measurements. Data sets were analysed by 2 sample t-test for single comparison to determine if means were significantly different at 95% level of confidence. All statistical analyses were performed using Minitab® 17.2.1 statistical software (Minitab, LLC, Pennsylvania, USA).

RESULTS AND DISCUSSION

Differences in the chemical composition

The main components of RO and TO are presented in Table 1. RO was found to be made up of approximately 1% β-glucan, 11% crude protein, 5% crude fat, and 77% total starch in dry basis. On the other hand, TO was made up of roughly 4% β-glucan, 12% crude protein, 9% crude fat, and 63% total starch. The crude protein and crude fat contents of RO and TO, as well as the β-glucan content of TO, are within the range based on previously reported studies. Total starch levels for both RO and TO were also similar to those previous reports on oat. The impact of thermal treatment on the percent composition of oat was evident. Compared with RO, TO had a lower level of total starch and higher levels of β-glucan, crude protein, and crude fat. The higher β-glucan content of TO compared to RO may be attributed to thermal processing effect wherein endogenous enzymes, including β-glucanase, an enzyme that hydrolyses β-glucan (Terra and Ferreira 2012), were inactivated.

Comparison of lipase and peroxidase activities

Table 1 shows that lipase and peroxidase activities were detected in RO. On the other hand, activities of both enzymes were not detected in TO. The result was expected and hence suggesting that the thermal processing steps applied in commercial oat line were effective in inactivating lipase and peroxidase enzymes, which makes TO more shelf stable than RO.

Morphological properties

SEM micrographs of RO and TO (data not shown) revealed irregularly-shaped polygonal starch granules that tend to exist in clusters of varying sizes. With thermal treatment of oat, no drastic change in starch granule morphology was observed. However, compared to RO, TO starch granules appeared less "edgy" or smoother and more enlarged individually. Moreover, prominent larger starch granule clumps were observed in TO than in RO. It is likely that thermal processing steps applied to oats caused such morphological change since hydrothermal processing typically promotes granule swelling, shape modification, and amylose leaching.

Differences in particle size distribution

Table 1 shows the particle size distribution of RO and TO. TO exhibited significantly larger particle sizes than RO, as indicated by higher d (0.1) and d (0.9) values. However, no significant difference in the volume mean diameter, indicated by d (0.5) value, was observed between RO and TO. Bigger particle size observed in TO can be attributed to agglomerates or clusters of small starch granules, which confirms previous observation in SEM analysis.

Thermal properties

Investigation of sample properties using hot-stage microscope allowed visual observation of the changes in the morphological characteristics of the sample with increasing temperature. On the other hand, examination of sample properties with DSC permitted an in-depth understanding of the starch gelatinization properties, as well as the characteristics of the amylose-lipid complex. Hot-stage microscopy data showed that as the temperature increases, starch granules of RO and TO exhibited swelling and deformation to some degree, which eventually led to total starch granule damage at high temperature of 90 °C. It is evident that at room temperature (30 °C), TO has bigger observable granules (or aggregates) than RO. Also, starch granules of TO and RO were found to exhibit visible swelling at around 50 °C and 55 °C, respectively. This indicates that the thermal treatment of TO altered the susceptibility of oat flour to heat treatment in the presence of moisture.

Table 1. Properties of raw (RO) and thermally processed (TO) oat

Property and parameters tested	RO	TO
β-glucan	1.04 ± 0.01	3.88 ± 0.06*
Crude protein	10.60 ± 0.04	11.51 ± 0.16*
Crude fat	5.12 ± 0.11	8.70 ± 0.18*
Total starch	76.83 ± 0.46	62.80 ± 0.70*
Lipase activity	Detected	Not detected
Peroxidase activity	Detected	Not detected
d (0.1), μm	6 – 8	7 – 11*
d (0.5), μm	38 – 183	92 – 349
d (0.9), μm	291 – 480	560 – 950*
T_o^1 (°C)	54.49 ± 0.04	56.76 ± 0.47*
T_p^1 (°C)	59.52 ± 0.08	62.89 ± 0.38*
T_c^1 (°C)	64.91 ± 0.08	72.17 ± 0.67
ΔH^1 (J/g)	7.74 ± 0.19	4.27 ± 0.15*
T_o^2 (°C)	85.30 ± 0.65	88.69 ± 0.13*
T_p^2 (°C)	96.52 ± 0.27	97.39 ± 0.35
T_c^2 (°C)	104.28 ± 0.12	101.41 ± 1.69
ΔH^2 (J/g)	1.68 ± 0.21	0.59 ± 0.03*
Peak viscosity (cP)	3287.33 ± 30.18	4452.00 ± 16.17*
Trough viscosity (cP)	1439.67 ± 17.84	3178.67 ± 16.70*
Breakdown viscosity (cP)	1847.67 ± 12.35	1273.33 ± 5.17*
Final viscosity (cP)	3377.00 ± 16.37	5358.33 ± 20.48*
Setback viscosity (cP)	1937.33 ± 14.17	2179.67 ± 4.70
Time to peak viscosity (min)	5.80 ± 0.00	5.93 ± 0.00

Results are presented as mean ± standard error of mean from independent replicates ($n = 3$, $n = 5^{\#}$).
Thermal properties: T_o refers to onset temperature, T_p refers to peak temperature, T_c refers to conclusion temperature, ΔH refers to enthalpy. Thermal properties labelled with superscript 1 indicate those parameters from low-temperature endotherm, which are associated with the melting of starch crystallites and gelatinization. Thermal properties labelled with superscript 2 indicate those parameters from high-temperature endotherm, which are associated with the melting of amylose-lipid complex. Significant difference between two types of oat, for a particular test parameter, is indicated by an asterisk (*). % Dry basis of chemical composition was calculated based on the following moisture content: RO–11% and TO–10%.

The DSC thermograms of oat samples exhibited two distinct endothermic peaks: (1) the low-temperature endotherm observed around 54 to 72 °C that is associated with the melting of starch crystallites and gelatinization and (2) the high-temperature endotherm observed around 85 to 104 °C that is related to the melting of the amylose-lipid complex. Specific parameters (T_o, T_p, T_c, ΔH) derived from these thermograms that are relevant for describing the thermal properties of RO and TO are presented in Table 1.

Pasting properties

Comparing RO and TO pasting profiles, higher peak viscosity was exhibited by TO than RO. This indicates that a thicker sample during cooking would be achieved with TO. Significant difference was observed between the trough viscosities of RO and TO. Trough viscosity refers to the reduction in peak viscosity during pasting, which is associated with starch degradation due to mechanical damage and high temperature (Zhou, Robards et al. 1998, Mukhtar, Shah et al. 2017). In comparison with RO, TO exhibited lower breakdown, which is derived from both peak and trough viscosities. The lower breakdown viscosity manifested by TO is suggestive of a more stable paste (Chatpapamon, Wandee et al. 2019), exhibiting greater stability of swollen granules with continuous shear during pasting. Among the two types of oat studied, TO displayed higher final viscosity than RO. The final viscosity provides an insight of the stability of cooked and cooled starch paste.

In general, comparing the pasting profile of two oat studied, TO demonstrated to produce a more viscous paste than RO. Lastly, the differences in the pasting properties of TO and RO highlight the structural modifications attributed to the initial thermal processing applied, which renders TO to be distinct from RO.

CONCLUSION

It is clear that thermal treatment (kilning at 115 °C for 30 min and steam-cooking at 100–104 °C for 18 min) of oat has led to modification of its physicochemical and functional properties. Compared with untreated oat (RO), thermally treated oat (TO) had higher amounts of β-glucan, crude protein, and crude fat contents. Commercial thermal treatment applied was also effeective in deactivating endogenous lipase and peroxidase enzymes. Aside from rendering oat to be commercially stable, this study also showed the concurrent impact of thermal treatment to other oat components, particularly starch. Based on the results presented in this research, the significant impact of thermal treatment in modifying the oat properties was demonstrated (more aggregated starch granule clusters, higher thermal transition temperatures, lower thermal enthalpies in TO). In the same context, this study was able to clearly establish that RO is structurally and functionally different from TO.

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MULTI-INDICATOR SPOILAGE MONITORING IN SKIN-ON TILAPIA FILLETS EXPOSED TO TEMPERATURE ABUSE

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Abstract: This study was conducted to determine various spoilage mechanisms in tilapia meat subjected to temperature abuse (27 °C) storage conditions. Microbiological populations including total aerobic mesophilic bacteria (TAMB), *Pseudomonas* spp., histamine-forming bacteria, and lactic acid bacteria continuously proliferated during the 10-h storage period. The very high initial TAMB of 6.72 log CFU/g was attributed to various intrinsic and extrinsic factors including species, composition, and harvesting and vending conditions. Chemical spoilage indicators including pH, histamine level, and total volatile basic nitrogen (TVB-N) were also monitored. The samples were found to exceed the recommended TVB-N limit of 25 mg/100g after 6 h storage period. Evaluation of various sensory attributes including overall acceptability, appearance, color, odor, and texture using Hedonic Scale rating and Focus Group Discussion showed that at such conditions, tilapia meat fillets have marketable and salable shelf-life of 4 h. Evaluation of a turmeric dye-based biosensor in detecting quality changes in tilapia meat in abused condition showed that while measurements of the Commission Internationale de l'Éclairage (CIE) *L**, *a** and *b** color space coordinates can distinguish fresh from abused samples, the sensitivity of the biosensor needs improvement to allow unaided eyes to judge color changes.

***Keywords*:** tilapia, microbial spoilage, chemical spoilage, sensory attribute deterioration, temperature abuse.

INTRODUCTION

The Philippines is a major fishing nation and the world's second-largest archipelagic state with more than 7,000 islands (FAO, 2014). Fish and other seafood are bountiful in the Philippine waters and Filipinos are fish consumers by tradition (Garcia *et al.*, 2005). Although the country has vast areas of aquatic resources, it is known that fish shortages for local consumption are perennially felt (DOST-PCAARRD, 2018) and thus the need for aquaculture (NASO, 2005). In the Philippines, seaweed, milkfish, tilapia, and giant tiger prawn were the main aquaculture species in 2012 (FAO, 2014). The Nile tilapia (*Oreochromis niloticus*) is a freshwater fish, which originated in Africa (Guerrero, 1985), but the farming of this species in freshwater lakes has been recognized as a lucrative business in the Philippines for a very long time (Bautista, 1984). Most commercial catches are traded in wholesale quantities at traditional landing centers or transported to major fish ports for auctioning (FAO, 2014). The fish go from producers to wholesalers who then distribute them to retailers (Gonzales, 1985). Ideally, fish should be marketed live, but there are instances when such condition is not met. Furthermore, Bari (2009) explained that for such a perishable food commodity, problems pertinent to temperature abuse arise too frequently. Exposure to inappropriate temperatures is common in the distribution chain and becomes almost routine in the domestic environment. Fish meat is susceptible to microbial and biochemical spoilage because of its high water activity and its suitable macro and micronutrient components (Gram and Huss, 1996). During spoilage, sensory attributes and nutritional value rapidly deteriorate, and toxic substances may be formed (Huss, 1995). Forsythe (2000) explained that although there has been much progress in the characterization of the total microbial flora and metabolite development during spoilage, not much is known about the specific contributions of specific microorganisms in the quality deterioration of food commodities. Furthermore, microbiological and chemical means of spoilage tracking are time-consuming and require technical expertise and technological inputs, which may not be possessed by relevant stakeholders. This can be addressed through the development of biosensors that may be used for rapid assessment of quality changes in food. Microbial and physicochemical quality deteriorations should also be compared with changes in the sensory attributes of the commodity, which are considered the ultimate measure of total quality (Bari, 2009). This study was conducted to monitor several microbiological, physicochemical, and sensorial deteriorative changes in tilapia meat subjected to extended temperature abuse. A rapid biosensor-based quality deterioration monitoring method was also evaluated based on natural dye color change. The results of this work provide baseline information on the mechanisms of quality changes in the tested commodity that may be useful in the control and maintenance of total quality.

MATERIALS AND METHODS

2.1. Tilapia meat. The fish samples used in the study were from Batangas Province which was purchased live in a wet market in Quezon City, Philippines. Live fish were sacrificed, eviscerated, descaled, and filleted in situ using vendor knives and equipment. Skin-on tilapia fillet samples were then placed in polyethylene bags and immediately transported to the laboratory. Upon arrival to the laboratory, samples were immediately subjected to analyses (time zero). Room temperature was not controlled (average temperature 27 °C), but were recorded to simulate temperature abuse normally encountered by the commodity. Fish temperature was similarly recorded throughout the study.

2.2. Microbiology-based assessment. Samples were obtained upon arrival to the laboratory and every hour for 10 h to monitor the changes in selected microbiological populations in the meat samples. Briefly, 30 g samples were aseptically weighed and homogenized with 270 mL 0.1% peptone water (PW, HiMedia, India) for 60 s using a food blender (HJB-115 Hanabishi, Philippines) previously sanitized with 200 ppm hypochlorite solution and hot water. The homogenate was thereafter subjected to serial 10-fold dilutions in PW prior to inoculations into specific growth media. Total aerobic mesophilic bacteria (TAMB) were determined by pour plating 1 mL aliquot of appropriate dilutions with tempered Plate Count Agar (PCA, HiMedia). To determine *Pseudomonas* spp., appropriate dilutions were pour-plated with Cetrimide Agar Base (CAB, HiMedia) supplemented with glycerol (Univar, Autralia). A medium described by Niven *et al.* (1981) was compounded and used to enumerate histamine-forming bacteria (HFB) from the fish samples. The HFB medium was composed of 0.5% tryptone (HiMedia), 0.5% yeast extract (HiMedia), 2.0% L-histidine-monohydrochloride, 0.5% NaCl (Ajax Finechem, Australia/New Zealand), 0.1% CaCO3 (Lola Chemie Laboratory Reagents and Fine Chemicals, India), 2.0% nutrient agar (HiMedia), and 0.006% bromocresol purple (HiMedia) at pH 5.3. Plates for TAMB, *Pseudomonas* spp., and HFB analyses were incubated aerobically at 35 °C for 24 to 48 h prior to colony enumerations. For HFB, a purple halo was deemed positive for amine production. Finally, Lactic Acid Bacteria (LAB) were determined using De Man, Rogosa, and Sharpe Agar (MRSA, HiMedia). Plates were incubated at 35 °C anaerobically using the Anaerobox and Anaeropack System (Mitsubishi Gas Chemicals, Tokyo, Japan) for up to 72 h prior to colony enumerations. All microbial populations enumerated within the storage time period were fitted to the Baranyi and Roberts (1994) model using the Dynamic Model Fit (DMFit) 3.0 (Institute of Food Research, UK) to determine the microbial growth kinetic parameters.

2.3. Chemistry-based assessment. Samples were withdrawn at predetermined time intervals within the 10-h temperature abuse exposure for monitoring the physicochemical property changes of the fish. The pH using a calibrated electrode (Horiba F-70, Tokyo, Japan). Histamine levels in the samples were determined following the AOAC 977.13 fluorometric method. The total volatile basic nitrogen (TVB-N) levels were determined following the procedures recommended by the fish products sub-committee of the Analytical Methods Committee (1979). Briefly, extracts or solutions are made alkaline with sodium hydroxide in a suitable semi-micro steam distillation set-up. The TVB-N are quantitatively distilled into standard acid. Formaldehyde is then added to render amines other than trimethylamine non-reactive and acids, which were finally titrated with a standard alkali.

2.4. Sensory attribute-based assessment. Sensory evaluations were conducted aided by a sensory panel composed of 9 members. They were presented with the fish samples in identical white plastic containers. Using a 9-Point Hedonic Scale, the overall acceptability of the meat, and acceptability of appearance, color, odor, and texture of the samples were rated. Panel members were also asked to write down specific characteristics of the samples they liked or did not like, which were used as pointers in an independent Focus Group Discussion (FGD). Acceptability ratings were independently conducted twice, while FGD was conducted only once.

2.5. Turmeric dye biosensor-based assessment. The study tested the utility of a turmeric dye biosensor for rapid assessment of fish quality deterioration based on dye color changes. The biosensor was developed by the Condensed Matter Physics Laboratory, National Institute of Physics (NIP), University of the Philippines, Diliman. For the analyses, 300 g skin-on tilapia fillets were placed in sterile 1.25 L, air-tight plastic containers with biosensors-installed covers. Color changes were monitored 0 to 10 h from 2 biosensors in each of 3 containers for a total of 6 replicates. It was photodocumented using a Canon EOS M10 digital camera and processed using the Image Color Summarizer (Krzywinski, 2006). Color changes were only reported in terms of CIE $L^* a^* b^*$ color space coordinates.

2.6. Statistical analyses. Data obtained from independently replicated experiments were subjected to single-factor analysis of variance (ANOVA) using the general linear model procedure (PROC GLM) of the SAS Statistical Software Package version 8.0 (Cary, NC, USA). The Duncan's Multiple Range Test (DMRT) was used for post-hoc determinations of significant differences at 95% level of significance.

RESULTS AND DISCUSSION

3.1. Microbiological populations in tilapia meat during temperature abuse. Table 1 summarizes the growth kinetic parameters of different microbial populations in the tilapia meat. Forsythe (2000) and Jay *et al.* (2005) explained that the bacterial microbiota of fish vary in the different parts of the animal. The slime coat on the skin

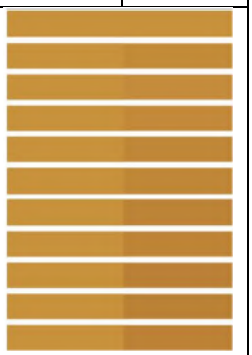
which is composed of mucopolysaccharides, free amino acids, trimethyl amine oxides, piperidine derivatives and other related compounds can have 3.0 to 5.0 log CFU/cm2, while the gills and intestines can have 3.0 to 4.0, and 2.0 to 9.0 log CFU/g, respectively. In a study conducted by Stenstrom and Molin (1990), spoiled fresh water fish had total aerobic biota of 8.0 log CFU/g, which were dominated by *Pseudomonas* and *Shewanella* spp. In this study, the TAMB populations reached 8.2 log CFU after 5 h of storage at ambient conditions. *Pseudomonas* spp. grew more slowly than the other monitored populations, and had the lowest final population, possibly due to the higher storage temperature tested. For HFB, Lopez-Sabater *et al.* (1996) explained that that tissues of gills and gut are a major source of HFB in fish. The populations isolated from the filleted meats must have come from the these sources during the evisceration and filleting processes. In the study conducted by da Silva *et al.* (2002) for vacuum packed cold-smoked fish, more isolates were able to produce histamine and tyramine in products that were stored at 25 °C than those at 5 °C. Similar to HFB, LAB are generally found in the gastrointestinal tract of fish (Ringø and Gatesoupe, 1998; Gatesoupe, 2008). da Silva *et al.* (2002) also reported that tyramines were produced by LAB isolates such as *Carnobacterium divergens* and *Lactococcus lactis* in vacuum packed cold-smoked salmon and trout.

Table 1. Growth kinetic parameters[1] and linear correlation coefficients (R^2)[2] of selected microbial populations in tilapia fillet at ambient (27.2 °C) storage conditions.

Microorganisms	Pop_{init} (t_0) (log CFU/g)	Pop_{fin} (t_{10}) (log CFU/g)	K_G (log CFU/h)	R^2
Total Plate Count	6.72 ±0.36^{a}	8.99 ±0.37^{a}	0.22 ±0.02^{b}	0.89-0.93
Pseudomonas spp.	3.64 ±0.28^{d}	6.12 ±0.14^{d}	0.25 ±0.04^{b}	0.76-0.94
Histamine-Forming Bacteria	4.67 ±0.23^{c}	7.68 ±0.24^{c}	0.34 ±0.04^{a}	0.91-0.97
Lactic Acid Bacteria	5.99 ±0.28^{b}	8.46 ±2.26^{b}	0.34 ±0.04^{a}	0.90-0.96

[1]Values are presented as mean ±SD obtained from 2 independently conducted experiments, with 2 internal replicates per experiment.
[2]R^2 values were obtained using Pearson's Correlation Calculator to determine strength of linear relationship between sensory scores and time. $R^2 \geq 0.67$: Substantial; ≥ 0.33: Moderate; ≥ 0.19: Weak.
[a,b,c…] Values on the same column followed by the same letters are not significantly different ($p > 0.05$).

Table 2. Mean physicochemical values[1], mean Hedonic ratings[2] for selected sensory attributes of skin-on tilapia meat fillets stored at abused conditions (27.2 °C) and biosensor color change through storage.

Storage Time (h)	Mean Physicochemical Values[1]			Mean Hedonic ratings[2] on Sensory Attributes		Visual Representation	
	pH	Histamine (mg/100g)	TVB-N (mg/100g)	Overall Acceptability	Odor	Reference	Observed
0	6.82 ±0.11^{a}	0.33 ±0.35^{b}	14.9 ±14.26^{c}	6.22 ±0.59^{a}	6.53 ±0.47^{a}		
1				5.53 ±1.20^{ab}	5.38 ±1.08^{b}		
2	6.62 ±0.27^{a}	0.23 ±0.25^{b}	17.65 ±10.88^{bc}	4.94 ±1.47^{bcd}	4.56 ±1.43$^{bcd, HS}$		
3				5.16 ±1.34^{bc}	4.75 ±0.93$^{bc, FGD}$		
4	6.61 ±0.13^{a}	0.20 ±0.20^{b}	14.45 ±1.74^{c}	4.41 ±0.89$^{cde, FGD}$	4.16 ±1.04^{cd}		
5				4.16 ±0.57$^{de, HS}$	3.72 ±0.69^{de}		
6	6.45 ±0.32^{a}	0.51 ±0.46^{ab}	23.15 ±14.26^{bc}	4.41 ±0.93^{cde}	4.00 ±0.63^{cd}		
7				3.53 ±0.71^{e}	3.00 ±0.83^{ef}		
8	6.52 ±0.31^{a}	1.58 ±1.04^{a}	40.90 ±12.01^{a}	2.25 ±0.30^{f}	2.25 ±0.46^{f}		
9							
10	6.54 ±0.14^{a}	1.53 ±1.11^{a}	34.15 ±11.39^{ab}				
R^2	**0.62**	**0.74**	**0.74**				

[1]Values are presented as mean ±SD obtained from 2 independently conducted experiments, with 2 internal replicates per experiment.
[2]Mean hedonic scores were obtained from two sessions of sensory evaluation by hired panelists. 1-Dislike Extremely; 2-Dislike Very Much; 3- Dislike Moderately; 4-Dislike Slightly; 5- Neither Like or Dislike; 6-Like Slightly; 7-Like Moderately; 8-Like Very Much; 9-Like Extremely
R^2 values were obtained using Pearson's Correlation Calculator to determine strength of linear relationship between sensory scores and time. $R^2 \geq 0.67$: Substantial; ≥ 0.33: Moderate; ≥ 0.19: Weak.
[a,b,c…] Values on the same column followed by the same letters are not significantly different ($p > 0.05$).
[FGD] End of shelf-life based on Focus Group Discussion with a consumer-type panel (n=9)
[HS] End of shelf-life based on 2-point reduction in the mean Hedonic scores obtained from 2 independent sensory evaluations of a consumer-type panel (n=9)

3.2. Chemical changes in tilapia meat during temperature abuse. Table 2 shows a general non-significant ($p > 0.05$) reduction in meat pH throughout the 10-h storage period. The lowering of pH could be attributed to the metabolism of glycogen in the muscle into lactic acid (Kyrana *et al.*, 1997; Huss, 1995). However, the very small change observed may be attributed to the simultaneous neutralization of the organic acid by the basic compounds formed in the later post-mortem changes as the fish meat begins to spoil (Huss, 1995). Histamine levels significantly ($p < 0.05$) increased within the 10-h storage period. This observed increase in the biogenic amine was parallel with the increase in the population of histamine-forming bacteria as well as other bacterial populations like *Pseudomonas* spp. and LAB. However, despite the observed increase in histamine the value did not exceed the maximum allowable level of 5 mg/100g and is safe from inflicting

scombroid poisoning (Alfonzo *et al.*, 2017). The initial total volatile basic nitrogen (TVB-N) content of the fillets was 14.9 mg/100g. This value is higher than that the initial levels in tilapia meat fillets reported by Cao *et al.* (2020), Khalafallah *et al.* (2015), who both reported initial TVB-N values of <10 mg/100g; and Shi *et al.* (2019) who reported an initial TVB-N of 10.02 mg/100g. Such disparities in the initial TVB-N levels may be attributed to a number of factors including variations in species, catching season and region, age, and sex of the fish (Khalafallah *et al.*, 2015). The TVB-N significantly ($p < 0.05$) increased during the 10-h storage period. By the end of the 10^{th} h, the initial level was more than doubled (34.15 to 40.90 mg/100g). Shi *et al.* (2019) reported a direct relationship between the rate of TVB-N production in tilapia meat and storage period. The TVB-N is produced in parallel with bacterial proliferation, endogenous enzymatic activities, and postmortem degradation of meat, and mainly consist of ammonia, dimethylamine, and trimethylamine. The faster TVB-N production observed in this current study may be attributed to the storage condition tested, which encouraged rapid microbial proliferation and optimized enzymatic activities. Cao *et al.* (2020) used 25 mg/100g as acceptable limit for fresh fish, while Khalafallah *et al.* (2015) used 30 mg/ 100g. From the results obtained in this study, samples had unacceptable TVB-N levels after 6 h of storage at temperature abuse condition.

3.4. Sensory attribute changes in tilapia meat during temperature abuse. As expected, all of the sensory attribute scores significantly ($p < 0.05$) decreased throughout the storage period, with odor and texture having faster significant change. By the end of the 8^{th} h, samples had Hedonic scores ranging from 2.13 to 2.34. Giménez *et al.* (2012) explained that sensory shelf-life is determined as the time required for overall liking scores of the product to fall below a predetermined value. In this study, the researchers initially set the reduction of Hedonic ratings by 2 points as indicator of the end of shelf-life. This was confirmed through independent FGD to determine the storage time at which the product is no longer acceptable or salable. Results showed that the 2-point Hedonic score reduction and FGD-determined end of shelf life only coincided for the appearance of the meat fillets (5 h). For overall acceptability, flesh color, and texture, the FGD-determined end of shelf-life came 1 h earlier than that when the Hedonic scores were used as bases. It should be emphasized that Hedonic scores between the 2 end of shelf-life indicators were not significantly ($p > 0.05$) different. The overall average of end of shelf-life at the test temperature abuse storage condition based on the 2 parameters was determined to be 4 h. This was shorter than the 6 h storage based on TVB-N levels in the meats.

3.5. Color changes in the turmeric-based biosensor during storage. Results showed that through time, the dye became less light, more red, and less yellow. These results are similar to those reported by Vergara *et al.* (2017) that measured color changes in the same turmeric-based dye adsorbed in titanium dioxide through reflectance spectroscopy. However, only the yellow to orange color shift was recorded, which was attributed to the lower concertation of ammonium hydroxide liberated in the meat. It should also be emphasized that statistically significant ($p < 0.05$) readings in CIE L*, a*, and b* values were only recorded on the 6th, 7th, and 8th h of storage, respectively. While these are comparable to the earlier established 6 h storage period for unacceptable TVB-N levels to be achieved, the 4-h limit of consumer acceptability was not characterized by any significant change in color reading. Furthermore, while the color change from 0^{th} to 1^{st} h of storage can easily be detected by unaided eyes, color changes from 1st to 10^{th} h are very difficult to judge without the use of color-measuring tool. These limitations can be addressed by improving the sensitivity of the biosensor to make color discrimination by a human judge easier.

CONCLUSIONS

Results suggest that tilapia meat fillet stored at abused temperature (27.2 °C) has a marketable and salable shelf-life of 4 h; but it can be deemed unacceptable as early as 2 h after purchase, due to foul odor. Results also show that the turmeric dye-based biosensor developed by NIP is not an efficient tool that can monitor fish freshness. Further study should be done to improve the sensing abilities reflected on color changes, as the change in color on the current biosensor is not easily perceived. The effect of container headspace on the color change can also be further observed. Moreover, the sensor's performance in monitoring the quality deterioration of other seafood may be investigated to further develop baseline data regarding its quality sensing capabilities. In conclusion, storage temperature is a major factor in the spoilage of tilapia fillets. Fish must be chilled immediately after catching. The integrity of the cold chain must be protected throughout all processing stages to control the rapid increase of microbial population and ensure maximum product shelf life (FSAI, 2018). Results obtained in this study provide a holistic overview of the various deteriorative mechanisms in the quality attributes of the test food product. These may be useful in the assurance and maintenance of quality attributes of a common food product that is often subjected to suboptimal storage conditions.

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SHELF-LIFE EXTENSION OF *SOTO BANJAR* INSTANT SEASONING

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Abstract: *Soto Banjar* is a popular traditional dish from Banjarmasin, Kalimantan, Indonesia. The preparation of *Soto Banjar* (spice mix and broth soup) is a long and complex process. A cottage industry in Samarinda runs a traditional *Soto Banjar* instant seasoning business. At the current time, no preservation techniques have been applied to the product. Despite its popularity, problems arise in the attempt of its business expansion to outside city due to its shelf-life at room temperature. This study begins with the selection of dried broth to be combined with the seasoning paste, followed by the selection of thermal heating techniques to be applied. Dried broth variations used were overripe tempe stock, block chicken broth, and mushroom stock, while thermal treatment variations were steam heating for approximately 100°C and retort sterilisation for 121°C. Based on sensory evaluation and FGD, the mushroom stock was selected to be combined with the spice mix. The *Soto Banjar* instant seasoning combined with dried broth can save time of serving for almost 2 hours. Retort sterilisation for 15 minutes was selected based on microbial content <10 CFU/g, undamaged packaging, lightness, and yellowish colour which are not significantly different, and similar taste with the original *Soto Banjar*.
Keywords: *Soto Banjar*, Instant Seasoning, Thermal Processing, Retort Sterilisation, Shelf-life

INTRODUCTION

Soto is known as a cuisine that exists in many regions in Indonesia which differ based on cultural diversity. *Soto* is one of the main traditional Indonesian culinary icons according to the Ministry of Tourism and Creative Economy of Indonesia in 2014. *Soto Banjar* is a popular dish which originates from Banjarmasin that differs from other types of *soto* that can be found throughout Indonesia (Alfisyah, 2019). It consists of several spices and condiments, with chicken as the protein source. The key characteristic of *Soto Banjar* is its traditional special paste spice mix. The preparation of *Soto Banjar* is a long and complex process (Hambali et al., 2005). Its paste seasoning and high protein content of natural broth soup affects the storage duration. Hence, it would be very practical if there is a paste instant seasoning combined with the dried broth addition. Evaluations such as the preference of dried broth, which was analysed, and efficiency of the product can be done. The traditional process of making *Soto Banjar* contributes to its short shelf life, in which inappropriate packaging procedure is done. Therefore, a preservation method is necessary to extend the shelf life and reduce the microbial content.

One cottage industry in Samarinda, East Kalimantan, experiences this problem. Their product can only be stored in freezing temperature. If it is not, the product will undergo damage caused by microorganism and oxidation reaction. This could prevent the business from expanding and compete outside the city, to modern retail, and/or abroad. The product needs in-packaged thermal heating technique to prolong the shelf life with reducing the microbial level and preventing further contaminations while storage. Steam heating and retort sterilisation were used as options for thermal heating technique applicable to products. The technique has a potential in prolonging the storage time and distribution at room temperature, so that the product can be more practical and has a lower cost of distribution. Various analyses were carried out on the effectiveness of shelf-life extension of *Soto Banjar* instant seasoning in terms of microbial contents, packaging damage, and sensory characters.

MATERIALS AND METHODS

This research was conducted from January 2023 until June 2023. There are three types of dried broths that are combined with spice mix. Samples were analysed with sensory evaluation and Focus Group Discussion with *Soto Banjar* stakeholders. These assessment techniques were used to select the preferred dried broth that can be applied to the product. Then, comparisons of total duration spent of using the *Soto Banjar* instant seasoning and traditional *Soto Banjar* were done to evaluate the product efficiency. Products were inserted to the sealed retort plastics packaging (PA+CPP). Steam heating (SH) (95º–100ºC) and retort sterilisation (RS) (121ºC, 15 psi) as thermal heating techniques were conducted with 3 different heating durations as independent variables, namely 5 minutes (S5 = steam sterilisation for 5 minutes and R5 = retort sterilisation for 5 minutes), 15 minutes (S15 and R15), as well as 25 minutes (S25 and

R25). After that, the resistance of the samples packaging was checked. After 14 days of quarantine time, microbial contents of samples were tested with TPC analysis to determine the best thermal heating technique and heating duration that can be applied. Packaging of samples were checked to evaluate whether there were swelling or volume addition. The microbial content of the original spice mix and its sample packaging were evaluated as the control data. Four benchmarking products of *Soto Banjar* seasoning were used to compare colourimetric values by colourimeter PCE-CSM 3. After the method was selected, and sensory intensity was conducted with sensory evaluation.

RESULTS AND DISCUSSION

- Dried Broth Determination and Efficiency Evaluation of *Soto Banjar* Instant Seasoning

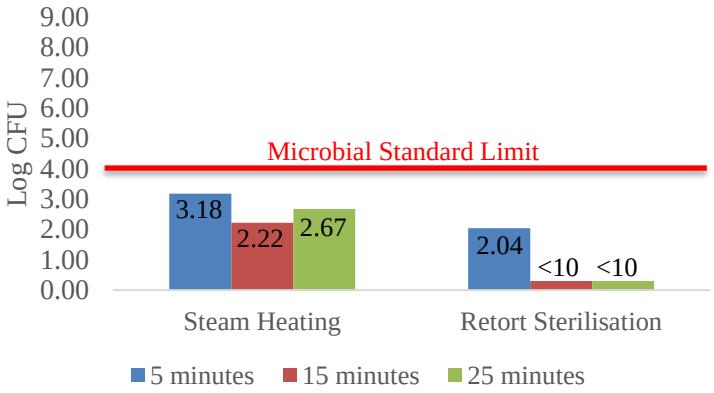
The function of dried broth is for practicality and shelf-life. This product needs to be made as practical as possible. In general, *Soto Banjar* requires natural chicken bouillon in the cooking process that has high content of protein that shorten the shelf-life. The high content of protein, water, and fat in meat and meat-derivative products will be conditions that have the potential for the growth of putrefactive microorganisms (Dirpan & Hidayat, 2023). Each panellist was presented with three samples which consist of the *Soto Banjar* soup that combined with either overripe tempe stock (OTS), block chicken broth (BCB), and mushroom stock (MS). On a parameter scale of 1 (extremely dislike) to 9 (extremely like), each of which has a rating category, panellists were asked to give a score according to their preferences. Parameters that were evaluated with stakeholders are colour, taste, and aroma. The MS had the highest rank mean compared to the OTS and BCB. There is a different taste and aroma between OTS and MS. Ingredients of MS produces a different flavour when compared to OTS. Mushrooms have 27% of protein content. This value is quite high for a plant-based food ingredient. Mushrooms which contain natural glutamic acid can give a savoury taste to dishes.

After the sensory test, FGD was executed with *Soto Banjar* stakeholders. MS is suitable and has the highest rank of preference because it can fulfil the original flavour and colour of *Soto Banjar* and can complement to the taste of *Soto Banjar*. Efficiency of product combined with dried broth addition was evaluated by comparing it to traditional *Soto Banjar* serving. If consumers use *Soto Banjar* instant seasoning, the product can save time up to 110 minutes or almost 2 hours, since the preparation only take 35 minutes, while the traditional serving can take time up to 145 minutes. This result concludes that *Soto Banjar* instant seasoning can be efficient in case of duration spent compared to traditional serving.

- Effect of Microbial Content Towards Heating Treatment and Heating Duration

In-packaged thermal heating processes decreases the value of total colonies in samples. Heating treatments that were applied successfully reduce the total microbial number and retain its number to lower than control (10^2 CFU/g) even after 14 days of storage (Figure 1). The employment of vacuum pressure in the retort treatment showed more effective microbial reduction of total microbial. Each microbe has an optimum temperature, minimum temperature, and maximum temperature for its growth (Susiwi, 2019). For example, *Clostridium botulinum* will destroy when the sterilising temperature has reached at 121.1°C. By reaching at that temperature, harmful pathogenic bacteria will disappear as well as bacteria with a lower maximum temperature. If the bacteria have disappeared, there will be no damage from the microbes during the 14 days of incubation.

Figure 1. Graph of Microbial Enumeration of Heat-Treated Spice Mix After Quarantine on 14 Days



According to BPOM (regulatory board to supervise food products in Indonesia) Regulation Number 13 regarding Maximum Limits of Microbial Contamination in Processed Food (2019), the maximum microbial limit for the type

of this product is 10^4 CFU/g. Both SH and RS in each duration do not exceed the limit. However, steam heating still results to a high of microbial contents that can damage dan inhibit the product during storage and distribution. Thus, it is not enough to sterilise the product. The sterilisation temperature conditions are not necessarily the same as the temperature that occurs in the sample because the increase or decrease in sample temperature occurs more slowly than the increase or decrease in sterilisation temperature (Azhari et al., 2023). This also indicates that R5 still has many microorganisms. The short 5-minute heating process makes the sample did not yet fully reach the desired temperature in sterilisation. Besides, heat propagation takes time to reach the coldest point (Praharasti et al., 2014). Then, temperatures above 100ºC are sufficient to remove bacterial spores (Azhari et al., 2023). Therefore, SH does not have the ability to get rid of spores. Most of the spore formers found in spices belong to the genera *Bacillus* and *Clostridium*. Raw materials such as pepper and turmeric can usually be contaminated by these bacteria. This is very common in herbs and spices, and sometimes the amount can reach very high levels, for example *Bacillus licheniformis* and *Bacillus cereus*.

The key process to choose the suitable method is the microbial enumeration result. Another consideration is the packaging condition after heating with two weeks of quarantine time. The best method that can be applied to products are R15 heating duration. *Soto Banjar* instant seasoning with SH processes had higher microbial count compared to those with RS. The gas formation after 14 days that was observed with the swelling of the packaging during quarantine time also confirmed the insufficiency of R5 heating treatment. On the other hand, R25 damaged the plastic packaging and caused exfoliation. It seems that combination of high pressure and heating in RS cause damaging impact in longer duration. The 14-day quarantine time is to provide incubation time for the microbes present in the product. Microbial growth is characterised by an increase in volume so that the packaging swells. This is caused by carbon dioxide. Lactic acid bacteria will break down the sugar from the *Soto Banjar* instant seasoning to produce lactic acid and carbon dioxide (Hofvendahl & Hahn-Hägerdal, 2000).

- Sensory and Physicochemical Characters of Selected *Soto Banjar* Instant Seasoning

Colour is an important factor to be analysed based on FGD results. Benchmarking was done to analyse colour differences of products. There are four products have different characteristics. Product 1 (P1) uses potassium sorbate as an additional preservative which has a shelf-life of up to 18 months. Product 2 (P2) which is in powder form, it does not use any preservatives. This product has a shelf-life of up to 12 months. Product 3 (P3) that must be stored in the freezer to maintain its quality. If stored properly in the freezer, it has a shelf-life of up to 6 months. Product 4 (P4) must also be stored in the freezer. If freezing condition, this product has a shelf-life of up to 12 months. Manufacturers choose to add preservative to preserve these products. Most existing *Soto Banjar* seasonings do not use a thermal process system for their commercially sterilisation. For lightness, there is no significant difference between control, and P2 as well as P4. For greenish colour, there is no significant difference between control and P1. However, all four brands have different yellowish colour compared to control. The control has a uniqueness in its yellowish colour. The yellow colour in the control comes from the use of turmeric. Meanwhile, other commercial products do not use turmeric. Dominant ingredients such as shallots and garlic will affect the colour of benchmarking products to be darker and not as yellow as the control.

The colour differences control and R15 were also compared (Table 1). When heat treatment was given to the food, its colour of the food has undergone changes caused by non-enzymatic reactions. The colour changed to brown and not greenish yellow as before. If two samples resulting from SH and RS were compared, then the sample resulting from retort sterilisation is darker than steam heating. Even though sterilisation has succeeded in eliminating many pathogenic bacteria, if it changes the colours of the product, this will reduce the quality of the product. Raw materials in *Soto Banjar* instant seasoning contain carotenoid compounds in them. This compound can be bound to oil and cause a brownish discolouration due to heating (Riyandi et al., 2022). For lightness and yellowish colour value, there is no significant difference, so it can be concluded that heating by R15 will not change the lightness and yellowish colour. However, this is inversely proportional to the greenish colour of the control and R15 because both has a significant difference. Control has a greener colour than R15 which is more towards a reddish colour. This is because the heating effect has made the colour of the sample a bit brownish which tends to be a red colour (darker).

Table 1. Colourimetry Result Between Control and Retort for 15 Minutes Samples (R15)

Type of Sample	*L (Dark-Light)	*a (Green- Red)	*b (Blue-Yellow)
Control	60.99 ± 2.94	5.18 ± 0.23	24.62 ± 3.53
R15	58.27 ± 4.34	6.74 ± 1.25	20.82 ± 6.24

The sensory evaluation was conducted with 34 untrained panellists with three samples which consisted of the control, R5, and R15. A score of 1 state that they choose very not strong and a score of 7 states very strong. Rating scores obtained from sensory evaluation were converted into ranking scores to ease the data analysis (Table 2). Parameters which evaluated are aroma and taste. Control reached the highest rank mean in both aroma and taste. These results

define that thermal heating techniques in *Soto Banjar* will change the aroma. This result supports the selected method and duration for R15 which are based on the minimal damages and total of bacteria colonies. This observational data is in line with the results of research conducted by Ansar (2006), where the quality of retorted products will deteriorate both in terms of sensory and nutritional content although the process makes microorganisms and spores inactive. Although R15 was considered to have a dominant just right taste by untrained panellists, the aroma and taste intensity had decreased after the retort process. The heating process can make the volatile compounds contained in the spice mix unstable (Riyandi et al., 2022).

Table 2. Rating Intensity Test Result

Type of Sample	Mean Rank of Intensity	
	Aroma	Taste
Control	2.6 ± 0.7^{a}	2.3 ± 0.7^{a}
R5	1.5 ± 0.7^{b}	1.5 ± 0.7^{b}
R15	1.9 ± 0.7^{b}	2.1 ± 0.8^{a}

$^{a\ \&\ b}$ *Different letters in the column indicate significant differences ($p<0.05$).*

CONCLUSIONS

(1) Dried broth that is suitable for *Soto Banjar* instant seasoning is mushroom stock. (2) The *Soto Banjar* instant seasoning combined with dried broth can save time of serving for almost 2 hours. (3) Retort sterilisation for 15 minutes (R15) was the effective method to sterilise the *Soto Banjar* instant seasoning compared to steam heating and other heating durations (5 minutes and 25 minutes). The number of colonies decreased even less than 10 CFU/g. (4) The heating by retort for 15 minutes did not change product colour characteristics which are lightness and yellowish colour. The heat-treated *Soto Banjar* instant seasoning changes the aroma, but there was no significant different of taste between control and R15. There are several recommendations: other heating techniques, the effect of packaging types on sterilisation, pre-heat thermal process before the spice mix is packaged (e.g., pasteurization), and retort sterilisation with automatic cooler system autoclave.

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PHYSICOCHEMICAL PROPERTIES AND SENSORY ACCEPTABILITY OF GAC ICE CREAM INCORPORATED WITH PALM SUGAR AS AN ALTERNATIVE SWEETENER

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Abstract: Commercialized ice cream commonly contains high fat and sugar contents, and excess consumption may lead to adverse health effects. Palm sugar has a low calorie and high fiber content, which will help to produce healthier ice cream. Therefore, this study aims to determine the effect of different ratios of white sugar and palm sugar on the physicochemical properties and sensory acceptability of gac ice cream. Five formulations of gac ice cream were prepared with different ratios of white sugar to palm sugar: 100:0, 90:10, 80:20, 70:30 and 60:40. Melting rate, overrun, hardness, colour profile, energy content and proximate analyses were carried out in triplicate. The sensory acceptability of gac ice cream was evaluated by 30 untrained panellists using a 7-point hedonic scale of an acceptance test. The result showed that increasing the percentage of palm sugar and decreasing the percentage of white sugar increased the melting rate, overrun, moisture content, and crude fibre content while reducing the L* value, energy content and hardness of gac ice cream. No significant difference (p>0.05) was found for sensory acceptability among control and treated samples. This study suggested that palm sugar has excellent potential to be used as an alternative sweetener as it increases the nutritional composition without compromising the sensory quality of dairy products.

Keywords: Dairy product, Fibre, Melting rate, Overrun, Sensory quality

INTRODUCTION

Malaysia's ice cream market increased by 3.43 percent in 2018, from RM 795.9 million in 2017 to RM 823.2 million in 2018 (Nik Hassan et al., 2021). This value strongly shows that ice cream is a popular dairy product for all ages throughout the year as it can increase feelings of enjoyment and happiness. However, continuous and high consumption of ice cream, which contains a high fat and sugar content, might contribute to the risk of type 2 diabetes, obesity, heart disease, and high blood pressure (Oli, 2020). Several studies have been done on the use of sucrose-alternative sweeteners in ice cream, such as stevia (Ahmed et al., 2023; Ozdemir et al., 2015) and combinations of honey, trehalose, and erythritol (Moriano and Alamprese, 2017). The addition of sucrose-alternative sweeteners can reduce the calories of ice cream by up to 45% (Moriano and Alamprese, 2017) and significantly affect the overrun, melting rate, and viscosity of ice cream (Ozdemir et al., 2015). Palm sugar is another potential sucrose-alternative sweetener that is

absorbed more slowly and has a lower calorie content (Sari Dewi et al., 2022). Palm sugar made from the sap of palm flowers is high in moisture content, dark in colour due to the Maillard reaction and caramelization during its production, contains antioxidants, has a low glycemic index (35), and has a fibre called inulin (Sari Dewi et al., 2022). The addition of 4% inulin reduced the hardness, extended the melting time, and increased the overrun without changing the product's sensory qualities (Narala et al., 2022). Gac fruit (*Momordica cochinchinensis* Spreng), also known as super fruit, is a native fruit of Southeast Asia and contains high levels of beneficial health compounds, particularly antioxidants. The aril, which is a membrane, surrounds the seeds of ripe gac fruit, has been reported to be an excellent source of fatty acids, bio-accessible pro-vitamin A, carotenoid (lycopene and β-carotene), and phenolic compounds, as well as a good natural red colourant (Thanh Do et al., 2019). Akkarachaneeyakorn et al. (2017) reported that a 1 : 1 ratio of soy protein and maltodextrin may reduce 50% of the fat content of gac ice cream. However, this rare gac fruit has a bland taste and is still not widely consumed as a food product. The development of popular dairy products like ice cream with the addition of gac fruit will add value to this fruit. Therefore, the objective of this study is to determine the effect of incorporating palm sugar on the physicochemical properties and sensory acceptability of gac ice cream.

MATERIALS AND METHODS

Fresh ripe gac fruit was collected from Taman Penyelidikan Alami, Universiti Malaysia Terengganu (UMT) at Bukit Kor, Marang, Terengganu, Malaysia. Palm sugar was purchased from local producer at Marang, Terengganu while non-dairy whipping cream, dairy whipping cream, maltodextrin, white sugar and fresh milk were purchased at Terengganu local market. The gac aril (seed membrane) was manually separated from the seeds and the preparation of gac ice cream was carried out according to modification method from Mohd Fauzi et al., (2022). Five formulations of gac ice cream were prepared with different ratios of white sugar and palm sugar (100:0, 90:10, 80:20, 30:70 and 40:60). Analyses of colour profile (Mohd Fauzi et al., 2022) hardness (modified method of Akkarachaneeyakorn et al., 2017), overrun and melting rate (Gonzalez et al., 2016), energy content (kJ/100 g) is according to Idris et al., (2019) as well as proximate composition of moisture, ash, fat, protein and fiber (AOAC, 2000) were carried out in triplicate. A 7-point hedonic scale of an acceptance test, with score 1 referring to dislike extremely and score 7 referring to like extremely, was used by 30 untrained sensory panellists to evaluate the attributes of colour, odour, sweetness, mouthfeel, flavour, and overall acceptance (Mohd Fauzi et al., 2022). One-way analysis of variance (ANOVA) with Fisher's LSD test was used to determine the significance of mean values at $p<0.05$.

RESULTS AND DISCUSSION

The physical properties of gac ice cream incorporated with different ratios of white sugar to palm sugar are shown in Table 1. As can be seen, by increasing the percentage of palm sugar in the formulation, the melting rate, hardness, and brightness (L*) values decreased while the overrun and b* values of gac ice cream significantly increased. Inulin binds water and forms a gel-like network (Kim et al., 2001). This immobilises the water molecules and prevents them from moving freely among the other molecules in the mixture, resulting in delayed melting of the product (El-Nagar et al., 2002). Palm sugar also contains fructose, which can promote the development of foaming and increase the overrun and reduce the hardness of ice cream (Maryani et al., 2021). The addition of palm sugar resulted in a darker colour (low in L* value) of the gac ice cream due to the Maillard reaction and caramelization process in producing of palm sugars (Saputro et al., 2020). As expected, the energy content of gac ice cream decreased with an increase in the percentage of palm sugar and a decrease in white sugar. The addition of 40% palm sugar with 60% white sugar contributes to reducing 12.9% of the energy content when compared to control ice cream (prepared with 100% of white sugar).

Table 1. Physical properties (n=3) of gac ice cream incorporated with palm sugar

Ice cream	Melting rate (g/min)	Overrun (%)	Hardness (kg)	L*	a*	b*
A	0.08 ± 0.001^{a}	26.12 ± 0.15^{e}	0.90 ± 0.03^{a}	84.44 ± 0.61^{a}	10.37 ± 0.63^{a}	27.11 ± 0.91^{c}
B	0.07 ± 0.002^{b}	27.40 ± 0.45^{d}	0.81 ± 0.02^{b}	83.74 ± 0.71^{ab}	10.08 ± 0.69^{a}	29.60 ± 0.36^{ab}
C	0.06 ± 0.001^{c}	28.07 ± 0.36^{c}	0.69 ± 0.03^{c}	83.19 ± 0.25^{b}	10.04 ± 0.15^{a}	29.55 ± 0.94^{ab}
D	0.04 ± 0.007^{d}	29.02 ± 0.09^{b}	0.54 ± 0.05^{d}	83.05 ± 0.71^{b}	9.82 ± 0.19^{a}	28.67 ± 0.51^{b}
E	0.02 ± 0.002^{e}	30.08 ± 0.55^{a}	0.44 ± 0.02^{e}	82.92 ± 0.08^{b}	10.23 ± 0.74^{a}	30.79 ± 1.11^{a}

Mean value ± standard deviation with different superscript letters in the same column are significantly different ($p<0.05$). Gac ice cream prepared by A (100% of white sugar and 0% of palm sugar), B (90% of white sugar and 10% of palm sugar), C (80% of white sugar and 20% of palm sugar), D (70% of white sugar and 30% of palm sugar) and E (60% of white sugar and 40% of palm sugar).

Table 2. Chemical properties (n=3) of gac ice cream incorporated with palm sugar

Ice cream	Moisture (%)	Ash (%)	Crude fat (%)	Protein (%)	Crude fibre (%)	Carbohydrate (%)
A	52.45 ± 0.25^{b}	0.22 ± 0.02^{a}	10.78 ± 0.61^{a}	0.81 ± 0.09^{a}	5.25 ± 1.08^{c}	30.49 ± 1.20^{a}
B	53.00 ± 2.27^{ab}	0.22 ± 0.06^{a}	11.37 ± 1.31^{a}	0.87 ± 0.09^{a}	8.05 ± 1.93^{b}	26.49 ± 1.38^{ab}
C	54.11 ± 0.77^{ab}	0.21 ± 0.02^{a}	12.43 ± 0.56^{a}	0.97 ± 0.11^{a}	10.39 ± 0.35^{a}	21.91 ± 0.21^{bc}
D	55.25 ± 2.88^{ab}	0.21 ± 0.02^{a}	11.53 ± 2.32^{a}	0.95 ± 0.09^{a}	10.87 ± 0.37^{a}	21.19 ± 4.69^{c}
E	55.90 ± 1.17^{a}	0.22 ± 0.04^{a}	12.80 ± 2.00^{a}	0.87 ± 0.15^{a}	11.47 ± 0.38^{a}	18.74 ± 3.08^{c}

Mean value ± standard deviation with different superscript letters in the same column are significantly different ($p<0.05$). Gac ice cream prepared by A (100% of white sugar and 0% of palm sugar), B (90% of white sugar and 10% of palm sugar), C (80% of white sugar and 20% of palm sugar), D (70% of white sugar and 30% of palm sugar) and E (60% of white sugar and 40% of palm sugar).

The ash, fat, and protein content of gac ice cream were not affected by the addition of palm sugar (Table 2), while, as expected, moisture and crude fibre increased significantly with the increase in palm sugar. Fructose in palm sugar contains a high water holding capacity (Maryani et al., 2021) and leads to an increase in the moisture content of ice cream, similar to the finding in dark chocolate with the addition of palm sugar (Saputro et al., 2020). Inulin in palm sugar significantly contributed to increase the fibre content of ice cream, and this result is in good agreement with Sari Dewi et al. (2022) on cookies. There is no significant difference ($p>0.05$) in the sensory mean score for all attributes, and the range was 5.13–5.40, 4.53-5.07, 4.67–5.08, 4.97–5.27, 4.80–5.30, and 5.03-5.28 for colour, odour, sweetness, mouthfeel, flavour, and overall acceptance, respectively. This result shows that the addition of palm sugar up to 40% did not affect the sensory quality of ice cream.

CONCLUSIONS

The development of gac ice cream using mixture of white sugar and palm sugar has proven successful. The general acceptability of palm sugar ice cream was found to be highly acceptable until up to 40%. Colour, hardness, melting rate, overrun, moisture content, and crude fibre content are all affected by addition of palm sugar. Palm sugar increases the melting rate, overrun, moisture content and crude fibre content of gac ice cream while reduced the L* value, energy content and hardness. Hence, the current study suggests that palm sugar has excellent potential to be use in the dairy product as it increases the nutritional composition without compromising the sensory quality. It would be interesting to run the calorie value analysis using a bomb calorimeter to provide precise data in supporting the calorific value of palm sugar as an excellent alternative sweetener for ice cream, as well as the sugar content analysis using high-performance liquid chromatography (HPLC). The study of the stability of low-calorie gac ice cream during storage is also great to explore.

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INTENTION TO ADOPT HALAL CONCEPT: EVIDENCE FROM ONLINE FOOD DELIVERY MEATBALL RESTAURANTS

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Abstract: During the Covid-19 pandemic, the increasing public awareness of hygienic, safe, nutritious, and healthy, symbolized by halal and tayyib, influences consumer preferences in food selection. Producers'efforts are to adopt halal concepts into their business processes. However, how does it relate to meatball partners of online food delivery (OFD)? The aim is to analyze the impact of engagement, personal norms, perceived organizational readiness, and behavioural controls on meatball partners'intentions to adopt the Halal concepts. The study focused on Meatball Partner OFD in Yogyakarta and Soloraya. The approach used is both quantitative and qualitative. The study distributes questionnaires on the intention to apply the halal concept to the owner or halal supervisor through purposive sampling, followed by interviews to complete the quantitative results. The results indicate that religious commitment, halal awareness, personal characteristics, perceived behavioural control, subjective norms, and attitudes do not affect the intentions of meatball partners to apply the halal concept, contrary to the TPB theory in influencing the intention to perform a behaviour. On the contrary, the obligation of halal certificates and organizational readiness has a positive effect on the intentions of meatball partners to apply the halal concept.
***Keywords*:** halal concept, meatball, online food delivery, theory planned behavior

INTRODUCTION

Research by Guney dan Sangun (2020) reports that new behavioral and habit changes due to the pandemic are associated with fears of price increases, food hoarding, increased awareness of moderation, and reduced consumption, the risk of food waste, to the consumption of raw food. , natural fruits and vegetables. or organic food and healthy, nutritious food. This shift was triggered by media reports that food safety was the source of the spread of the Covid-19 virus, not from food, but from viruses that grow in raw materials. derived from wild animals are consumed directly, because they have not yet paid attention to food safety factors (Ceniti *et al*., 2021). While the symbol of clean, safe, nutritious and healthy food is halal food (Billah *et al*., 2020). Because of concerns about the impact of the pandemic, the public is increasingly aware of the need to consume and purchase halal foods (Hidayat *et al*., 2021). To meet these criteria, some local restaurants that sell halal food are adapting to keep their distance from consumers, protect their food products from direct contact with the outside air, Prioritize cleanliness, redirect transactions through online ordering and delivery using digital payments (Amri, 2020). Faced with commercial competition, each manufacturer strives to enhance consumer confidence in the products offered (Zakia et al., 2020). Having a halal certificate is one way to gain consumer trust. This study aimed to determine the intention of manufacturers to adopt the concept of halal certification as a step towards achieving halal certification in order to gain public recognition. If the meatball partners have halal status information in the food delivery service app, the service quality and sales will be improved and the built online food ordering system will be best (Indraswari dan Kusuma, 2018).

MATERIALS AND METHODS

This reseach, development of the theory of planned behavior is carried out on the treatment of independent variable data in questionair by using least squares structural equation modeling (PLS-SEM) to analyze the data. Many researchers recommend using PLS SEM as a good statistical path modeling tool to solve complex multivariate models (Hair *et al.*, 2014) and more flexible, powerful, and superior statistical tool for prediction and theory testing (Henseler *et al*. 2015).

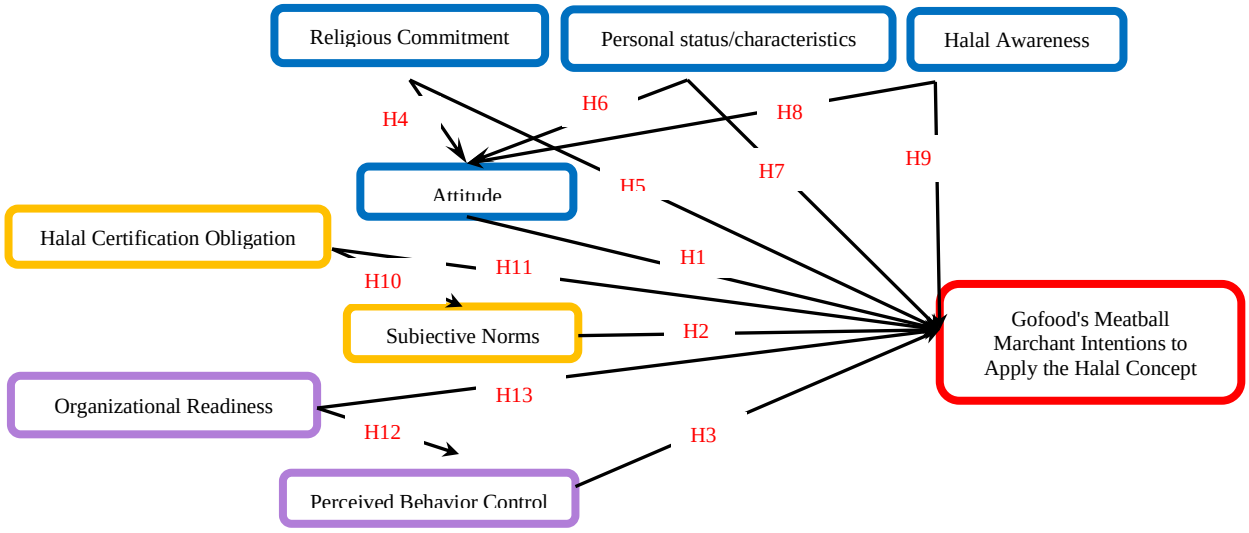

Figure 1 Research Model

RESULTS AND DISCUSSION

The criteria of the search model, after checking the load factor, a valid search model is obtained. The load factor is the value generated by each index to measure the structure. We remove the non-response flags to get an external load value that meets the requirement. If the structure is measured by the decrease indicator, the external load value will also change, because the value divider is distributed across all the indicators. The value of the load factor contributes to a clear explanation of the latent structure (Hair *et al.*, 2014).

Table 1 Hipothesis Test

Hipotesis	Relationships	Path coefficient	Standard Deviation (STDEV)	T Statistics (|O/STDEV|)	P Values	Supported
H5	KA -> NMKH	0,012	0,093	0,125	**0,901**	No
H4	KA -> SK	0,438	0,095	4,621	**0,000**	Yes
H9	KH -> NMKH	0,135	0,088	1,532	**0,126**	No
H8	KH -> SK	0,253	0,092	2,737	**0,006**	Yes
H12	KO -> KPR	0,488	0,110	4,452	**0,000**	Yes
H13	KO -> NMKH	0,262	0,105	2,500	**0,013**	Yes
H7	KP -> NMKH	0,032	0,101	0,315	**0,753**	No
H6	KP -> SK	0,111	0,084	1,334	**0,183**	No
H3	KPR -> NMKH	0,109	0,081	1,347	**0,179**	No
H11	KSH -> NMKH	0,405	0,105	3,867	**0,000**	Yes
H10	KSH -> NS	0,587	0,084	6,966	**0,000**	Yes
H2	NS -> NMKH	0,007	0,101	0,073	**0,942**	No
H1	SK -> NMKH	0,056	0,111	0,503	**0,615**	No

Religious commitment (KA) has a positive and significant effect on attitude (SK). Commit to living life as a follower of a religion, of course, following the rules and guidelines of life that shape attitudes and behaviors in everyday life. According to a study by Setyawati *et al.* (2020) who also found that religion plays an important role in implementing Halal practices in MSME and green marketing. This finding is also consistent with research findings on intention to use halal vaccines (Sudarsono *et al.*, 2023) and purchase of halal products (Al-Banna, 2019) unaffected by religious commitment. It turns out that at the level of performance intent, religion has no effect because there are other contexts that influence willingness to perform, such as administrative, physical, and funding readiness (Ikawati & Rahman, 2022). Halal awareness (KH) has a positive and significant effect on attitude (SK). Consistent with previous research on halal perception has a positive effect on perception of MSMEs implementing halal supply chain (Azmi *et al.*, 2018) dan halal logistic (Izzati Ab Ghani *et al.*, 2020) in Malaysia. Residents of a city are more exposed to information related to halal implementation through exhibitions, training and halal certification process (Nusran *et al.*, 2018). However, they have not yet applied for halal certification because of difficulties in meeting the existing requirements. Personal characteristics (KP) did not affect attitude (SK) and personal characteristics (KP) did not affect intention to adopt the halal concept of meatball partners. This result is consistent with the finding (Al-Nahdi *et al.*,

2009) that attitude does not affect intention to operate a halal restaurant. The personal trait evoked in this study is the leader's personality as a decision maker. The individual characteristics mentioned were related to age, education level, leadership attitude and motivation to run the business.

Organizational readiness (KO) has a positive and significant effect on perceived behavioral control (KPR). Organizational readiness (KO) had a positive and significant effect on the intention to adopt the halal concept of meatball partners. The challenge in implementing the halal concept for manufacturers is preparing for change (Mohamad Husny *et al.*, 2016), one of which is related to the administrative order. This is reflected in the sales reports are not well organized, still follow the traditional system. Cognitive behavioral control (KPR) did not affect the intention to perform halal concept (NMKH) of meatball partners. This differs from previous research on the consumer side (Aisyah et al., 2019) where perceived behavioral control has a significant influence on purchase intention and decision. Subjective norm (NS) did not affect the intention to adopt the halal concept (NMKH) of the meatball partner and the attitude (SK) did not affect the intention to adopt the halal concept (NMKH) of the meatball partner pellets. NS and SK have no effect on NMKH because although they already have knowledge and awareness about the implementation of the halal concept, the organizational capacity has not been able to modify it at the level of implementing the artwork. art. Because the intrinsic motivation of the producer plays a very important role in the implementation of the halal concept in the business process (Soltanian *et al.*, 2016). Similarly, the finding that attitude has no effect is consistent with previous research which suggested that risky attitudes do not affect the motivation of MSMEs to become halal entrepreneurs. Contrary to the findings (Othman *et al.*, 2017) this attitude plays an important role in the Halal certification process. Claiming a Halal Certificate (KSH) has a positive and significant impact on Subjective Standards (NS) and Requesting a Halal Certificate (KSH) has a positive and significant impact on intention to implement the halal concept of meatball partners. Information about the Halal certification requirements is widely available from the government and supporting organizations. Therefore, a positive NS must be developed in society in the hope of creating a mutually supportive environment for this purpose. Consistent with previous research (Soltanian *et al.*, 2016) the government support factor has a positive effect on the increasing motivation of MSMEs to become entrepreneurs halal.

CONCLUSIONS AND RECOMMENDATIONS

Research results show that due to a theoretical decline in planned behavior, religious commitment, perception of halal, personal characteristics, perceived behavioral control, subjective norms and attitudes are not influence the intention to adopt the halal concept of meatball partners. Unlike the theory of planned behavior about attitudes, subjective norms, perceived behavioral control affects the intention to perform the behavior. On the other hand, the obligation for halal certification and organizational readiness had a positive influence on the intention to implement the halal concept of the meatball partners. Organizational willingness to play a positive role in controlling perceived behavior in adopting halal concepts and requirements for halal certification also had a positive effect on subjective norms, although the control subjective norms and behaviors do not affect the intention of the partner to apply halal concept. In terms of managerial implications, it can be suggested that the factors of religious commitment and halal awareness for entrepreneurs, need to be improved. Halal obligation and organizational readiness are two factors that have a strong influence on the intention to implement the halal concept of partners. Therefore, strengthening the organization becomes a source of capital for manufacturers, because fulfilling the obligation to issue halal certification is not the job of each person in the organization but the joint work of all members. part of the organization.

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Polygonum minus HUDS. AS A NATURAL-BASED SOLUTION FOR OBESITY MANAGEMENT: *IN VITRO* AND *IN VIVO* EVALUATION

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Abstract: Obesity is a detrimental condition that promotes the onset of non-communicable diseases. Current pharmaceutical interventions to treat obesity had side effects and limitations. *Polygonum minus* is a plant genus that has been used in traditional medicine due to its phytomedicinal metabolites content. The variation between different growth stages (8, 10 and 12 weeks) was studied using proton nuclear magnetic resonance (^{1}H-NMR) combined with multivariate data analysis (MVDA). The total phenolic content (TPC), pancreatic lipase (PL), α-glucosidase (AG) inhibitory activities, and DPPH radical scavenging activity of *P. minus* aerial extracts (PM) were also evaluated and correlated with their phytochemical constituents. PM at 12 weeks (12w) exhibited the highest ferric-reducing antioxidant power and enzyme inhibition activities against PL and AG. Metabolomics analysis identified nine compounds correlated with PM bioactivities at 12w, including glutamine, quercetin, isorhamnetin, quercetin 3-*O*-rhamnoside, rutin, myricetin derivatives, α-glucose, β-glucose, and ascorbic acid. Additionally, we evaluated the *in vivo* anti-obesity effects of PM in Sprague Dawley (SD) rats fed a high fat diet (HFD). Treatment with PM (200 and 400 mg/kg) attenuated HFD-induced body weight gain in rats by 24.83-44.20% compared to HFD control group, with the 200 mg/kg group showing lower fasting blood glucose level ($p<0.05$). Furthermore, the faecal fat content and serum lipid profile improved towards the positive control group. The acute toxicity study showed that a single oral administration of 2000 mg/kg of PM produced no mortality and signs of abnormalities observed during the 14-day clinical observations in female SD) rats. These findings highlight the potential of 12w PM as a safe natural-based solution in obesity management.

Keywords: Obesity prevention, Enzyme inhibition, High fat diet, Kesum, Plant metabolomics

INTRODUCTION

Obesity is now regarded as an epidemic, with over 1 billion including adults, adolescents, and children suffering from excessive body fat accumulation. There has been a significant surge in the economic burden associated with obesity-related complications with the increasing prevalence of obesity (Spanggaard et al., 2022). The escalating treatment expenses have put a significant strain on both patients and society, prompting the development of cost-effective treatment methods. Medicinal plants have emerged as one of the potential fields of study for research and development in obesity management, owing to their promising pharmacological properties and valuable metabolite compositions. Plant polyphenols are known for their antioxidant properties, which play significant role in disease prevention and treatments (Margină et al., 2020). Characterising bioactive compounds from medicinal plants is a challenging process due to the complex composition of plants (Salem et al., 2020). Additionally, there are various factors that can influence the metabolites composition of plants, including the harvesting age. Understanding the factors that affect metabolite compositions based on the agricultural practice, specifically harvesting ages, is highly necessary to ensure sustainability and maintain a high-quality standard of raw materials and final products with the most optimal medicinal properties. In Malaysia, *P. minus* is one of the commonly used herbs in the preparation of various national cuisine. This plant has been reported to exhibit a wide range of phytochemicals, including flavonoids, phenolic acids, and amino acids (Hussin et al., 2019). However, the knowledge of metabolite composition and bioactivities of *P. minus* at different harvesting ages is currently lacking, particularly in the aspect of obesity management. The present study aims

to assess the *in vitro* antioxidant and enzyme inhibition activities (pancreatic lipase and α-glucosidase) of *P. minus* at different harvesting ages (8, 10, and 12 weeks). This study also aims to profile the chemical constituents of *P. minus* and correlate with the biological activities using ^{1}H NMR-based metabolomics. Finally, the effect of *P. minus* in obesity prevention was assessed in Sprague Dawley rats fed with a high fat diet.

MATERIALS AND METHODS

Plant materials and extraction: *P. minus* was planted using a stem-cutting propagation technique at Kompleks Rumah Tumbuhan, Universiti Kebangsaan Malaysia, Bangi. The aerial parts of *P. minus* were harvested in six replicates after 8, 10, and 12 weeks. The sample was cleaned before being dried using a freeze dryer (Freeze Dryer Alpha 1-2 LD Plus, Germany) to a constant weight. *P. minus* extracts were produced based on method by Rahman et al., (2017).

Chemical profile and bioactivities of *P. minus* at different harvesting ages

Total phenolic content (TPC): The TPC was determined using the Folin Ciocalteu (FC) method in accordance with Wan Nasir et al., (2021) with some modifications.

Antioxidant assays: The DPPH free radical scavenging activity was determined, and ferric reducing antioxidant assay (FRAP) assay was carried out according to Ain Ibrahim et al., (2023).

Pancreatic lipase and α-glucosidase inhibition assay: Inhibition of pancreatic lipase by plant extracts was determined using a method modified from Roh & Jung (2012). The α-glucosidase inhibitory activity was determined using a method adapted from Wan Nasir et al., (2021) with some modifications.

^{1}H NMR analysis: ^{1}H NMR analysis was carried out according to method described by Ain Ibrahim et al., (2023). Two-dimensional (2D) J-resolved ^{1}H NMR was used to assist with the assignment and confirmation of compounds.

***In vivo* anti-obesity evaluation**

Animal model: Male Sprague Dawley rats (8-10 weeks), weighing 200-250g were obtained from Animal House, Universiti Kebangsaan Malaysia. The rats were housed in individual cages (430 x 290 x 201 mm), with a stainless-steel top. Prior to treatment, the rats were acclimatised for ten days at room temperature (26-28°C) with a relative humidity environment of 55-60% and 12-hour dark/12-hour light cycle. The rats had free access to normal rat chow (Specialty Feeds, Australia) and water. The animal procedures received approval from the UKM Animal Ethical Committee (UKMAEC) at Universiti Kebangsaan Malaysia (Approval No. UKM.PPI.AEC.800-4/3/1).

Experimental design: Rats were divided into five groups (n=5): (1) Normal Diet (ND), (2) High Fat Diet (HFD), (3) High Fat Diet and 200 mg/kg body weight of PM (HFD + 200 mg/kg PM), (4) High Fat Diet and 400 mg/kg body weight of PM (HFD + 400 mg/kg PM) and (5) High Fat Diet and 25 mg/kg body weight of orlistat (HFD + orlistat). Rats in the normal diet group were given a standard rat pellet (Specialty Feeds. Australia), whereas rats in group 2, 3, 4 and 5 were fed a high fat diet prepared from full cream milk powder (20%), sugar (20%), standard rat pellet (14%), corn starch (13%), wheat flour (13%), and ghee (10%). Faeces were collected during the fourth and eighth weeks for faecal fat content analysis. Fasting blood glucose was measured during the baseline (week 0) and final week (week 8) of study. After eight weeks of treatment, the rats were fasted overnight and sacrificed using carbon dioxide asphyxiation. The blood was withdrawn, and the serum samples were subjected to biochemistry analysis (lipid profile, liver, and kidney function biomarker tests). The acute toxicity study was conducted in accordance with the limit test of OECD guidelines (Test no: 425) for 14 days.

Statistical analysis: Data were analysed using Minitab 21.1.1. One-way ANOVA with Pos Hoc Tukey HSD test was conducted to compare the means across different harvesting ages (n=6). A significant difference is defined as $p<0.05$. SIMCA 14.1 software was used to run multivariate data analysis on the NMR binning data.

RESULTS AND DISCUSSION

Bioactivities and chemical profiling of *P. minus* at different harvesting ages.

The 12-week harvested PM demonstrated a significantly higher ($p<0.05$) TPC, FRAP score, and enzyme inhibitory activity against PL and AG compared to the 10-, and 8-week samples (Table 1). Additionally, both the 12- and 10-week harvested PM exhibited a significant difference ($p<0.05$) in the DPPH activity compared to the 8-week sample. Harvesting age can reflect the maturity of plants during harvest and it is one of the key factors in determining the quality and quantity of phytochemicals since biochemical, physiological, and structural changes occur during maturation (Adegbaju et al., 2020). Increased phenolic content with age as demonstrated in this study may be the result of their active production and accumulation in plant cells during plant development (Moradi et al., 2020). Antioxidant activity in plants is commonly attributed to the presence of plant polyphenols (Stagos 2020). Previous study also indicated that digestive enzyme inhibition effects of plant extracts may be attributed to their high concentration of polyphenolics and flavonoid constituents (Song et al., 2019). The findings of this study demonstrated that PM exhibits significantly higher bioactivities as the plant matures. Hence, it is proposed that the accumulation of

bioactive chemicals compounds in PM increases with maturity and harvesting age, leading to enhanced biological activities *in vitro*. The results of this study are consistent with a report by Nobossé et al. (2018) who noted that as the leaf extract of *Moringa L*. matured, their TPC and flavonoid content increases.

Table 1. Total phenolic content, antioxidant and enzyme inhibitory activities of *P. minus* at different harvesting ages.

Harvesting age	TPC (mg GAE/g extract)	FRAP (µmol TE/g extract)	Inhibitory activity, IC_{50} (µg/mL)		
			DPPH	Pancreatic lipase	α-glucosidase
8 weeks	265.90 ± 34.60^{b}	975.60 ± 45.30^{d}	7.64 ± 1.03^{a}	65.50 ± 5.38^{a}	1.98 ± 0.28^{a}
10 weeks	288.05 ± 20.61^{b}	1175.80 ± 74.20^{c}	5.90 ± 0.56^{b}	62.92 ± 6.43^{a}	1.85 ± 0.16^{a}
12 weeks	377.10 ± 28.40^{a}	1547.40 ± 58.30^{b}	6.13 ± 0.60^{b}	40.40 ± 5.15^{b}	1.29 ± 0.15^{b}
Ascorbic acid	NA	3174.80 ± 143.20^{a}	4.11 ± 0.14^{c}	NA	NA
Orlistat	NA	NA	NA	4.25 ± 0.14^{c}	NA
Quercetin	NA	NA	NA	NA	4.11 ± 0.14^{c}

All values are presented as the mean ± standard deviation (n=6), using one-way ANOVA in Minitab 21.1.1 software. Different lowercase letters indicate a statistically significant difference between samples in the same group ($p<0.05$). NA=Not available.

Based on ^{1}H NMR-based metabolomics, distinct metabolite patterns across different harvesting ages were observed, with the 12-week harvested sample clustering away from the other sample. The PLS regression biplot revealed a positive correlation between the 12-week harvested samples with DPPH radical scavenging activity and enzyme inhibitory activity (Figure 1). Nine metabolites, including glutamine, quercetin, isorhamnetin, quercetin 3-*O*-rhamnoside, rutin, α-glucose, β-glucose, myricetin derivatives and ascorbic acid were identified in the most active region (12 weeks), each having VIP scores of more than 0.7.

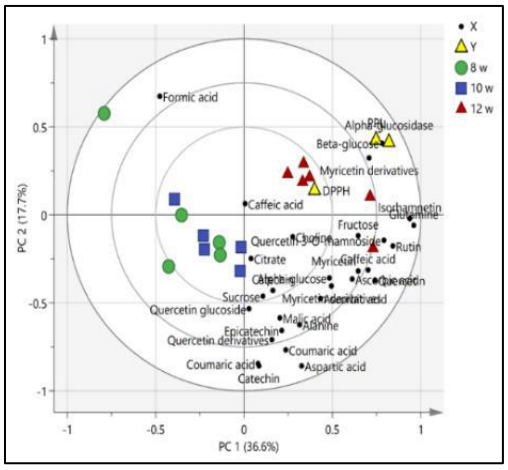

Figure 2. The PLS regression biplot illustrating the correlation between *P. minus* at different harvesting ages and bioactivities.

***In vivo* anti-obesity evaluation**

Administration of 12-week harvested PM for 8 weeks to Sprague Dawley rats fed a HFD prevented the body weight gain by 24.83–44.20% lower than the untreated HFD group, with significantly lower fasting blood glucose levels observed in the low dosage PM-treated group ($p<0.05$). There was an improving trend in the faecal fat content and serum lipid profile, however these changes were not statistically significant ($p>0.05$). Besides, although PM-treated group exhibit a slightly reduced abdominal fat percentage (3.91 and 4.52% in 200 mg/kg and 400 mg/kg PM group, respectively) compared to the negative control (4.62%), this outcome also did not achieve a statistical significance ($p>0.05$). Based on the faecal fat content, food and calorie intake, this suppression of body weight gain was not primarily attributed to *in vivo* gastrointestinal pancreatic lipase inhibition, or any significant changes in the food and calorie intake pattern. This implies that there may be additional factors contributing to the observed reduction in body weight gain, such as through the regulation of the intestinal or gut microbiota (Liu et al., 2022)(ref). Further research and a larger sample size may be required to improve the statistical power and assess the true impact of the intervention on this parameter in this study. Nevertheless, it is worth noting that even a small weight reduction, around 5% may help in alleviating the condition of existing obesity-related disorders (Fruh, 2017). Additionally, a single dose administration of 12 week-harvested PM at 2000 mg/kg dosage appears to not cause any mortality and show clinical signs of toxicity based on the observations made throughout the 14 days experimental period.

CONCLUSIONS:

The present study highlights the promising potential of 12-week harvested *P. minus* as a natural ingredient with antioxidant properties and obesity preventive effects through *in vitro* and *in vivo* assessments. The *in vitro* bioactivities of *P. minus* may be influenced by the harvesting ages, suggesting the need to consider this factor in agricultural practices and future research to ensure standardized and high-quality raw materials. Although *P. minus* exhibits *in vitro* enzyme inhibitory activity, this effect is not strongly evident in the in *vivo* study. The anti-obesity study on *P. minus* would benefit from a more comprehensive approach to pre-clinical evaluation using animal models with an obesity phenotype, to confirm its efficacy in treating obesity. Additionally, conducting metabolite fingerprinting analysis with serum, urine, and faeces may identify biomarkers and metabolic pathways related to the anti-obesity effects of *P. minus*.

ACKNOWLEDGEMENT: This study was financially supported by Geran Galakan Penyelidik Muda (GGPM 2019-067).

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COMPARATIVE ANALYSIS OF PROCESS INTENSIFICATION TECHNOLOGIES (PIT) FOR IMPROVED CELL DISRUPTION AND LIPID RECOVERY IN *Aurantiochytrium* sp. SW1 MICROALGAE

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Abstract: A locally isolated marine microalgae *Aurantiochytrium* sp. SW1 exhibits a high proportion of lipid. However, the recovery of high yields of intracellular lipid poses a challenge due to the relatively robust cell wall of microalgae, which necessitates energy-intensive cell-disruption. This study investigated the effect of different process intensification technologies (PIT), namely microwave treatment, autoclave treatment, and ultrasonication on the disruption of wet *Aurantiochytrium* sp. SW1 cells, facilitating the recovery of intracellular lipids. Effectiveness of cell disruption was assessed by measuring lipid content, suspension turbidity and size distribution. Additionally, scanning electron microscopy (SEM) was utilized to observe changes in cellular morphology following PIT treatments. The results showed that all three PIT methods led to a reduction in particle size as treatment time increased, suggesting successful disruption of cell walls and subsequent release of intracellular components. Ultrasonication demonstrated the highest effectiveness, particularly at 50 W power for 10 minutes, resulting in a lipid content of 67.76 ± 0.03 %. Microscopic analysis revealed ultrasonication- induced cell shrinkage and loss of rigidity, supporting its efficacy in facilitating solvent penetration through cell membranes for improved lipid recovery. This study highlights the potential of PIT techniques, as sustainable and energy-efficient strategies for lipid extraction from *Aurantiochytrium* sp. SW1 microalgae.

Keywords:: autoclave, cell disruption, intracellular lipid, microwave, ultrasonication

INTRODUCTION

Microalgae have diverse applications, including biofuels, nutraceuticals, and components for food and feed. They are primary eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) producers in marine food chain, offering a sustainable alternative source for polyunsaturated fatty acids (PUFAs). DHA and EPA have been reported to confer various health benefits, including maintaining proper retinal and neurological development, as well as defense against inflammatory, cardiovascular, and neurodegenerative illnesses, along with cancer (Jesionowska et al., 2023). Omega-3s may lower COVID-19 risk by enhancing macrophage virus clearance, reducing inflammation, and boosting anti-SARS-CoV-2 antibody production (Fadiyah et al., 2022).

Fish oil is a known omega-3 source, but sustainability concerns and pollutants drive interest in microalgae oil (Sun et al. 2018). *Aurantiochytrium* sp SW1, a marine Thraustochytrid, has been discovered to accumulate more than 50% (w/w biomass) of lipid comprising 50% DHA (of total fatty acids) (Manikan et al., 2015). However, handling cells that are only a few microns in size, the presence of robust cell walls surrounding the algal cells, and the prevalence of moisture that affects with extraction solvents make it challenging to extract lipids from algal cells. Therefore, process intensification technology (PIT) plays a crucial role in lipid extraction to reduce energy costs and minimizing downstream processing steps while maximizing yield (Jeevan Kumar et al., 2017). Wet *Aurantiochytrium* sp. biomass (moisture content 74-77%) was employed in this study in order to eradicate the need for drying processes. Three types of PIT were used: electromagnetic radiation by microwave, thermal treatment via autoclave, and mechanical process via ultrasonication. Cell disruption effectiveness was determined by measuring lipid content and particle size distribution (PSD). Scanning electron microscopy (SEM) was also used to evaluate changes in cellular morphology after PIT treatments.

MATERIALS AND METHODS

2.1 Culture condition and harvesting

Aurantiochytrium sp. SW1 (GenBank: KF500513) was provided by the Microbial Physiology Laboratory, Faculty Science and Technology, National University of Malaysia. The stock culture, seed culture and production culture was prepared based on Manikan et al., (2015). The cells were harvested by centrifugation at 6000 rpm for 10 min. The wet microalgae biomass with moisture content 74 -77 % approximately was used in this study.

2.3 Process Intensification Technologies (PIT)

Three types of PIT treatment were used: microwave, thermal treatment and ultrasonication. Microalgae (0.5 ± 0.1 g) was suspended in 30 ml of distilled water. The sample suspension was treated in microwave oven (SHARP R213CST) for 30, 60, 90 and 120 seconds at 800 W and 2.45 GHz. For thermal treatment, the sample suspension were autoclaved at 121 ºC and 108 kPa for 10, 20 and 30 minutes. Ultrasonication (50W, 25 KHz) was done using a probe sonicator (TFT Lab Sonicator) for 5, 10, and 15 minutes with 30-second breaks between each minute, with samples in an ice bath to avoid overheating.

2.4 Particle Size Distribution (PSD) and Cellular Morphology Observation

A laser scattering particle size distribution analyzer (HORIBA LA-960) and refractive index of 1.46 was used to determined the PSD of disrupted microalgae suspensions (Rivera et al., 2018). The cellular morphology of microalgae were observed using scanning electron microscopy (SEM) (ZEISS VP-SEM LEO 1450VP). The samples were freeze dried before observed using SEM. The applied voltage value is set at 10 kV.

2.5 Lipid Extraction Efficiency

Lipid extraction was carried by using the chloroform/methanol method (2:1, v/v) as described by Folch, Lees, and Stanley (1957) with slightly modification depends on type of PIT. Wet microalgae (5 g) was used for this test. The solution were left for overnight before added with 0.9% NaCl. The bottom phase which comprised of lipid dissolved in chloroform was recovered and dried using rotary evaporator. The oil was weighed and the lipid content (% w/w) was calculated using Equation (1):

$$\text{Lipid content (\%w/w biomass)} = \frac{Weight\ of\ oil\ extracted\ (g)}{Weight\ of\ biomass\ (g)} \times 100 \quad \textbf{(1)}$$

2.6 Statistical Analysis

All analyses were carried out in triplicate. Minitab version 19.0 statistical software (Minitab Pty Ltd., Sydney, Australia) was used to analyze the data and find significant differences in the mean ($p \leq 0.05$) using one-way ANOVA and Fischer's multiple range test.

RESULTS AND DISCUSSION

3.1 Particle Size Distribution (PSD)

Figure 1 show the PSD of untreated *Aurantiochytrium* sp. SW1 was right-skewed unimodal, with a median particle size (D_{50}) = 8.278 µm, indicating that 50% of the particles were ≤ 8.278 µm. The majority of particles in the untreated sample are 8.826 µm in size, accounting for 12% of the total particle volume. According to Manikan et al., (2015) the vegetative cells of *Aurantiochytrium* sp. SW1 were sphere-shaped and ranged in size from 8 to 14 µm in diameter. Figure 1 also show that the untreated sample's right-skewed distribution indicates that the sample contains more coarse particles. Following ultrasonication, the distribution became bimodal, with two population ranges of 0.1 - 0.4 µm and 2-15 µm and the sonicated sample has smaller size particles compared to untreated as shown in the distribution move further from untreated sample. The bimodal distribution formation from the data reveal that the proportion of smaller particles increases following ultrasonication. The most abundant size particle observed after 5 and 10 minutes of treatment is 6.720 µm (which accounted for 19% and 18% of the total volume of particle after 5 and 10 minutes, respectively). The appearance of an elevated amount of particles smaller than the size of a single cell indicates the presence of cell debris or other cell components released by the force given (Bauer et al., 2023).

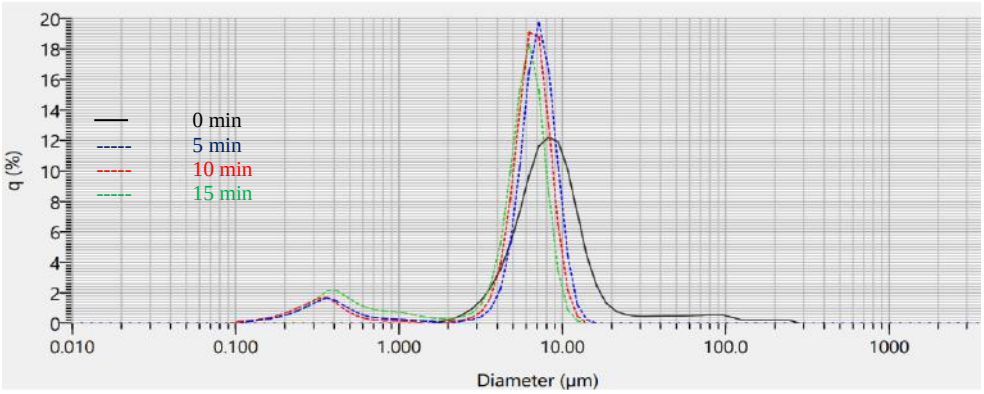

Fig. 1 Volume particle size distribution of samples *Aurantiochytrium* sp. SW1 suspensions treated ultrasonication. q (%): volume percentage of the corresponding particles relative to the total volume of measured particles.

3.3 Cellular Morphology Observation

Based on Figure 2, the findings reveal that raw *Aurantiochytrium* sp. SW 1 has a rough sphere surface, the cell surface is still intact, and the cell membrane is not damaged. The cells' natural form collapsed after ultrasonication. The intact round cells were lost, and the majority of the cells shrank. External shear stress caused by cavitation during ultrasonication treatment may develop interspaces, holes, and microfractures in cells, resulting in shrinkage (Zhang et al., 2019).

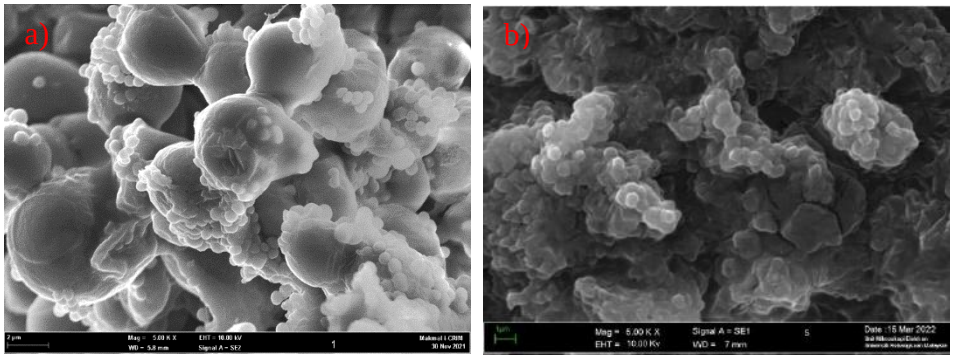

Fig 2. Representative scanning electron microscopy images of *Aurantiochytrium* sp. SW1 a) untreated suspension b) treated with ultrasonication for 5 min

***3.4** Lipid Extraction Efficiency*

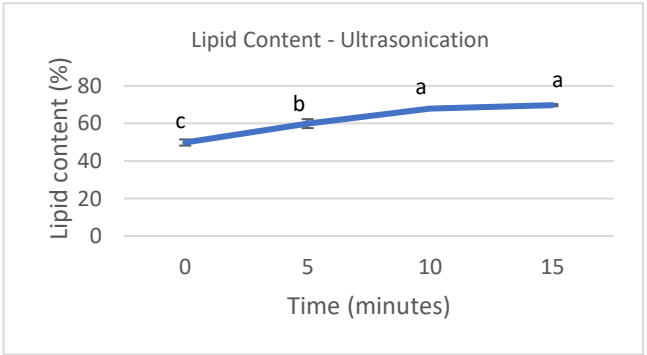

Fig 3. Lipid content of *Aurantiochytrium* sp. SW1 suspensions treated with microwave, thermal and ultrasonication at different time. [a–c] Means with different alphabets are significantly different at $p \leq 0.05$.

According to previous study, *Aurantiochytrium* sp.SW1 can accumulate more than 50% lipid (Manikan et al., 2015; Prabhakaran et al., 2023). Lipid content of untreated sample (0 mins) is 50.61 ± 1.49%. Figure 3 demonstrates that the

lipid content of *Aurantiochytrium* sp. SW1 increased considerably after being exposed to ultrasonication treatment. Lipid content at 10 and 15 mins treatment did not show significant difference, showed that ultrasonication for 10 minutes was sufficient. At 10 minutes of ultrasonic treatment, the lipid content was 67.76 ± 0.03%. Ultrasonication has also been proven to be effective in assisting with the extraction of lipids from various species, including *Chlorella* sp., *Nostoc* sp and *Tolypothrix* sp. (Prabakaran & Ravindran, 2011).

CONCLUSION

The study highlights *Aurantiochytrium* sp. SW1 as a promising alternative of oil rich in beneficial omega-3 PUFAs. Process intensification technologies (PIT) play an important role in minimizing downstream processing in microalgae lipid extraction. From this study, ultrasonication demonstrated the highest effectiveness, particularly at 50 W power for 10 minutes, resulting in a lipid content of 67.76 ± 0.03 %.

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RESPONSE SURFACE OPTIMIZATION OF POLYTETRAFLUOROETHYLENE (PTFE) COATING ON DRYING CHAMBER WALL OF SPRAY DRYER

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Abstract: This study was carried out by response surface methodology (RSM) along with central composite rotatable design (CCRD) to optimize the polytetrafluoroethylene (PTFE) coating on dryer wall of spray dryer in order to minimize the stickiness problem during spray drying process. The concentration and exposure time of PTFE were examined as independent variables while surface free energy (SFE) was evaluated as response. Statistical analysis showed that regression coefficient values greater than 0.75 for the response which shows a good fit. The optimum conditions were discovered at PTFE concentration of 8.86% and PTFE exposure time of 6 minutes. These conditions would result in low SFE. The optimum parameters were applied into spray dryer system and it enhances the product recovery of the powder. This study showed that PTFE application was effectively optimized to enhance the efficiency of spray drying process.
Keywords: Response surface methodology, stickiness, polytetrafluoroethylene, spray drying

INTRODUCTION

Spray drying is a widely used technique in various industries, including food, pharmaceutical and manufacturing. The process of spray drying involves introducing liquid raw materials into a drying chamber where the droplets come into contact with a drying gas, leading to the evaporation of the solvent and the subsequent transformation of the liquid into powder form (Dantas et al., 2018). Spray drying is a well-established drying method that still holds considerable promise for ongoing development (Bellinghausen, 2018).

Stickiness plays a vital role in food products, offering both advantageous and disadvantageous effects for manufacturers and consumers. For spray drying, stickiness can be a common problem that affects the process and the quality of the final product. Food products especially juices with high levels of low-molecular-weight sugars like sucrose, glucose, and fructose can lead to issues related to stickiness when the juices are subjected to spray drying (Farías-Cervantes et al., 2020).

There are many approaches for mitigating the issue of stickiness encompass both process-related methods (low humidity air) and material science-oriented methods (use drying aids) (Jayasundera et al., 2009). But these approaches have some drawbacks. An innovative approach can be implemented for addressing the stickiness issue by altering the surface properties of the spray dryer's drying chamber, utilizing PTFE which has unique attributes such as chemical inertness, and mechanical strength (Dhanumalayan & Joshi, 2018).

This study aims to decrease the occurrence of powder stickiness and improve the effectiveness of the spray dryer by augmenting product recovery. This is achieved through a chemical surface treatment applied to the glass surfaces of the drying chamber using PTFE solution. The RSM was utilized to determine optimal parameters for PTFE application. The parameters were PTFE concentration and PTFE exposure time while the response was SFE. Then, the product recovery was examined using the optimized parameters of PTFE by spray drying of sucrose-maltodextrin solution.

MATERIALS AND METHODS

Sample preparation

A dispersion of PTFE (Sigma-Aldrich, USA) was appropriately diluted to achieve the desired concentration. Borosilicate glass microscope slides (Labchem Sdn. Bhd., Malaysia) were used as substrates to mimic the drying chamber wall of spray dryer. Following the surface treatment process based on (Wang et al., 2012) with slightly modification, a dipping technique was applied to coat the borosilicate glass at room temperature based on experimental design generated from RSM (Table 1). The treated substrates were then cured on the substrate surface and the carrying solution evaporated by placing them in a 180°C oven dryer. These treated substrates were referred as PTFE-coated plates.

Surface free energy evaluation

Using the approach outlined by (M. Pelagade et al., 2012), the surface free energy (γ_s) of the sample, along with its polar (γ_s^p) and dispersion (γ_s^d) components, were evaluated using two different sets of contact angles (water and glycerin). This calculation was performed using the equation (1) known as the Owens-Wendt-Kaelble equation. The SFE values for the test liquids are as follows: dispersive component (γ_l^d), polar component (γ_l^p) and total surface energy (γ_l) for glycerin are 33.6°, 29.7° and 63.4°, respectively; dispersive component (γ_l^d), polar component (γ_l^p) and total surface energy (γ_l) for water are 21.8°, 51.0° and 72.8°, respectively.

$$\sqrt{\gamma_s^d \gamma_l^d} + \sqrt{\gamma_s^p \gamma_l^p} = \frac{1}{2}\gamma_l (1 + \cos\theta) \quad (1)$$

γ_l= total SFE,

γ_l^p= polar component of the SFE of the liquid,

γ_l^d= dispersion component of the SFE of the liquid.

Spray drying process

The sucrose-maltodextrin solution was introduced into a laboratory-scale spray dryer (Buchi Mini Spray Dryer B-290, Büchi, Switzerland) based on method by (Abidin et al., 2019) with a slightly modification. For effective separation and retrieval of the product, a cyclone air separator/powder recovery system was utilized. The drying chamber were treated with optimum PTFE coating. The spray dryer was designed in a way that the resulting outlet and inlet air temperatures were determined by a combination of factors, including the inlet temperature, aspirator setting and pump setting.

Product recovery

The assessment of product recovery for the spray-dried liquid samples was conducted following the method by (Ramlan et al., 2017), with minor adjustments. The product recovery was determined after the spray drying process by calculating it using equation (2).

$$\text{Product recovery (\%)} = (\text{mass of powder (g)}/\text{solid mass of feed solution (g)}) \times 100\% \quad (2)$$

Statistical analysis

The experimental design and statistical analysis were applied using RSM with CCRD via Design Expert version 13.0 software (Stat Ease, 2021, USA). To find the optimum parameters, the range for PTFE concentration and exposure time are fixed. A set of criteria is established to either maximize or minimize the desired outcome. The software then generates an optimum point that indicates the suggesting PTFE concentration and exposure time, along with a predictive value. Subsequently, experiments are conducted to confirm the effectiveness of the optimized value.

RESULTS AND DISCUSSION

The RSM was employed to assess the effect of two parameters which is PTFE concentration and PTFE exposure time on the surface properties of the PTFE-coated plate in terms of SFE. The collected experimental data were subjected to software analysis, and the outcomes regarding the response are presented in Table 1.

Table 2 presents the analysis of variance (ANOVA) results. Based on the findings, the model was significant. The coefficient of determination (R^2) exceeded 0.75, signifying a good fit. The insignificance of lack-of-fit test confirmed the model accurately fits the experimental data. Besides the findings indicated significance ($p < 0.05$) for both the PTFE concentration and its squared term. A significant p-value was also observed for the interaction between PTFE concentration and PTFE exposure time. However, the PTFE exposure time and its squared term showed p-value of 0.5144 and 0,9376, respectively, which indicates that there is no significant effect to the response.

The response was most influenced by the PTFE concentration, as indicated by the highest absolute coefficient value of 14.52. The coefficient was in negative value, showing that an increase in the variable led to a decrease in the SFE value. The equation describing the response surface used to fit the data of SFE based on quadratic models, is shown in equation (3).

$$Y = 7.6078 - 14.5215A - 0.377011B + 2.0295AB + 11.2797A^2 - 0.0477759B^2 \quad (3)$$

Y indicates the SFE, with A and B denoting PTFE concentration and PTFE exposure time, respectively. This regression coefficient model (mathematical equation) enables the computation and assessment of how factors impact the SFE on the PTFE-coated plate.

Table 1: Actual and coded (in parentheses) values of the variables used to design the experiment and the response values for the SFE test.

Run	PTFE concentration, %	PTFE exposure time, minutes	SFE, mN/m
1	12.00 (0.000)	18.00 (0.000)	8.34
2	4.00 (-1.000)	6.00 (-1.000)	35.64
3	12.00 (0.000)	18.00 (0.000)	7.13
4	12.00 (0.000)	18.00 (0.000)	6.49
5	20.00 (1.000)	6.00 (-1.000)	4.49
6	20.00 (1.000)	30.00 (1.000)	7.13
7	12.00 (0.000)	18.00 (0.000)	6.63
8	12.00 (0.000)	18.00 (0.000)	9.44
9	0.69 (-1.414)	18.00 (0.000)	51.56
10	4.00 (-1.000)	30.00 (1.000)	30.17
11	12.00 (0.000)	1.03 (-1.414)	7.06
12	12.00 (0.000)	34.97 (1.414)	6.93
13	23.31 (1.414)	18.00 (0.000)	7.74

Table 2: Regression model for SFE.

Term	Coefficient	F-value	p-value
A-PTFE concentration	-14.52	699.36	< 0.0001
B-PTFE exposure time	-0.3770	0.4714	0.5144
AB	2.03	6.83	0.0347
A^2	11.28	366.92	< 0.0001
B^2	-0.0478	0.0066	0.9376
Model significance: <0.0001 (Significant)	R^2: 0.9936 R^2(adj): 0.9890	Lack of fit: 0.2271 (Not significant)	

Surface plot was used to visually representing the influence of PTFE concentration and PTFE exposure time on the SFE of the PTFE-coated plates (Figure 1). According to the plot, an elevation in PTFE concentration corresponded to a reduction in SFE. This trend aligns with the findings of previous studies (Lee et al., 2022), which point out that increased PTFE content leads to lower SFE.

The analysis revealed that the optimum PTFE coating conditions could be achieved by applying PTFE concentration of 8.86% and PTFE exposure time of 6 minutes. Through the optimization process, the predicted SFE was 16.17 mN/m, along with a desirability value of 0.806. To assess the validity of the optimum point, the actual values obtained from experimental runs were compared. It was observed that the experimental response values closely matched the predicted values, with no significant difference found ($p > 0.05$). This suggests that the applied model was appropriate for the optimization of the PTFE coating process.

The findings indicated an increase in product recovery through spray drying after the application of PTFE solution to the surface of drying chamber, compared to before the treatment. The yield post-treatment reached 49.49%, surpassing the 34.73% yield prior to treatment ($p < 0.05$). This signifies that the chemical utilized for surface treatment resulting in low surface energy and effectively enhanced the hydrophobicity of the glass (Mondal et al., 2022), thereby mitigating the issue of powder stickiness during the spray drying process.

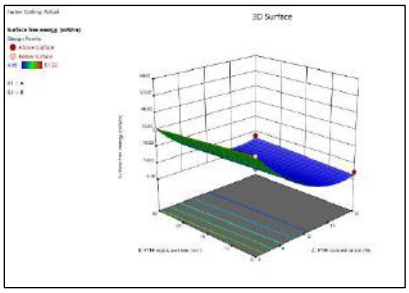

Figure 1: Surface plot of PTFE concentration vs. PTFE exposure time on SFE.

CONCLUSION Thirteen experimental runs, as determined by the RSM-CCRD methodology, were employed to explore how PTFE coating influenced SFE of glass surface, across varying levels of PTFE concentration and PTFE exposure time. PTFE concentration and its squared term were significant ($p<0.05$) to the response. Optimum condition for the PTFE coating were established. A PTFE concentration of 8.86% and PTFE exposure time of 6 minutes were shown to be the most optimum conditions for PTFE coating and it can be applied to enhance the product recovery during spray drying processes.

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FISH OIL EXTRACTION VIA ENZYMATIC PROTEOLYSIS OF JADE PERCH FISH VISCERA

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Abstract: Fish oil rich in polyunsaturated fatty acids (PUFAs) has been widely used as supplement due to its health benefits. *Jade Perch*, a fatty fish originally from Australia, has recently made significant strides in aquaculture industry in Malaysia. This work focuses on optimizing the extraction of fish oil from *Jade Perch* viscera. The viscera were then hydrolyzed using Alcalase® enzyme (2.4 AU-A/g) to facilitate oil extraction. The proteolysis parameters varied were pH, Alcalase concentration and temperature. By employing Response Surface Methodology (RSM), the study determined the optimal conditions for extraction as follows: pH 7.37, Alcalase concentration of 2.72%, and a temperature of 57.22°C. Under these conditions, a remarkable oil yield of 90.81% was achieved, showcasing the efficiency of the extraction process. These findings highlight the promising potential of *Jade Perch* viscera as a rich source of fish oil. Furthermore, the validation of RSM model demonstrated excellent agreement between the experimental and predicted results, confirming the reliability of the optimization approach. In conclusion, this study showcases the optimization of fish oil extraction from *Jade Perch* viscera, unlocking the potential of this natural health treasure.
Keywords: Viscera, enzymatic hydrolysis, Jade Perch

INTRODUCTION

Jade Perch (*Scortum Barcoo*) is a fast-growing freshwater fish from Australia, gaining popularity in the aquaculture industry over the past decade (Gang et al., 2010). Its flesh is known for its delicious taste, abundance of flesh with few bones, and high nutritional value. Recently, Jade Perch has become a favorite in Asian markets, especially among the Chinese community, driving increased breeding in aquaculture systems to meet demand (Wim et al., 2011; Liu et al., 2013).

Fish oil is a source that contain high omega-3 polyunsaturated fatty acid (PUFAs) which mainly composed of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which getting lot of attention from the world due to the positive feedback as the source of nutrients for the human health (Kosasih et al. 2019). Omega-3 PUFAs are associated with numerous health benefits, including the prevention of various diseases like hypertension, cardiovascular disease, arthritis, and cancer, as well as supporting neuronal and eye health (Wall et al., 2010). Preliminary studies have revealed that Jade Perch contains a high concentration of omega-3 fish oil compared to other species, making it a promising alternative source of these beneficial fatty acids (Izzati et al., 2018; Iberahim et al., 2020).

Conventional fish oil extraction methods involve high temperatures or pressures, leading to potential loss or degradation of thermally unstable components (Mbatia et al., 2010; Bonilla-Mendez et al., 2018; Eshari et al., 2022). Enzymatic hydrolysis extraction, on the other hand, offers a more environmentally friendly and efficient approach. It utilizes enzymes to facilitate tissue disruption and lipid release, making it safer and more ecologically friendly for effective oil extraction (Van, 2016; Hu et al., 2022). Factors like substrates, neutral protease activity, temperature, pH, and enzyme concentration play crucial roles in enzymatic fish oil extraction from Jade Perch viscera. Researchers aim to optimize the enzymatic hydrolysis process and enhance neutral protease activity to increase fish oil yield.

Response surface methodology (RSM) was a statistical method for the modeling and optimization of numerous variables that identified the ideal process conditions using sequential testing and first- or second-order polynomial equations (Amruth et al. 2018;). According to Linder et al. (2005), the method had already been used to optimize the enzymatic hydrolysis of a number of substrates, including fish by-products.In this study, the enzymatic hydrolysis of *Jade perch* fish viscera was investigated using experimental design of a response surface methodology (RSM) of central composite rotatable design (CCRD) as statistical problem-solving techniques.

MATERIALS AND METHODS

Chemicals and raw materials

Jade Perch was purchased from Aqua Narque Sdn. Bhd. ponds located at Seremban 2, Negeri Sembilan. The fishes were freshly caught on the same day of purchased, carried in a polystyrene box filled with ice and processed (cleaned, degutted and viscera removed) immediately upon arriving at the laboratory. Alcalase® 2.4 L FG (2.4 AU-A/g of enzyme activity) protease from *Bacillus licheniformis* was purchased from Novozyzymes. All reagents used were analytical grade.

2.1 Sample Preparations

Jade Perch viscera was kept in freezer (-18^{o}C) at least overnight and blended using the blender (*Grindomix-GM 300*) to reduce the size. The blended viscera were freeze dried for 48 hours (-47^{o}C under vacuum less than 1 bar). The sample were stored in freezer (-20^{o}C) before used (Qi-Yuan et al.2016; Kosasih et al. 2021).

2.2 Enzymatic Hydrolysis Extraction

Jade Perch fish viscera (10g) was weighed into 250 mL Erlenmeyer conical flask and heated for 5 minutes at 95^{o}C to activate the enzyme activity. Next, 100 mL of potassium phosphate buffer was added, and the pH was adjusted using either 0.5 M hydrochloric acid (HCl) or 0.5 M sodium hydroxide (NaOH). The mixture was then incubated for 2 hours in a water bath shaker at the specified conditions (pH, Alcalase concentration, and temperature from the RSM). Alcalase 2.4 L enzyme was added at the desired concentration to initiate the reaction. After the enzymatic hydrolysis process, the enzyme was deactivated by heating at 95°C for 5 minutes. The mixture was then centrifuged for 30 minutes at 5000 rpm, separating it into four layers: oil, emulsion, protein hydrolysate, and sediment. The top oil layer was carefully pipetted out and stored in the freezer at -4°C to prevent oxidation (Qi-Yuan et al., 2016; Kosasih et al., 2022).

2.3 Central Composite Rotatable Design (CCRD) in Response Surface Methodology (RSM) for optimization

The central composite rotatable design (CCRD) was used to optimize the enzymatic fish oil extraction from the viscera of *Jade Perch* by response surface methodology (RSM). The three independent variables chosen were pH, concentration of Alcalse (%) and temperature (^{o}C), as shown in Table 1 below. The range of each independent variables were determined according to the results from preliminary study. The percentage of oil yield (%) is the dependent variable. The central composite design contained 20 treatments including 23 factorial points, six axial points (α=1.68) and six replications of the central points.

Table 1. Independent variables, three factors with five levels central composite rotatable design

Independent Variables	**Symbol**	**Codel level**				
		-1.68 (-α)	-1	0	1	1.68 (α)
pH	X_1	5.82	6.50	7.50	8.50	9.18
Concentration of Alcalase (%)	X_2	0.82	1.50	2.50	3.50	4.18
Temperature (^{o}C)	X_3	38.18	45.00	55.00	65.00	71.82

Statistical analysis

The response surface methodology (RSM) was statistically analyzed using the Design Expert version 7.1.5 software (Stat-ease Inc., Minneapolis, Minn., U.S.A.). The multiple regressions analysis was done by involving the quadratic and interaction effects to develop a quadratic polynomial equation. A second order polynomial equation is assumed to describe the estimated behavioral model of both dependent variables. Equation 1 below is the second order polynomial equation to estimate the behavioral model.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11}\beta_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad \textbf{(1)}$$

Where Y depict the estimated dependent variable (oil yield, %); β_0 is the constant term for β_i, β_j, β_{ij} which showed the linear, quadratic and interaction terms as shown.

The R^2 and lack of fit value were determined from the ANOVA table in RSM. After the data analysis, the optimum conditions for fish oil extraction with the highest yield was obtained in the desirability function used (numerical). Then, the response surface plots were analyzed to describe the relationship of the two independent variables while another one independent variable is kept at optimal value (fixed).

RESULTS AND DISCUSSION

Optimization of enzymatic hydrolysis for Jade Perch viscera fish oil extraction

The combined effect of three factors (pH, concentration of Alcalase, and temperature) on the responses, fish oil yield (%) was investigated using a central composite rotatable design (CCRD) in response surface methodology (RSM). Table 2 shows the predicted and experimental results of the 3-factor, 5-level central composite design.

The determining coefficient (R^2) was 0.9974 (data from ANOVA in RSM), indicating that this quadratic model accurately represented the genuine relationships between the hydrolysis parameters. As demonstrated in Table 2, the model's validity is supported in the current study using analysis of variance (ANOVA). The quadratic polynomial model equation's statistical significance was evaluated using ANOVA. At a 95 percent confidence level ($p<0.05$), the model was found to be significant which means that the quadratic model was significant from of the variance in the data. All of the linear model terms (X_1, X_2, X_3) were significant ($P<0.05$) in this scenario. X_1^2, X_2^2 and X_3^2 had significant effects ($P<0.05$) among the quadratic coefficients. As for the model interactions (X_1X_2, X_1X_3, and X_2X_3), the connections for those variables were significant effect ($p<0.05$) among the quadratic coefficients shown.

Table 2. The design of experiments and dependent variable values predicted and experimental results of the oil extraction optimization from *Jade Perch* viscera

Std order	Type	Code level of variable			Lipid Yield (%)	
		X_1	X_2 (%)	X_3 (°C)	Experimental	Predicted
1	Factorial	-1	-1	-1	68.36	68.14
2	Factorial	1	-1	-1	57.18	56.43
3	Factorial	-1	1	-1	89.24	89.60
4	Factorial	1	1	-1	68.43	68.59
5	Factorial	-1	-1	1	75.34	75.12
6	Factorial	1	-1	1	75.29	74.88
7	Factorial	-1	1	1	84.94	85.63
8	Factorial	1	1	1	75.92	76.09
9	Axial	-1.682	0	0	75.03	74.64
10	Axial	1.682	0	0	56.31	56.78
11	Axial	0	-1.682	0	66.64	67.56
12	Axial	0	1.682	0	87.47	86.62
13	Axial	0	0	-1.682	76.48	76.72
14	Axial	0	0	1.682	89.06	88.90
15	Center	0	0	0	90.76	89.92
16	Center	0	0	0	89.89	89.92
17	Center	0	0	0	90.81	89.92
18	Center	0	0	0	89.24	89.92
19	Center	0	0	0	89.83	89.92
20	Center	0	0	0	89.01	89.92

The model's fitness was assessed using the lack of fit test. The lack of fit test had a non-significant P-value ($P>0.05$), indicating that the model was appropriate for predicting the yield of oil from the *Jade Perch* viscera. The projected quadratic model for enzymatic extraction conditions of *Jade Perch* viscera was statistically valid, according to the analysis of variance. RSM came up with the following final response surface regression Equation 2 below.

$$Y = 89.92 - 5.31X_1 + 5.67X_2 + 3.62X_3 - 2.33X_1X_2 + 2.86X_1X_3 - 2.74X_2X_3 - 8.56X_1^2 - 4.54X_2^2 - 2.51X_3^2$$ **(2)**

where Y, X_1, X_2, and X_3 are oil yield (%), pH, concentration of Alcalase (%) and temperature (°C) each.

The fitted models were examined to confirm that the data provided a good approximation to the underlying system and that the least squares regression assumption was not broken (Zhou and Regenstein, 2004; Bhaskar et al. 2008; Qi-Yuan et al 2016; Kosasih et al. 2021).

CONCLUSION

The study highlights Jade Perch viscera as a promising source of fish oil rich in beneficial omega-3 PUFAs. Enzymatic hydrolysis conditions significantly impacted fish oil production and were represented by second-order polynomials. Optimal parameters for maximum fish oil yield (90.81%) were identified as 57.22°C temperature, pH 7.37, 2.72% Alcalase concentration, 1:10 buffer/sample ratio, 160 rpm agitation, and 120 minutes incubation. Model predictions closely matched experimental findings, validating the optimization approach.

Acknowledgement
We acknowledge financial support from National University of Malaysia with the Research Grant of University (GUP-2018-1111).

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THE APPLICATION OF DOUBLE EMULSION IN THE DEVELOPMENT OF LOW SALT CHILLI SAUCE

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Abstract: Overconsumption of salt from foods such as condiments has led to proactive action by policymakers and the industry to find the most suitable salt reduction strategy. Thus, this study was conducted to study the application of double emulsion for salt reduction strategy in sauce. The effect of salt encapsulation on the saltiness perception and the physicochemical properties of chilli sauce was determined. The encapsulated salt solution was prepared by using the two-step homogenization method producing double emulsions with salt solutions at different concentrations. The prepared emulsions containing salt were then formulated in the production of chilli sauce. Sensory analysis revealed that chilli sauces formulated with double emulsion were significantly ($p<0.05$) saltier than the sauces produced with single emulsion at all levels of salt concentrations. Physicochemical analyses showed a significantly higher viscosity value ($p<0.05$) for chilli sauce samples formulated with double emulsion as compared to single emulsion samples. In addition, a significant effect ($p<0.05$) in ash and crude fat was also observed for double emulsion-formulated samples while no significant effect was observed in terms of fibre and protein content. This study reveals the potential application of double emulsion for salt reduction strategy in food products that can be beneficial for the food industry.

***Keywords*:** Chilli sauce, salt reduction, double emulsion, low-salt foods, sensory perception

INTRODUCTION

The overconsumption of sodium has been a source of concern in recent years (Dunteman et al., 2021). According to the World Health Organisation, 99.4% of the world's population consumes more salt than the recommended daily intake of 2g sodium/day (World Health Organization, 2012). Sauce and condiments is a significant source of sodium. According to the study conducted by Shahar et al. (2019) on the amount of sodium for various sauces available in the Malaysian market, only 21.7% from 44 brands of chilli sauces showed values that is below the 2017 UK salt guidelines. Chilli sauce is one of the most popular chilli-based sauces which is prepared using a variety of ingredients, including chilli, garlic, water, sugar, salt, vinegar, hydrocolloids, and preservatives. The addition of NaCl to foods increases the product's quality and palatability, as well as its shelf life (Vinitha et al., 2022).. Thus reducing salt in foods is a great challenge to food industry.

According to Kongstad & Giacalone (2020), strategies to reduce salt content in food includes salt reduction, salt replacement, and physical modifications. However, the removal of sodium is associated with reduced saltiness but increased bitterness and reduced sourness, due to the altered taste-taste interactions (Kongstad & Giacalone, 2020). Since double emulsion enables the encapsulation of flavoring compound such as salt, it has the potential to aid in increasing the taste intensity.According to lyasoglu Buyukkestelli & El (2021), using double emulsions (DE) to minimise the salt concentration is possible to achieve by adding salt to the outer aqueous phase of a W/O/W double emulsion. Moreover,from the study of Wang et al. (2021), double emulsions modify the degree of contact between the water phase and the oral surface. However, the targeted delivery of salt on the taste buds cannot be achieved by the inclusion of salt in the outer aqueous phase thus prevents the maximum perception of salt from emulsion based food products. In this study, the application of DE for producing low salt chilli sauce was investigated. DE incorporated chilli sauce was formulated by encapsulating salt in the inner phase of DE for targeted delivery and maximum perception that can be beneficial for the development of emulsion based food products such as sauces.

MATERIALS AND METHODS

Emulsion preparation

Emulsion containing salt at different concentration was prepared in the form of single and double emulsions. The single oil-in-water (O/W) emulsion was prepared by homogenizing oil and water by using a homogenizer. The generation of double emulsion was conducted by using the two-step homogenization method. Firstly, the primary water-in-oil (W_1/O) emulsion was prepared by homogenizing the inner aqueous phase with oil phase. The produced W_1/O emulsion was then further homogenized with the outer aqueous phase forming water-in-oil-in-water emulsion. Distilled water containing different concentration of salt was used as the outer and inner aqueous phase of single and

double emulsion respectively. The concentration of salt was set at 8.68/100ml, 0.45g/100ml, 0.3g/100ml and 0.15g/100ml for both single and double emulsions.

Chilli sauce preparation

Chilli paste was prepared by mixing blanched chillies with tomatoes, sugar, garlic and water by using an industrial blender. The mixture was cooked for 30 minutes with low heat and the pH was adjusted to 3.5 using 25% acetic acid (Ali et al., 2019). The chilli paste was then combined with the salt emulsion and corn starch and warmed at 80°C for 10 minutes before being placed in a sterile jar for storage. The final salt concentration for the prepared chilli sauces were 0.15g of salt/100mL, 0.10g of salt/10mL and 0.05g of salt/100mL for formulations with reduced amount of salt while sample with 2.86g of salt/100mL was used as control (salt concentration for commercial sauces).

Determination of droplet size and encapsulation efficiency

Droplet size determination was conducted for both single and double emulsion according to Mohd Isa et al. (2022). The acquired photomicrographs were analysed by MATLAB software using the circular Hough transform. Encapsulation efficiency was determined by measuring the salt content in the outer aqueous phase of double emulsion immediately after preparation. The samples were left in upright position to allow for creaming before 1 mL of the outer aqueous phase was carefully withdrawn. The salt concentrations were determined by Mohr's titration and the encapsulation efficiency was calculated following the equation:

$$Encapsulation\ efficiency\ (\%) = \frac{C_0 - C}{C_0} \times 100$$

Where C_0 is the concentration of salt before encapsulation into $W_1/O/W_2$ while C is the concentration of salt in W_2 immediately after encapsulation.

Sensory analysis of chilli sauce

The sensory evaluation was performed by using a simple paired comparison test (2-Alternate Forced Choice tests, BS ISO 5495:2007). 30 panelists were recruited among the staff and students of Universiti Malaysia Terengganu. Data was collected and analyzed with chi-square using the following equation:

$$\chi^2 = \frac{(\Theta 1 - \mathrm{E}1)^2}{E1} + \frac{(\Theta 2 - \mathrm{E}2)^2}{E2}$$

Rheological measurement of chilli sauce

A rheometer with cone and plate geometry was used to measure the rheological properties of the sauce samples (DH3, TA Instruments, USA). The measurements were conducted at a temperature of 25℃ with a shear rate ranging from 0.1 to 100s-1.

Chemical analysis

pH of chilli sauces was determined using pH metre. Proximate analysis involving moisture, crude protein, crude fat, crude fiber, and ash content was conducted on the chilli sauce sample according to the standard procedure (AOAC, 2016). Carbohydrate estimation was calculated with the following equation (FAO, 2003):

Carbohydrate (%) = 100 – (%∑ moisture + ash + fat + protein + crude fiber)

Statistical analysis

The data was statistically analysed by using one-way Analysis of Variance (ANOVA) with Tukey's HSD by using Minitab software. The differences were considered significant at $p < 0$.

RESULTS AND DISCUSSION

Effects of salt concentration on droplet size

Figure 1 showed the average droplet size for single and double emulsions containing different concentration of salt. From the data obtained, increase in salt concentration leads to smaller droplet size (oil globule) for double emulsion samples. However, based on the data for single emulsion, the increase in salt concentration leads to increase in droplet size. This result is in agreement with previous study whereby the presence of salt in the inner aqueous phase creates a hypoosmotic condition that leads to reduce droplet size and increase in stabilty as the Laplace pressure was stabilized (Mohd Isa et al., 2021). However, for single emulsion, larger droplet size can be attributed to the unfavourable interactions between the surfcatant and salt that may lead to coalescence. In addition, high encapsulation efficiency was also achieved for double emulsions ranging from 95-99%.

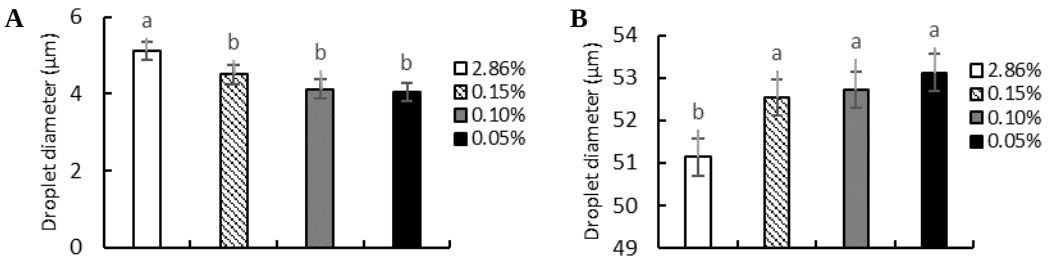

Figure 1. Droplet diameter for single emulsions (A) and double emulsions (B). Bar represents mean±standard deviation with same letters indicating no significant different at p>0.05.

Sensory evaluation

Sensory evaluation was conducted to compare the saltiness perception between samples prepared with single and double emulsion. From the results obtained, it is determined that the majority of the panels were able to differentiate the level of saltiness between the samples although the amount of the salt content was similar for both of the presented samples with double emulsion samples were perceived as significantly saltier as compared to single emulsion at all salt concentration. The reduction of the aqueous phase leads to increase in salt concentration and altered how saltiness was perceived regardless of the amount (Ilyasoglu Buyukkestelli & El, 2019). The encapsulation of salt in double emulsions leads to highly concentrated salt within the inner phase that was released in burst during oral processing and leads to increase in saltiness perception even at a lower salt concentration.

Table 1: Sample pairs presented to assessors in sensory evaluation

Set	Samples	g salt/100g	Number of assessors choosing the samples as saltier	Chi-square, χ^2
1	O/W	2.86	3	19.2 > critical χ^2 (3.84)
	W/O/W		27	
2	O/W	0.15	8	6.53> critical χ^2 (3.84)
	W/O/W		22	
3	O/W	0.10	6	10.8> critical χ^2 (3.84)
	W/O/W		24	
4	O/W	0.05	8	6.53> critical χ^2 (3.84)
	W/O/W		22	

Samples for with calculated χ^2 > critical χ^2 indicates significant difference ($p<0.05$)between two samples at df=1 and a=0.05.

Rheological properties of chilli sauce

In general, chilli sauce produced with double emulsions showed a higher viscosity as compared to samples produced with single emulsions. Increase in salt concentrations also leads to increase in viscosity for sauce produced with double emulsion with samples containing 0.28g salt/100ml had highest viscosity of 0.251 Pa.S (Figure 2). Previous study reported that the viscosity of an emulsion depended on the mass fraction of the dispersed phase whereby higher fraction leads to increase in viscosity (Yildirim et al., 2016; Ilyasoglu Buyukkestelli & El, 2019). The increase in viscosity is also due to the higher amount of surfactant as reported by Zhang et al. (2021) thus, DE containing both PGPR and Tween 80 showed a higher viscosity as compared to SE that only contain Tween 80.

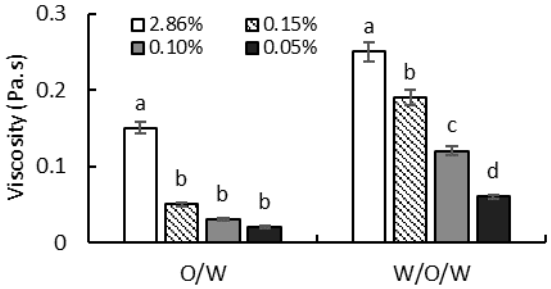

Figure 2. Viscosity value of emulsions samples at different salt concentration. Bars represents mean ± standard deviation with same letters indicating no significant different at p>0.05.

Chemical characteristics of chilli sauce

Based on Table 1, the different emulsions used in the production of chilli sauce does not significantly affect the the amount of proximate content except for ash, fat and carbohydrate content. The significant difference in ash is attributed

to the different amount of salt where increase in salt leads to increase in ash content that also indicates the increase in mineral content. The chilli, salt and corn oil are the sources of the minerals found in the chilli sauce such as sodium, phosphorus, iron, and vitamin E (Deepa et al., 2013). In general, the amount of fat in the chilli sauce samples was higher as compared to the commercial chilli sauces which has been reported to contain 0.4g per 100g by MyFCD. Whereby amount was similar to salad dressings. The use of double emulsion leads to significant reduction in fat as reported previously by Yildrim et al. (2016).

Table 1. Chemical characteristics of chilli sauce made with single and double emulsions. Mean±standard deviation with different letters within the same row indicates significant different at $p<0.05$.

	O/W				W/O/W			
	2.86%	**0.15%**	**0.10%**	**0.05%**	**2.86%**	**0.15%**	**0.10%**	**0.05%**
pH	3.43 ± 0.01^{a}	3.61 ± 0.03^{a}	3.59 ± 0.03^{a}	3.61 ± 0.02^{a}	3.27 ± 0.03^{b}	3.25 ± 0.05^{b}	3.31 ± 0.05^{b}	3.32 ± 0.02^{b}
Moisture	75.58 ± 0.42^{b}	73.16 ± 0.71^{c}	69.15 ± 0.95^{d}	73.21 ± 1.37^{c}	76.89 ± 0.53^{b}	81.30 ± 0.25^{a}	80.89 ± 0.36^{a}	80.91 ± 0.30^{a}
Ash	1.59 ± 0.01^{b}	0.53 ± 0.01^{c}	0.45 ± 0.1^{d}	0.31 ± 0.1^{e}	1.82 ± 0.05^{a}	0.55 ± 0.01^{c}	0.47 ± 0.01^{d}	0.33 ± 0.01^{e}
Protein	1.55 ± 0.05^{a}	1.02 ± 0.03^{a}	1.17 ± 0.08^{a}	1.34 ± 0.03^{a}	1.28 ± 0.05^{a}	1.29 ± 0.04^{a}	1.29 ± 0.04^{a}	1.23 ± 0.03^{a}
Fat	4.7 ± 1.03^{ab}	4.7 ± 0.52^{ab}	5.3 ± 0.71^{a}	4.3 ± 0.60^{b}	2.5 ± 0.58^{d}	2.7 ± 0.16^{d}	3.3 ± 0.57^{c}	3.0 ± 0.10^{cd}
Carbohydrate	16.09 ± 0.70^{c}	20.03 ± 0.51^{b}	23.05 ± 0.53^{a}	20.53 ± 0.65^{b}	17.13 ± 0.71^{d}	14.04 ± 0.53^{e}	14.34 ± 0.51^{e}	14.21 ± 0.71^{e}
Fiber	0.51 ± 0.05^{a}	0.54 ± 0.02^{a}	0.55 ± 0.01^{a}	0.53 ± 0.03^{a}	0.51 ± 0.04^{a}	0.52 ± 0.05^{a}	0.51 ± 0.02^{a}	0.50 ± 0.02^{a}

CONCLUSIONS

In conclusion, the application of double emulsion for the production of low-salt chilli sauce help to significantly improve its saltiness perception. Different salt content significantly affects the emulsion characteristics in terms of droplet size. The increase in salt also resulted in increase in viscosity which may indicate better emulsion stability. Future study should be conducted in order to understand the mechanism involves during oral processing that leads to increase in perception in order to widens its application in various food products

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THE EFFECT OF PHYTOCHEMICAL CONTENT AND ANTIOXIDANT ACTIVITIES OF PERIA PANTAI (*Colubrina asiatica*) LEAVES EXTRACTS ON THE SHELF LIFE QUALITY OF COOKED CHICKEN BURGERS

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Abstract: This study determined the phytochemicals and bioactivities of *Colubrina asiatica* leaves extracts and their effect on the shelf-life quality of cooked chicken burgers. *C. asiatica* leaves were oven-dried (OD) at 40°C or freeze-dried (FD) at −50°C and extracted with 0, 20, 40, 60, 80 and 100% ethanol. Results showed OD 100% ethanol extract (OD100) had the highest ($p<0.05$) phenolic, flavonoid, vitamin C, and ferric reducing power. Meanwhile, OD 80% ethanol (OD80), OD100, FD 80% ethanol (FD80) and FD 100% ethanol extracts (FD100) reported with the highest ($p<0.05$) DPPH scavenging activities. UHPLC-QTOF-MS confirmed that OD100 and FD100 extracts contained rutin, kaempferol-3-O-rutinoside (K3R) and quercetin-3-O-rhamnoside (Q3R) while HPLC-PDA demonstrated that FD100 extract had higher rutin and K3R content than OD100 extract. The shelf-life quality of a commercial chicken burger, chicken burgers without antioxidant (control) and chicken burgers with OD100 and FD100 extracts stored at 4°C using aerobic packaging were analysed on day 1, 6, and 12. The optimal fat content and extract concentration of the burgers were generated with response surface methodology (RSM). The findings indicated that the addition of *C. asiatica* extract decreased ($p<0.05$) malondialdehyde (MDA) and peroxide content of the burger compared to control.
***Keywords*:** Natural antioxidant, high performance liquid chromatography (HPLC), lipid oxidation, phenolic compounds, response surface methodology (RSM)

INTRODUCTION

Lipid oxidation is one of the major causes of meat deterioration due to production of hydroperoxides, aldehydes and ketones which may affect the flavour, colour and structure of meat protein (Kumar et al., 2015). The decline in meat quality and nutritional value caused by oxidative rancidity leads to a shorter shelf life for the meat and adds to the issue of food waste (Reddy et al., 2018). The susceptibility of chicken meat to lipid oxidation, owing to its high polyunsaturated fatty acid (PUFA) content, means that the fat composition of processed chicken significantly impacts the rate of this oxidation within the product (Sohaib et al., 2017). Synthetic antioxidant such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are frequently utilised to inhibit or delay lipid oxidation in meat (Bianchin et al., 2020). However, the risks of consuming synthetic antioxidants on health have led the increase in demand for natural antioxidants (Altemimi et al., 2017). *Colubrina asiatica* leaves extract was reported to possessed high phenolic content and antioxidant activities (Somdee et al., 2016). Antioxidant analyses of *C. asiatica* leaves extract shown inhibition towards DPPH, hydrogen peroxide, nitric oxide and superoxide anion radical activities (Desai & Gaikwad 2014).

The earlier part of this study determined the effect of different drying methods and solvent polarities towards phytochemical and antioxidant activities of *C. asiatica* leaves extracts. *C. asiatica* leaves were oven-dried (OD) at 40°C or freeze-dried (FD) at −50°C and extracted with 0, 20, 40, 60, 80 and 100% ethanol. The dried extracts were analysed for total phenolic content (TPC), total flavonoid content (TFC), vitamin C, DPPH scavenging activity (IC_{50}) and ferric reducing power (FRAP). The extracts with high TPC, TFC, vitamin C and antioxidant activities were further analysed for qualification of phytochemicals with UHPLC-QTOF-MS and quantification of rutin, K3R and Q3R with HPLC-PDA. The selected extracts were also used to determine the effect of *C. asiatica* extracts towards color (L^*, a^*, b^*) and lipid oxidation of cooked chicken burger stored at 4°C using aerobic packaging. Many studies reported the

effect of using plant extract as antioxidant agent but the optimal extract concentration rarely reported. Thus, RSM analysis was applied to obtain the optimal fat content of burger and extract concentration added to the burger for the shelf-life study.

MATERIALS AND METHODS

The *C. asiatica* leaves were dried with two different drying methods: oven dried at 40°C and freeze dried at -50°C prior to extraction with six different polarities of solvent mixture (ethanol to water: 0:100, 20:80, 40:60, 60:40, 80:20, 100:0). The sample was extracted for 24 hours at 27°C using an incubator shaker set to 200 rpm. Aqueous extract was filtered with Whatman No. 1 filter paper and the excess solvent was removed using a rotary evaporator. The total phenolic content (TPC), total flavonoid content (TFC), vitamin C content, DPPH scavenging activity (IC_{50}) and ferric reducing power (FRAP) of the *C. asiatica* extracts were analysed. Extracts with high phytochemical content and antioxidant activities were selected for further analyses. The phytochemicals in OD100, FD80 and FD100 extracts were identified using ultra-high perfomance liquid chromatography paired to quadrupole time of flight mass spectrometer (UHPLC-QTOF-MS) in negative mode. The quantification of rutin, K3R and Q3R in OD100 and FD100 extracts were performed using HPLC coupled to photodiode array (HPLC-PDA).

The optimisation of fat content in burger and OD100 and FD100 extract concentration was carried out using RSM with central composite rotatable design (CCRD) in Design Expert 7.1.5. The $-\alpha$ and $+\alpha$ values for fat content were set at 10% and 25%, respectively while, the $-\alpha$ and $+\alpha$ values for extract concentration were set at 10 mg/kg and 1000 mg/kg, respectively. The analysis was carried out following the 13 RSM combinations from Design Expert and the responses for color (L^*, a^*, b^*), peroxide value (PV) and thiobarbituric acid reactive substance (TBARS) values were measured. The optimal fat content and extract concentration obtained based on the desirability of the responses and root mean squared deviation (RMSD) was calculated to validate the results from the RSM model. Chicken burgers with OD100 and FD100 extracts, chicken burgers without extract/antioxidant (control), and commercial chicken burger were stored at 4°C using aerobic packaging. The optimal fat content and extract concentration from RSM analysis were used to formulate the burgers (RSM1: 17.07% fat and 811.45 mg/kg extract; RSM2: 22.80% fat and 330.17 mg/kg extract). The colour (L^*, a^*, b^*), PV and TBARS value of burgers were analysed on day 1, 6, and 12.

RESULTS AND DISCUSSION

Table 1 shows the TPC, TFC, vitamin C, DPPH (IC_{50}) and FRAP values of *C. asiatica* extracts with different drying methods and solvent polarities. The results obtained shows that OD100 extract had significantly higher ($p<0.05$) TPC, TFC, vitamin C and FRAP value than the other extracts. Meanwhile, OD80, OD100, FD80 and FD100 reported with the highest ($p<0.05$) DPPH scavenging activities than the other OD (0%, 40% and 60% ethanol) and FD (0% and 60% ethanol) extracts. Thus, the results showed that oven drying method with 100% ethanol would give extract with highest phytochemical content and antioxidant activities.

Table 1 The phytochemical properties and antioxidant activities of *C. asiatica* leaves extracts.

Drying Method	Water : Ethanol Ratio	TPC (mg GAE/g)	TFC (mg QE/g)	Vitamin C (mg/g)	DPPH (IC_{50}) (mg/mL)	FRAP (mg TE/g)
Oven drying	0:100	15.74 ± 1.61^{bcd}	36.56 ± 1.82^{e}	67.03 ± 8.32^{e}	3.07 ± 0.35^{a}	34.12 ± 2.41^{cde}
	20:80	10.71 ± 1.53^{e}	41.83 ± 1.73^{de}	75.71 ± 5.48^{e}	ND	16.96 ± 2.94^{f}
	40:60	11.36 ± 2.14^{de}	32.51 ± 0.59^{ef}	94.80 ± 12.09^{d}	2.34 ± 0.99^{abc}	22.99 ± 5.67^{ef}
	60:40	12.74 ± 1.87^{de}	32.61 ± 1.39^{ef}	109.33 ± 14.74^{cd}	2.42 ± 0.36^{ab}	35.32 ± 15.26^{cde}
	80:20	20.02 ± 2.29^{b}	51.25 ± 3.75^{d}	152.24 ± 18.19^{b}	0.59 ± 0.12^{e}	62.51 ± 10.25^{b}
	100:0	25.15 ± 0.87^{a}	169.48 ± 9.99^{a}	179.85 ± 7.44^{a}	0.62 ± 0.06^{e}	81.57 ± 3.36^{a}
Freeze drying	0:100	17.04 ± 1.85^{bc}	41.74 ± 0.28^{de}	64.02 ± 7.48^{e}	2.12 ± 0.70^{bc}	31.06 ± 2.02^{def}
	20:80	12.42 ± 3.77^{de}	22.07 ± 3.58^{fg}	66.04 ± 2.92^{e}	ND	21.15 ± 12.31^{ef}
	40:60	12.47 ± 3.12^{de}	31.86 ± 5.39^{ef}	71.60 ± 10.07^{e}	2.27 ± 0.28^{abc}	21.29 ± 6.88^{ef}
	60:40	14.31 ± 1.47^{cde}	14.63 ± 1.02^{g}	94.34 ± 14.02^{d}	1.52 ± 0.51^{cd}	34.98 ± 6.59^{cde}
	80:20	17.67 ± 1.31^{bc}	86.17 ± 4.10^{c}	122.18 ± 3.51^{c}	0.95 ± 0.04^{ed}	44.34 ± 7.75^{cd}
	100:0	18.37 ± 4.02^{bc}	155.64 ± 21.02^{b}	142.43 ± 4.17^{b}	1.00 ± 0.37^{ed}	48.27 ± 13.75^{bc}

ND: not determined (failed to achieve 50% inhibition of DPPH).
Value shows mean ± standard deviation, mean (n = 3).
[a-f] Mean with different superscript in each analysis indicates there was significant difference ($p<0.05$) between extracts with different drying method and solvent polarity.

Heat treatment during oven drying would deactivate polyphenol oxidase (PPO) enzyme and inhibit microbial growth that could affect stability and phytochemical content in the extract (Minatel et al., 2017; Mphahlele et al., 2016). Ethanol solution has both polar hydroxyl group (-OH) and non-polar ethyl group ($-CH_2CH_3$) which allow ethanol to extract phytochemicals more effectively than water. Ethanol also has low toxicity risk and sound absorption

which make it favourable for solvent extraction (Suharni et al., 2021). Thus, OD100 extract and both FD80 and FD100 were selected for qualification analysis due to their significant phytochemical content and antioxidant activities.

The UHPLC-QTOF-MS analysis of OD100, FD80 and FD100 extracts identified the presence of phenolic acid, flavonoid, alkaloid, terpenoid and saponin that could contribute to antioxidant activities of the extracts. Rutin, quercetin, kaempferol, K3R, Q3R and asiaticoside were among the compounds identified in the extracts. These compounds were reported by previous studies with high antioxidant activities (Cui et al., 2022; Liana et al., 2019; Mohamed Isa et al., 2018; Yahaya et al., 2019). The HPLC-PDA analysis showed that rutin and K3R in FD100 extract (rutin: 51.06 µg/mL; K3R: 144.73 µg/mL) was higher than OD100 extract (rutin: 17.22 µg/mL; K3R: 118.43 µg/mL). Thermal effect of oven drying would deteriorate the stability and bioactivity of rutin. Heat could affect K3R similarly since they are from the same group of compounds (flavonoid) and share almost similar structure (Chaaban et al., 2016; Lv et al., 2020).

RSM generated the optimised fat and extract content for OD100 extract was 17.07% fat and 811.45 mg/kg extract (RSM1) while for FD100 the optimal for fat and extract was 22.80% and 330.17 mg/kg extract (RSM2). Both RSM1 and RSM2 were used to formulate burgers for shelf-life study. The small RMSD values (0.0208 – 1.1143) obtained for both extracts showed validity of the optimal values obtained from the RSM model. The shelf life study of cooked chicken burgers stored at 4°C in aerobic packaging showed the effect of OD100 and FD100 extracts to the color (L^*, a^*, b^*) and lipid oxidation of the burgers. Addition of OD100 and FD100 extracts decreased ($p<0.05$) the lightness (L^*) and increased the greenness ($-a^*$) and yellowness (b^*) of the burgers.

Table 2 showed that the commercial burger and burgers with OD100 and FD100 extracts had lower ($p<0.05$) peroxide content than the control burgers throughout the storage (except for FD100/RSM1 on day 6 and FD100/RSM2 on day 6 and 12). Similarly, the commercial burger and the burgers with OD100 and FD100 extracts also reported with lower ($p<0.05$) MDA content than the control burgers (except for OD100/RSM1 on day 6 and FD100/RSM2 on day 1 and day 6). From the PV and TBARS data, it can be deduced that *C. asiatica* extracts able to decrease the formation of primary (hydroperoxides) and secondary (MDA) products of lipid oxidation owing to the presence hydroxyl groups of the phenolic compounds in the extract which capable to stabilised free radicals by donating hydrogen atoms (Yahaya et al., 2019).

Table 2 PV (meq peroxide/kg) and TBARS value (mg MDA/kg) of cooked chicken burgers stored at 4°C in aerobic packaging.

Treatment	Days of Storage		
	1	6	12
		PV (meq peroxide/kg)	
Commercial	0.00 ± 0.00^{Ac}	0.00 ± 0.00^{Ae}	0.00 ± 0.00^{Ad}
C/RSM1	2.25 ± 0.12^{Aa}	2.83 ± 0.47^{Aa}	2.75 ± 0.35^{Aa}
C/RSM2	2.58 ± 0.59^{Aa}	2.17 ± 0.24^{Ab}	3.00 ± 0.00^{Aa}
OD100/RSM1	1.25 ± 0.35^{Ab}	1.17 ± 0.00^{Ad}	1.25 ± 0.12^{Abc}
OD100/RSM2	1.00 ± 0.00^{Bb}	1.33 ± 0.24^{ABcd}	1.75 ± 0.12^{Ab}
FD100/RSM1	0.67 ± 0.24^{Bbc}	1.83 ± 0.24^{Abc}	1.00 ± 0.47^{ABc}
FD100/RSM2	1.33 ± 0.24^{Bb}	1.83 ± 0.24^{Bbc}	2.83 ± 0.24^{Aa}
		TBARS (mg MDA/kg)	
Commercial	0.44 ± 0.09^{Ad}	0.42 ± 0.03^{Ad}	0.40 ± 0.03^{Af}
C/RSM1	2.30 ± 0.00^{Ca}	2.56 ± 0.07^{Bb}	3.00 ± 0.12^{Aa}
C/RSM2	2.33 ± 0.03^{Ba}	2.76 ± 0.00^{Aa}	2.84 ± 0.12^{Ab}
OD100/RSM1	1.49 ± 0.15^{Cc}	2.48 ± 0.05^{Ab}	1.87 ± 0.04^{Be}
OD100/RSM2	1.75 ± 0.10^{Bb}	2.10 ± 0.01^{Ac}	2.17 ± 0.01^{Ad}
FD100/RSM1	1.65 ± 0.10^{Bbc}	2.13 ± 0.12^{Ac}	2.23 ± 0.03^{Ad}
FD100/RSM2	2.15 ± 0.04^{Ba}	2.56 ± 0.05^{Ab}	2.64 ± 0.03^{Ac}

C: control (without extract but, has fat content as RSM1 and RSM2)
OD100: oven-dried, 100% ethanol; FD100: freeze-dried, 100% ethanol
RSM1: 17.07% fat and 811.45 mg/kg extract; RSM2: 22.80% fat and 330.17 mg/kg extract
Value shows mean ± standard deviation, mean (n = 2)
$^{A-C}$ Mean value with different alphabet for each treatment indicates there was significant difference ($p<0.05$) between burger of the same treatment on different day of storage.
$^{a-f}$ Mean value with different alphabet on each day of storage indicates there was significant difference ($p<0.05$) between burger with different treatment on the same storage day.

CONCLUSIONS

C. asiatica extracts had significant antioxidant compounds that would contribute towards the antioxidant activities of the extract thus, reducing the level of lipid oxidation of cooked chicken burgers stored at refrigerated storage.

ACKNOWLEDGMENTS

This research was supported by Universiti Kebangsaan Malaysia research grant (GUP-2018-114).

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A LONG SHORT TERM MEMORY (LSTM)-BASED MODEL FOR THE PREDCITION OF BIOACTIVE PEPTIDES IN FOOD PRODUCTS

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Abstract: Bioactive peptides (BPs) are short chain amino acids that have been shown to have several health benefits. Laboratory-based identification of BPs is a time-consuming and labor-intensive process hence multiple in-silico methods have been developed and used to determine BPs from various sources. In this study, a model, based on a Long Short Term Memory (LSTM) binary classification model, was created and trained on existing bioactive peptides from literature and available databases to determine if an input peptide is bioactive or not. Peptide sequences used were from plant and animal sources. In addition, a set of non-bioactive peptides were used as the null set. The study used word encoding on each sequence wherein each sequence was preprocessed to have spaces in between each amino acid before being tokenized. Results showed an 89.13% validation Accuracy and a 0.8174 f-measure value for the model that utilized the dataset containing all peptides used. Model performance can be improved by adding to the dataset and trying different encoding methods which retains more information. The constructed model and its prediction can be used as a baseline for further studies in the predictive determination of bioactive peptide sequences from food products.
***Keywords*:** bioactive peptides, predictive modeling, LSTM

INTRODUCTION

Bioactive peptides (BPs) are short-chain amino acids that have shown activity in terms of providing health benefits to the consumer. BPs are produced during the enzymatic breakdown that proteins undergo during digestion, or in other cases, food processing (Daliri et. al, 2017; Toldra et. al, 2018). There is an increasing interest in these macromolecules in different fields and industries due to the fact that they can provide several benefits not just in terms of health, but also in terms of food safety and quality. Several studies have shown that bioactive peptides can provide antihypertensive, antioxidative, and immunomodulatory health benefits to name a few. Likewise, in terms of food product quality, there are bioactive peptides that have shown antimicrobial properties. (Daliri et. al, 2017; Sanchez and Vasquez, 2017; Udenigwe and Aluko, 2012). This interest resulted in numerous laboratory-based studies focusing on identifying new bioactive peptide sequences and deducing their functions. However, one of the major drawbacks of these studies is the labor-intensive and time-consuming process of experimentation (Li et al., 2022).

Machine learning is a field that uses different computational algorithms designed to copy the human cognitive process. One approach of machine learning is neural networks wherein several layers of interconnected algorithms are used to analyze inputs and generate outputs similar to how the human brain processes data (Naga and Murphy, 2015; Ma et. al, 2022). There are numerous neural network models that have been developed, and some of these have already been used in predicting bioactive peptide sequences. An example of which is the bidirectional recurrent neural network (BRNN) model that uses sequential data used in predicting bioactive peptide sequences from larger protein sequences and the protein's predicted structural features (Schuster and Paliwal, 1997; Mooney et. al, 2013). A disadvantage of this model is the need to have the original protein sequence available which may not be possible for pre-digested samples. Another model used in bioactive peptide research is the convolutional neural network (CNN). CNN uses kernels that allow it to extract features on its own. The model has been used in predicting the anti-hypertensive activity of certain peptides given several properties of the peptide as inputs (Shi and Zhang, 2022).

Recurrent neural networks (RNN) are most commonly used in time series, or also known as, sequential data. RNN has a node which stores the data from the previous state to use in current predictions. Although RNN has problems in creating long term dependencies, in BPs' case, it might not recognize an amino acid 20 positions before the current state. Long Short Term Memory (LSTM) uses additional gates that tells the node if it should forget, store, or output the information it has which alleviates the weights being too small or too large (Hochreiter & Schmidhuber, 1997).
This work aimed to address the lack of predictive models that only uses the peptide sequence to determine whether or not it has bioactivity. LSTM is most fit in this application due to the possible dependencies between longer chains of

amino acids, with only the sequence data being the input of the model. It is important to form these relationships unlike models that consider other peptide properties.

MATERIALS AND METHODS

The peptide sequences utilized in this study were all obtained from existing literature, and all came from both plant and animal food sources only. Peptide sequences were obtained from the BIOPEP-UWM database (Minkiewicz et. al, 2019). Non-bioactive protein sequences were obtained using the protein sequences available within the database and fragmenting it via the enzymatic digestion function of BIOPEP-UWM. Bioactive peptide sequences on the other hand were obtained using both the BIOPEP-UWM database and those pre-existing in published literature. Fragments are then labeled as "nonbioactive" or "bioactive" accordingly. A total of 4672 bioactive peptides and 1123 non-bioactive were extracted, 2731 of which were of lengths 5 or less and of that, 2036 were bioactive. In the 3064 sequences with length of more than 5, 2637 of which are bioactive.

The data were separated into three (3) sets: peptide chains with 5 or less amino acids (s1), more than 5 (s2), and a set of all peptides (s3). The peptide sequences were then run in an Excel function to add spaces in between each amino acid and saved as csv. The labeled dataset created was imported to MATLAB and separated into training and validation datasets at a 8:2 ratio, respectively. Datasets then underwent the MATLAB function *tokenizedDocument* to treat each of the data as a document. *wordEncoding* is then applied which maps each amino acid to numeric indices, then converting the documents into sequences with length 5 for the s1 and 20 for s2 and s3 to prepare as input for the model.

The model has 6 layers consisting of a sequence input layer, a word embedding layer with *embeddingDimension* of 50, LSTM layer with 10 hidden units, Fully connected layer, softmax layer, and a Classification Output layer. This approach is often used to process text documents, in this case each amino acid is being treated as a word in a sentence or document. Similar to one hot encoding, the position of each amino acid is not considered. The model is then trained using the training settings in Table 1. The training dataset was used to train the model directly, while validation dataset is used every 50 iterations to evaluate performance during training.

Table 1. Setting used to train the predictive model

Solver	adam
MiniBatchSize	128
GradientThreshold	2
Shuffle	every-epoch

The model was evaluated using the f-measure and Accuracy, derived from the confusion matrix from the validation split. Accuracy is the amount of correctly classified peptides over the total number of peptides. F-measure is the weighted average of precision and recall. Accuracy is highest at 100% while f-measure is highest at 1.

RESULTS AND DISCUSSION

S1 and s3 were trained to 200 epochs, while the s2 was trained up to 50 epochs. S1 and s3 accuracy stabilized at around 50 epochs and maintained that accuracy. Figure 1 shows the training graph of s3. The s2 was only trained until 50 as after that point accuracy started to drop due to possible overfitting due to the size of the dataset. The representative evaluation metrics of s3 is shown in Table 2. The macro average accuracy and macro average f-measure of the model under each dataset is seen in Figure 2 where s3 achieved the highest Accuracy and f-measure at 89.13% and 0.8174 respectively. This proves that there are dependencies that the LSTM model can see with only the identity the of amino acid and how many of it exists without looking at the position and neighboring amino acids. The way the model works is similar to how documents are encoded, where certain keywords are the triggers of those that are, in this case, bioactive or not. Improvements can still be made to the model as it is necessary to increase the amount of non-bioactive peptides to match the number of bioactive peptides. Even if the accuracy of the model is decent without information such as position of each peptide, it would improve the performance of the model if such information was simultaneously fed through a different type of amino acid encoding method.

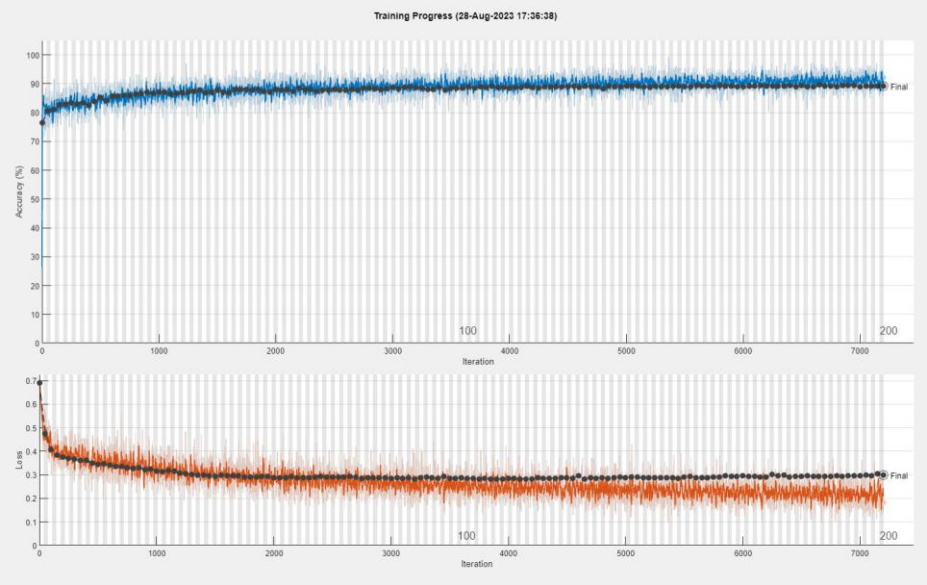

Figure 1. Training graph of the s3 dataset

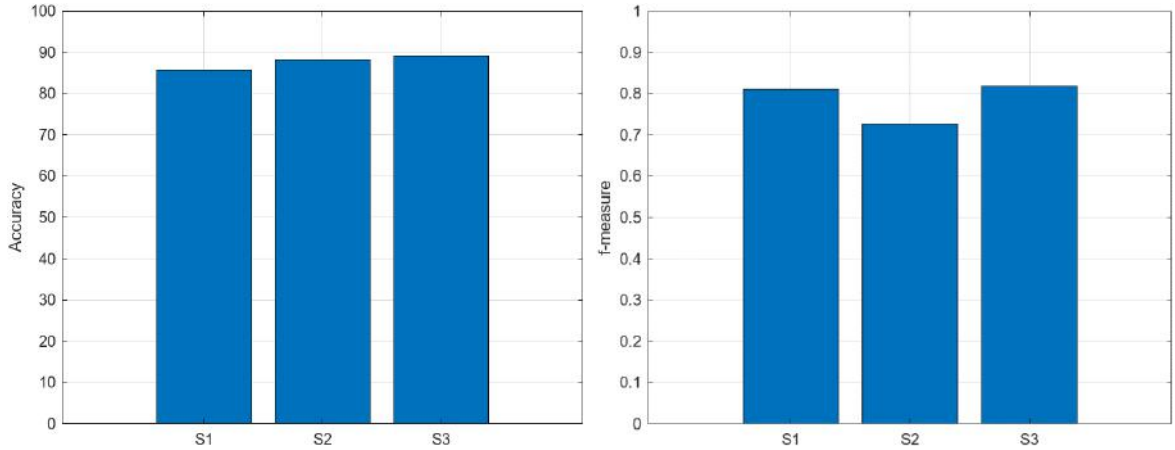

Figure 2. Macro average of the Accuracy (L) and the f-measure (R) between models

Table 2. Evaluation metrics of S3

S3	Bioactive	Nonbioactive	Macroaverage	Microaverage
true_positive	885	148	516.5	516.5
false_positive	76	50	63	63
false_negative	50	76	63	63
true_negative	148	885	516.5	516.5
precision	0.9209157128	0.7474747475	0.8341952301	0.891285591
sensitivity	0.9465240642	0.6607142857	0.8036191749	0.891285591
specificity	0.6607142857	0.9465240642	0.8036191749	0.891285591
accuracy	89.13%	89.13%	89.13%	89.13%
F-measure	0.9335443038	0.7014218009	0.8174830524	0.891285591

CONCLUSIONS

In this study, LSTM-based binary classification model was built using a combined total of 5795 peptide sequences. The dataset underwent an encoding method similar to the ones used for analyzing documents and was split 8:2 training and validation dataset, respectively. Two other datasets were also created to compare performance when isolating different lengths of peptides, one where it only consisted of those of length 5 and below and the second for the rest. After training and evaluating the models, the model that was trained using the dataset that consisted of all the peptides with an accuracy of 89.13% after 200 epochs. The study demonstrated the possibility of predicting peptide activity just from its sequence. Moreover, this study can be used as a baseline model in creating other frameworks which are capable of predicting peptide activity even in the absence of the original protein sequence, which is a common occurrence in food systems.

Separation of dataset is not as necessary as initially conceptualized as the model showed similar performance between the three datasets and the best performing model is the one trained on all the BPs without splitting. It is recommended to build the labeled dataset further by adding more non-bioactive peptide sequences to match the number of bioactive peptides for a more robust model. Giving the model more information, such as amino acid positioning should also improve the performance of the model. This could be done by using a different type of encoding that preserves more information. Finally, the number of hidden units on the LSTM layer underwent preliminary testing and showed no significant difference between 10, 20, 100, and 200 hidden units, the study opt to stick with 10 hidden units for resource efficiency as it can predict faster. Although fine tuning the number of hidden units can be used to increase model performance by a few points. The improved model can be used to predict the activity of a peptide even before it is tested experimentally.

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PHYTOCHEMICAL DIVERSITY OF ALPINIA MUTICA ROXB. LEAVES EXTRACTS AND THEIR BIOACTIVITY CORRELATIONS ELUCIDATED BY NMR-BASED METABOLOMICS

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Abstract: Alpinia mutica Roxb. have long been used for traditional medicinal purposes due to the presence of some active metabolites, particularly polyphenols. However, there is limited information on the chemical constituents and their relationship with the bioactivities of the herb. The choice of extraction solvent will greatly influence the metabolite profile and bioactivities of an herbal extract. Using nuclear magnetic resonance (NMR) based metabolomics approach, we carried out discriminative analysis of the metabolite profiles when different extraction solvents (ethanol:water - 100:0, 80:20 60:40, 40:60, 20:80 and 0:100) were used and correlate the metabolite profiles with the phenolic content, antioxidant, anti-hyperglycemic and antiobesity activities. Identification of primary and secondary metabolites was performed using NMR and liquid chromatography tandem mass spectroscopy (LC-MS/MS). The 40:60 extract showed high phenolic content, strong power in reduction of the ferric ion and low IC50 value in scavenging DPPH free radical, inhibition of pancreatic lipase (PL) and α-glucosidase (AG). From the NMR metabolomic analysis, the PLS biplot indicated that the presence of quercetin, apigenin, tryptophan, curcumin, ascorbic acid, catechin, choline, alanine, phenylpropanoid and isorhamnetin in the 40:60 extract were responsible for the bioactivities measured. This study exposed information on the chemistry and biology of the herb that may be useful for the development of natural antioxidant and antiobesity agent.
***Keywords*:** Extraction, Multivariate data analysis, Phytomedicine, Metabolomics, Alpinia mutica Roxb

INTRODUCTION

Alpinia mutica (*A. mutica)* is a perennial aromatic herb native to Malaysia and Thailand that produces a horizontal, underground stem. Traditionally, this plant has long been used as local remedies for various ailments such as flatulence and to reduce inflammation (Ibrahim et al., 2014). Phytochemical investigations revealed the presence of phenolic compounds identified as flavokawin B, pinocembrin, 5-6 dehydrokawain, pinostrobin and β-sitosterol (Sirat et al., 2013). Previous studies have demonstrated that the bioactive components present in *A. mutica* are responsible for most of the biological activities of the plant, including antioxidant, antimicrobial, antibacterial, and anti-cancerous properties (Mustahil et al., 2013). The use of different extraction solvents substantially affects the metabolite content and biological activities that could be advantageous in the fields of health, food, and cosmetics (Zengin & Baysal, 2014). This is because bioactive metabolites exist in different polarities and chemical characteristics due to their stability in different solvent. Therefore, the most suitable extraction solvent must be optimised to ensure the presence of sufficient bioactive secondary metabolites levels in the extract. Metabolomics is a powerful tool to investigate organism's metabolites both qualitatively and quantitatively. However, due to abundance of datasets, multivariate data analysis (MVDA) is needed to identify the pattern and correlate between the variables. Principal component analysis (PCA) and partial least square (PLS) regression are commonly used in MVDA to separate samples based on their metabolites and chemical properties. Hence, the objectives of this study were to evaluate the metabolite differences of *A. mutica* leaves based on their chemical profiles and to investigate the optimal extraction solvent in order to produce extracts with the most desirable and potent antioxidant as well as α-glucosidase and pancreatic lipase inhibitory activities.

MATERIALS AND METHODS

Sampling and plant extraction

Extraction of plant extract was based on the protocol by Ain Ibrahim et al., (2023) with some modifications. Six replications of *A. mutica* leaves were freshly collected at Taman Herba, Universiti Kebangsaan Malaysia consistently

at 8.30 am - 9.30 am at six different days. The samples were extracted with different ethanol-to-water ratio (100:0, 80:20, 60:40, 40:60, 20:80 and 0:100) with six replicates in each ratio.

Bioactivities of *A. mutica* leaves extract and metabolomics profiling by ^{1}H-NMR

Total phenolic content (TPC): The TPC was determined based on the method of Ain Ibrahim et al., (2023) with some modifications.

Antioxidant assays: The DPPH free radical scavenging activity was based on the method reported by Sin et al., (2018) and ferric reducing antioxidant assay (FRAP) was carried out according to Mirghani et al., (2018).

α-glucosidase and pancreatic lipase inhibition assay: The assays were performed according to the method of Ain Ibrahim et al., (2023) and Roh & Jung (2012), with some modifications.

1H-NMR analysis: ^{1}H-NMR analysis was carried out according to method described by Ain Ibrahim et al., (2023) using 600 MHz Bruker Avance III NMR spectrometer.

Liquid chromatography–mass spectroscopy analysis: Analysis was carried out using Linear Ion Trap Quadrupole LC/MS/MS Mass Spectrometer (AB Sciex 3200QTrap LCMS/MS with Perkin Elmer FX 15 UHPLC system).

Multivariate data and statistical analysis: Data were analyzed using SPSS version 20.0. Difference in means were determined using one-way ANOVA. The correlation among the tested parameters and variation among the samples were determined using multivariate data analyses through SIMCA software (v. 13.0, Umetrics, Umeå, Sweden).

RESULTS AND DISCUSSION

Bioactivities of *A. mutica* leaves extract at different solvent ratio

The experimental results indicated the samples 40:60 and 20:80 have significantly higher ($p<0.05$) TPC and FRAP value compared to samples 100:0 and 0:100 (Table 1). The results of TPC was in accordance with previous research, which reported both TPC and flavonoid content were increased as the ethanol concentration was increased up to 50% of ethanol (Liao et al., 2022). Previous research by Nasir et al. (2023) indicate that ethanol-to-water ratio (50:50) provide highest phenolic content and antioxidant as compared to other solvent. The results demostrated that the binary-solvent system could provide the maximum extraction of phenolic compounds compared to single-solvent system. For DPPH, extracts containing ethanol as solvent showed lower IC_{50} values ($p<0.05$) compared to water extract (0:100). The results were supported by Özbek et al., (2018) indicate that ethanol and water mixture samples were significantly produce high antioxidant activity in Pistachio hull extracts compared to water extracted samples.

Table 1. Total phenolic content, antioxidant and enzyme inhibitory activities of *A. mutica* leaves extract

Solvent ratio (ethanol: water)	TPC (mg GAE/g extract)	FRAP (µmol TE/g extract)	Inhibitory activity, IC_{50} (µg/mL)		
			DPPH	α-glucosidase	Pancreatic lipase
100:0	195.34 ± 6.28^{b}	1226.49 ± 173.51^{c}	44.40 ± 5.06^{bc}	28.70 ± 3.67^{b}	162.84 ± 16.97^{c}
80:20	213.46 ± 22.99^{ab}	2061.65 ± 227.99^{b}	32.66 ± 3.80^{c}	65.15 ± 6.92^{a}	195.78 ± 18.02^{c}
60:40	218.11 ± 29.08^{ab}	2287.60 ± 213.43^{b}	49.62 ± 5.02^{b}	28.48 ± 4.09^{b}	290.97 ± 24.46^{b}
40:60	235.03 ± 27.65^{a}	2320.58 ± 172.25^{b}	39.55 ± 5.52^{bc}	15.51 ± 2.31^{c}	157.27 ± 16.91^{c}
20:80	238.68 ± 28.14^{a}	2657. 68± 193.16^{a}	42.99 ± 5.72^{bc}	71.58 ± 4.56^{a}	470.52 ± 40.54^{a}
0:100	173.39 ± 8.97^{c}	793.06 ± 109.29^{d}	104.87 ± 12.38^{a}	67.43 ± 4.30^{a}	NA
Ascorbic acid	NA	2734.54 ± 201.01^{a}	17.16 ± 1.63^{d}	NA	NA
Quercetin	NA	NA	NA	24.07 ± 3.60^{b}	NA
Orlistat	NA	NA	NA	NA	15.14± 1.94^{d}

Values represent the mean ± standard deviation (n = 6). $^{a-d}$ Different letters show significant differences ($p<0.05$) analyzed by Tukey's. NA=Not available.

In addition, α-glucosidase assay showed that samples 40:60 has significantly lowest IC_{50} value ($p<0.05$) compared to other extracts including quercetin. The results indicated 40:60 can be the best solvent ratios to inhibit α-glucosidase activity as it exhibits the lowest value compared to other samples. While pancreatic lipase assay showed that 40:60, 80:100 and 100:0 has significantly lower IC_{50} value compared to other extracts. This is supported by research done by Vangoori et al., (2019), stating that the increased percentage of ethanolic extract of *Myristica fragrans* possess a good inhibition of the enzymes compared to other solvents. The strong pancreatic lipase inhibition may be due to its chemical composition that exist in plant-based samples including phenolics, flavonoids and alkaloids compounds.

Identification of metabolites and bioactivities of relationship by ^{1}H-NMR

The metabolites identified in the extracts of these plant samples include both primary and secondary metabolites such as carbohydrates, amino acids, phenolics and flavonoids. Quercetin was found, including other metabolites such as apigenin, tryptophan, formic acid, ascorbic acid, cathechin, choline, alanine, phenylpropanoid and isohamnetin. Other metabolites that were found including diterpenes group such as curcumanggoside, and zerumin B. Sesquiterpernes also were found including furanodiones and curcuzederone. In the aromatic regions, some phenolics compound were found including 1-(4-hydroxy-3,5-dimethoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-(Le,6e)-1,6-heptadiene-3,4-dione and demethoxycurcumin. For metabolomic profiling, most of the samples extracted with ethanol and water were separated away from the extracts 100:0 and 0:100. The PLS regression biplot exhibited a positive correlation in the samples extracted with ethanol and water against DPPH radical scavenging activity, pancreatic lipase and and α-glucosidase inhibitory activity (Figure 1). It was also observed that TPC and FRAP were positively correlated to the samples extracted with ethanol and water, suggesting that the high phenolic content contributes to high antioxidant activity in the extracts (Zhang et al. 2014). In addition, the phenolics such as demethoxycurcumin were identified in the samples extracted with ethanol and water. For α-glucosidase inhibitory activity, 0:100 sample showed the lowest α-glucosidase in contrast to other samples. The difference may be due to the active compounds present in samples extracted with ethanol and water such as curcumanggoside and zerumin B compared to water extract. According to previous research, Zerumin B was stated as anticancer activity, while other compound referred to as important compounds for medicinal drugs purposes (Pande & Chanda, 2020).

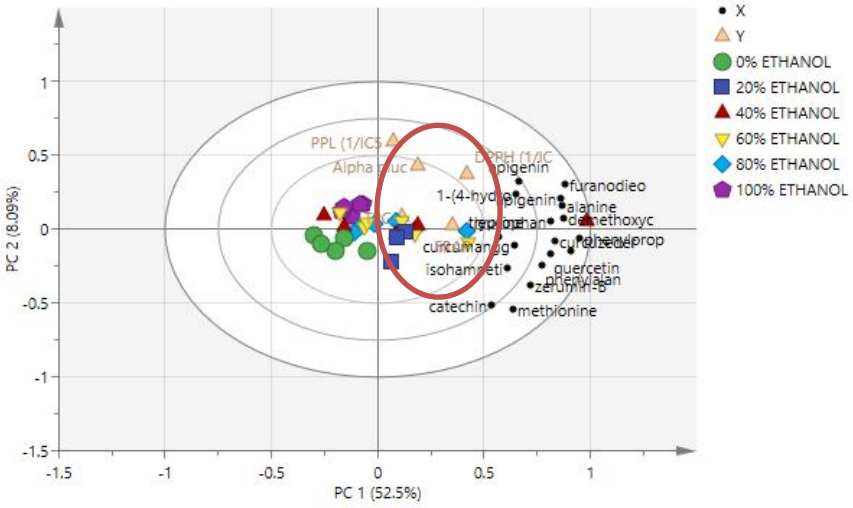

Figure 1. The biplot obtained from PLS describing the variation between the sample extracts and the correlation with the bioactivities.

Identification of active plant metabolites using LC-MS/MS

Further identification by LC-MS/MS revealed the presence of bioactive metabolites which includes rutin, gallic acid, kaempferol, triamcinolone benetonide, 4-Phenyl-3- furoxancarbonitrile, dichloroplatinum, Oxyphyllenone B and carbanilic acid among others. These are among the phenolic compounds that can be used as glucosidase inhibitors due to their bond with the protein and helps in controlling the blood glucose level (Lin et al., 2019). Gallic acid derived from phenolic acid compounds, originally from the plants that contain phenolic ring and carboxyl functional group. Compound oxyphyllenone B also identified in the sample belongs to the class of natural phenolic compound found in plants and marine organisms. It can help in the treatment of diabetes and should have been studied extensively for its potential medicinal and pharmacological applications.

CONCLUSIONS

The approach of using metabolomics as a tool to identify the diversity of metabolite profiles of the extracts with six different solvent ratios was successfully applied. NMR fingerprinting including PCA and PLS analysis allow the discovery of the best solvent according to their relative levels of metabolites. However, the information obtained in this study may not be fully understandable and accurately predict the ability of this extract to affect a whole organism. Hence, for better illustration and interpretation of the obtained results, further analysis such as in-vivo studies are recommended to acquire a clearer and detailed evidence (in progress). The study can be used to develop potential impact of phytochemical in *A. mutica* leaves extract through biomarker discovery and the elucidation of mechanisms related to obesity and diabetes which are important for diagnostics, prevention and treatment.

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THERMAL STABILITY KINETICS OF LYCOPENE AND B-CAROTENE IN GAC FRUIT ARIL PASTE (Momordica cochinchinensis)

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Abstract: Gac fruit is renowned for its health-promoting bioactive compounds, including lycopene and carotene. This study explored the impact of heating on gac aril paste at temperatures from 70°C to 90°C for up to 14 hours. Carotenoids were extracted using low-volume hexane assisted by ultrasonic extraction. Findings unveiled the first-order reaction kinetics governing lycopene and β-carotene degradation in gac aril, supported by high correlation coefficients (0.96 to 0.97). Lycopene exhibited accelerated degradation at 90°C ($k = 12.05 \times 10^2\ h^{-1}$), twice the rate observed at 70°C ($5.41 \times 10^2\ h^{-1}$). In contrast, β-carotene displayed slightly slower degradation rates, even at 90°C ($7.31 \times 10^2\ h^{-1}$). Corresponding half-life ($t_{1/2}$) values for lycopene ranged from 5.75 to 12.81 hrs, while β-carotene displayed values of 9.48 to 20.2 hrs. Notably, β-carotene exhibited better thermal stability across all heating temperatures when compared to lycopene. The kinetic parameters (k, $t_{1/2}$, and D values) indicated that increasing the heating temperature augmented the degradation rates of both lycopene and β-carotene, albeit with a moderate effect, as demonstrated by their respective activation energy (Ea) values of 33.7 and 27.5 kJ/mol. In summary, this study underscores the temperature-driven decline in lycopene and β-carotene levels in gac fruit aril paste.
***Keywords*:** Carotenoids, degradation, half-life, heating, kinetic parameters

INTRODUCTION

Gac (Momordica cochinchinensis) fruit, a unique tropical fruit originating from South-Eastern Asia and first discovered in Vietnam (Mai & Debaste, 2019), exhibits a charming orange-reddish exocarp upon ripening, covered by loosely packed soft thorns. Inside, beneath the spongy mesocarp, lies the vibrant red, sticky, and soft aril flesh enveloping the hard seeds (Vuong, 2000). This rich red hue is attributed to the abundance of carotenoids in its arils, particularly lycopene and β-carotene, the two key pigments responsible for its striking coloration (Baç et al., 2023). Notably, the aril surpasses the mesocarp and exocarp in carotenoid content (Aoki et al., 2002).

Research indicates lycopene and β-carotene's protective potential against cancer and chronic diseases such as prostate cancer (Tanambell, 2019), liver cancer (Navarro-Gonzalez et al., 2018), and cardiovascular diseases (Kulczynski et al., 2017). Moreover, their ability to combat singlet oxygen and peroxyl radicals positions them as effective agents against oxidation and UV radiation. Additionally, they serve as dietary precursors to vitamin A and contribute as natural orange-red pigments (Jafari et al., 2021).

The processing of Gac fruit's red aril flesh generally entails physical preparation integrated with heat treatments to ensure safety and edibility. However, this process could lead to a reduction in valuable carotenoids due to heat and oxidation-induced degradation (Baç et al., 2023). Multiple studies highlight the direct impact of thermal processing on carotenoid stability in food systems (Nkhata et al., 2020; Sarungallo et al., 2020). Hence, this study aims to unravel the kinetics of lycopene and β-carotene's thermal stability in Gac fruit aril paste across a temperature range of 70°C to 90°C.

MATERIALS AND METHODS

Materials

Gac fruits were obtained from Superfruit Valley, Chuping, Perlis. All chemicals used were analytical grade unless stated otherwise.

Gac arils and sample preparation

Gac fruits were cut in half to collect the red arils flesh whereas the exocarp and mesocarp was removed. The soft red arils were separated from the seeds and blended to obtained homogenized slurry.

Thermal treatment

The procedure were performed using Baç et al., (2023) method. Thermal inactivation tubes made from heat-resistant borosilicate glass were used for the heating experiments. Gac arils sample (7 mL) was pipetted into the tubes and the top were sealed by flame. Tubes were then placed in a water bath at 70, 80, and 90°C (Memmert WB 14, Schwabach, Germany) for 2-hour intervals (0, 2, 4, 6, 8, 10, 12, 14 hours). Extended heating times encouraged lycopene and β-carotene changes. Post-heating, tubes were cooled in icy water, opened, and reflectance at 25°C measured lycopene and β-carotene content (mg/g), loss (%), and remaining (%) for each interval.

Lycopene and β-carotene analysis

Ultrasound assisted extraction of lycopene and β-carotene was carried out according to the method described by Yilmaz et al. (2017) with the modification during sample preparation. Lycopene and β-carotene were then measured spectrophotometrically at absorbance of 453, 505 and 663 nm (Akter et al. 2020).

Degradation kinetic parameters

In this study, the degradation kinetics of lycopene and β-carotene in Gac aril paste were studied at various temperatures during heating. At all temperatures, the degradation of lycopene and β-carotene followed the first-order reaction kinetics. First-order reaction rate constants (k), half-life periods (t1/2) and D (Decimal reduction times) values for the degradation of these carotenoids were calculated from the following equations (1) to (3) respectively:

$\ln (Ct/Co) = -k\,t$ (1)

$t_{1/2} = -ln\ 0.5/k$ (2)

$D = 2.303/k$ (3)

Where t is heating time (h), k is the reaction rate constant (h−1), C0 is the initial concentration (mg/100 g) of the lycopene and β-carotene prior to heating. Temperature dependence of these degradations were determined by calculating the activation energy (Ea) and temperature coefficient (Q_{10}) values.Where k_0 is the frequency factor (h^{-1}), R is the universal gas constant (8.314 J/(mole K)), T is the absolute temperature (K).

Statistical analyses

Experimental data from lycopene and β-carotene contents were subjected to one-way analysis of variance (ANOVA) using the Minitab statistical software version 20. Duncan's multiple range test at 5% significance was run to determine the statistical differences among means.

RESULTS AND DISCUSSION

The stability of both pigments in Gac arils were compared with percentage losses and kinetic parameters. The increase in temperature from 70 to 90°C and time 0 to 14 hours has cause substantial losses in lycopene and β-carotene of Gac arils (Figure 1). The percentage loss of lycopene (77.1%; $p<0.05$) was higher and much severed compared to β-carotene (63.2%; $p<0.05$) at 90°C after over 14 hours of heating. In facts, even at 70°C half of lycopene percentage in Gac arils has already diminished (51.4%; 14 hours) compared to β-carotene at same time (36.7%; 14 hours). This shows that as the heating time increased on each temperature (70°C, 80°C, 90°C) both lycopene and β-carotene concentration gradually decrease, and percentage losses increases.

Determination coefficients (R^2) were calculated for zero-, first- and second-order models for lycopene and β-carotene and the highest R^2 values ($R^2 = 0.9511– 0.9816$) suggesting that the degradation of carotenoids in Gac arils followed first-order kinetic model. This findings was similar with another study showing decrease of total content of carotenoids in red fruit oil was directly proportional to the rise in temperature and heating time (Sarungallo et al., 2020). This probably due to absence of other supporting and protective antioxidants such as tocopherols, ascorbic acid and polyphenol after carotenoid extraction (Baç et al., 2023).

Temperature dependence of lycopene and β-carotene degradations in Gac arils was evaluated by comparing the Ea and Q_{10} values. It is interesting to note that the highest Ea for lycopene (Ea=54.1505 kJ/mol) reflecting its highest sensitivity on temperature elevations starts at temperature of 70°C compared to β-carotene with highest (Ea=31.3685 kJ/mol) at temperature setting of 80°C.

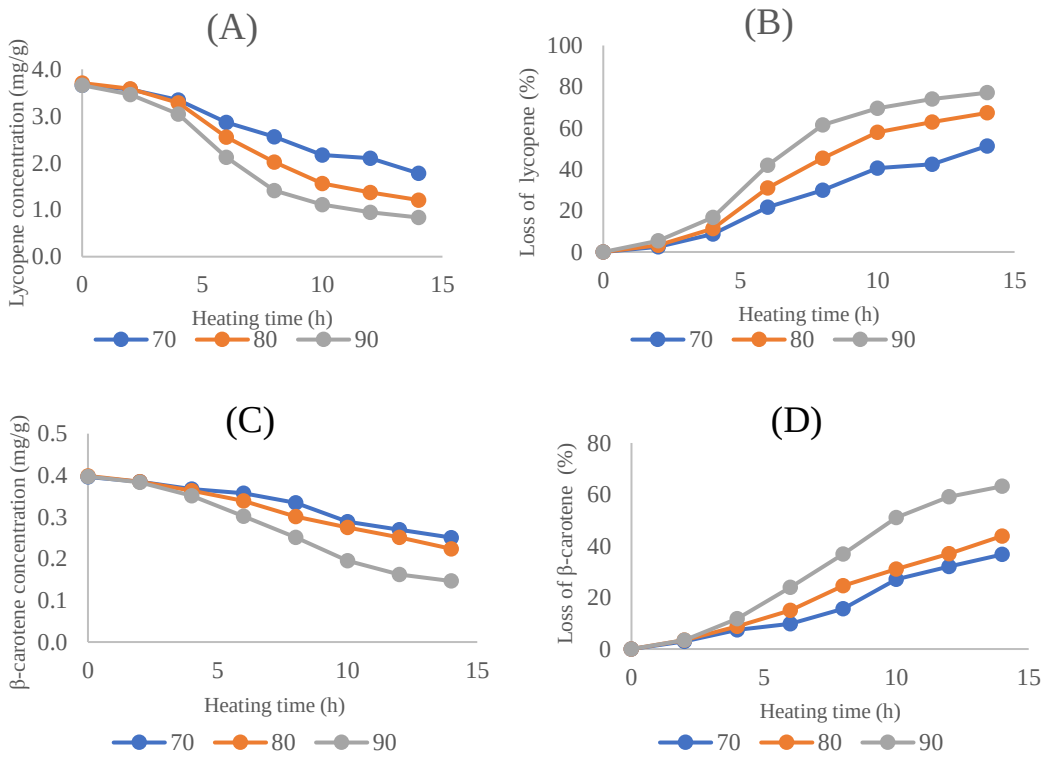

Figure 1. Changes of lycopene concentration (A), lycopene percentage loss (B), β-carotene concentration (C), and β-carotene percentage loss (D) in Gac aril fruits at different temperatures and heating times

Lycopene also exhibited higher and accelerated degradation at 90°C ($k = 12.05 \times 10^2\ h^{-1}$) doubled the rate observed at 70°C ($k = 5.41 \times 10^2\ h^{-1}$) while β-carotene has slightly slower degradation rates even at 90°C ($k=7.31 \times 10^2\ h^{-1}$). This shows that β-carotene able to tolerate higher heat treatment and less sensitive to degradation whereas lycopene begins to degrade at 70°C and progressively declining as heating increases. This heightens sensitiveness towards elevated temperature contribute to higher losses percentage of lycopene as the temperature and time increases as seen in Figure 1(B).

Both lycopene and β-carotene have highest Q_{10} values at 90°C (Q_{10} lycopene=2.2274; Q_{10} β-carotene =2.1312) indicating they were sensitive to an elevation of temperature ranging 70 to 90°C resulting similar pattern of degradation as demonstrated in Figure 2(A) and Figure 2(B). It is safe to mention that β-carotene in Gac arils was generally more preserved and highly stable during heating compared to lycopene. Lycopene has twice the singlet oxygen quenching activity makes it better antioxidant compound and more reactive compared to other carotenoids. In opposite, the two double bonds of β-ionone rings in β-carotene structure do not take part in the polyene chain therefore reduces the reactivity and results in higher stability of β-carotene (Baç et al., 2023; Ferreira and Rodriguez-Amaya 2008). Higher losses of lycopene may also be contributed by the tendency of lycopene isomerization. Moderate heat treatment (i.e; 80 °C for 4 h) can lead to isomerization of lycopene from a fraction of all trans-isomer to different cis-isomer (Phan-Thi and Waché 2014).

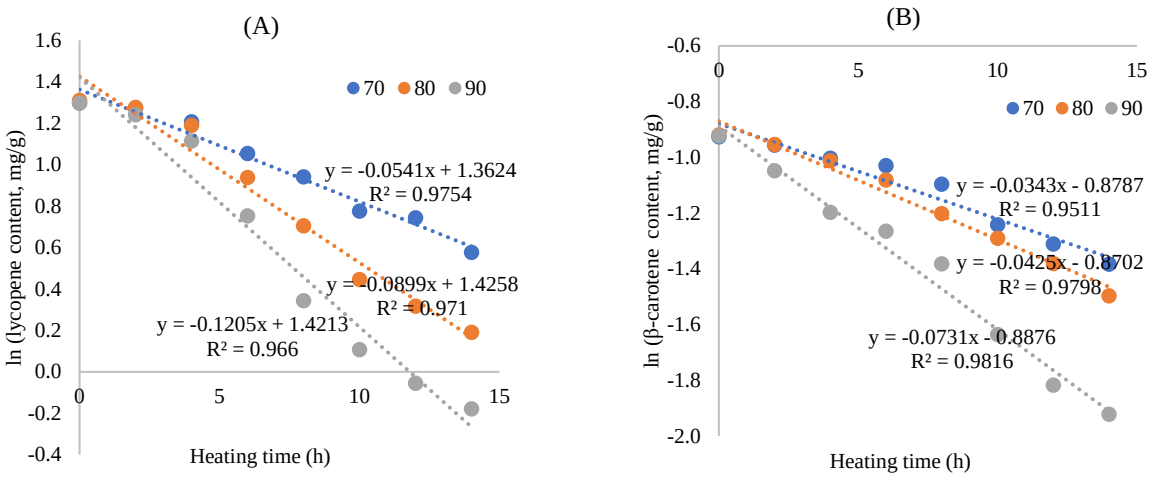

Figure 2. Changes in the lycopene (A) and β-carotene (B) contents of Gac at different temperatures

CONCLUSIONS

Overall, thermal degradation of lycopene and β-carotene in Gac aril were greatly relies on temperature and time. The stabilities of both lycopene and β-carotene were decreased as k values increases, $t_{1/2}$ and D values decreases. The degradation of lycopene and β-carotene followed first-order kinetic model curves with heating effect and percentage losses observed was more pronounced in lycopene.

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IN VITRO PREBIOTIC EVALUATION OF EDIBLE BIRD'S NEST AND HYDROLYSATE BY USING HUMAN FAECAL SLURRY

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Abstract: Edible Bird's Nest (EBN) is the dried salivary secretion from the swiftlets. EBN was reported to consist unique and high valued glycoprotein, sialic acid, epidermal growth factor (EGF), and other components that promote health condition. Today, EBN is utilized in many industries yet bounded due to limitation of insolubility and low extraction rate. This study thus produces soluble EBN hydrolysate using enzymatic hydrolysis. However, information of EBN towards the human gastrointestinal health is scarce. **Objective:** This study aimed to investigate the digestibility and prebiotic activity of EBN as a food ingredient. **Method:** *in vitro* upper gastrointestinal digestion was performed followed by *in vitro* fermentation using colon model. Prebiotic evaluation was assessed through bacterial enumeration by fluorescent *in situ* hybridisation (FISH); and quantification of beneficial short-chain fatty acids (SCFA) using HPLC. **Findings:** This present study revealed that population of probiotics, *Bifidobacterium* and *Lactobacillus* have demonstrated a positive response similar to fructooligosaccharides (FOS). Both raw EBN and hydrolysate also highlighted a significantly higher inhibition for pathogenic *Clostridium histolyticum* group. The metabolites analyzed also showed a production similar to FOS. **Future Recommendation:** These findings thus summarized that consumption of EBN is beneficial to the human gastrointestinal health by means of preventing colonic diseases such as colon cancer and other related disorders. However, further study regarding to EBN as a functional food in relation to colon cancer is still required for confirmation.
***Keywords*:** bioactive glycopeptide, edible swiftlet's nest (ESN), functional food, *in vitro* fermentation, colon model study.

INTRODUCTION

Edible bird's nest (EBN) is dried gelatinised salivary secretion from swiftlet during the breeding season (Guo *et al.*, 2006). In Malaysia, the common source of consumable EBN is mainly the white and black EBN produced from *Aerodramus fuciphagus* and *Aerodramus maximus* respectively. Consumption of EBN helps in treating several health disorders and consumptive diseases such as autoimmune diseases, coughs, tuberculosis, asthma, stomach ulcer, gastrointestinal disorders, promoting physical and mental strength, and anti-aging thus maintaining youthfulness (Hui Yan et al., 2021). Yet, the application has been limited in various industries due to the physico-chemical properties such as low solubility and extraction rate. Therefore, this study applies enzymatic biotechnology on EBN for improvement. This biotechnology is also a low cost, eco-friendly alternative to reduce processing wastage, pollution and economic cost through utilization and bioconversion into high-grade product. For instances, enzymatic hydrolysis on many plants and animal proteins has been used to produce bioactive peptide (BAP) from milk, casein, whey, egg, meat, fish, soy beans, sesame bran and oil byproducts, peas, and even food waste like fish skin. In terms of consumption, the process of enzymatic technology applied may ease the absorption and assimilation of its nutrition in the human gastrointestinal system due to the production of simpler EBN molecules. Based on previous research, the glycan part in glycoprotein enhances solubility and provides ability in resisting proteolytic enzymatic digestion which provides them possible strength to remain intact until reaching the colon and lead to the prebiotic potential of EBN itself. However, the study of digestibility is scarce and no study was done regarding to prebiotic potential of EBN. Therefore, this study investigates the digestibility and the prebiotic potential of EBN and hydrolysate, as well as the composition changes through *in vitro* colon model that mimic the human gastrointestinal environment. The evaluation of SCFA production and bacteria composition changes due to the input of EBN as substrate is therefore the precursor for the investigation towards colon health.

MATERIALS AND METHODS

Preparation of Sample
Cleaned edible bird's nest (EBN) sample was purchased from Mobile Harvester Sdn. Bhd., Shah Alam, Selangor, Malaysia. Fructo-oligosaccharide (FOS) is used as the positive control in this study.

Production of EBN Hydrolysate
Sample (1%) was soaked in distilled water overnight at 4°C prior to double-boiling process as described in previous report *(Hui Yan et al., 2022)*. Generally, sample was heated for 30 minutes in a water-bath then left to cool down. Protease (1%) was used to perform enzymatic hydrolysis on the double-boiled EBN sample at pH 8.0 and 60°C for 1 hour. Sample was then brought to boil for deactivation purposes. Sample underwent freeze-drying for storage.

***In vitro* Upper Gastrointestinal Digestion**
Digestion process was performed as described in *Lee-Ling et al., (Lee-Ling et al., 2022)*. Simulated digestion fluid for oral, gastric, and duodenal-intestinal phase was prepared and warmed to 37°C prior to the addition of digestive enzymes which will initiate the digestion process. Sample (1.5g) in 15 mL distilled water underwent oral phase digestion using heat-stable α-amylase (75 U/mL) at pH 7.0 for 2 minutes in a shaking incubator. Sample mixture was then adjusted to pH 3.0 for gastric phase digestion using pepsin (2000 U/mL) for 2 hours at 170 rpm, followed by the duodenal-intestinal digestion using bile salt (10 mM) and pancreatin (2000 U/mL

Preparation of Human Faecal Slurry
Fresh human faecal was obtained from healthy volunteers with BMI 19-23 kg/m^2; age between 21-30 years old *(Nashri et al., 2023)*. Volunteer was required to have no prescribed antibiotics for at least 6 months, no history of gastrointestinal diseases, and no intake of any supplements including prebiotic and probiotics. Fresh faecal sample (25 g) was obtained on-site and diluted with 250 mL sterile phosphate buffered solution (PBS) in an aerobic chamber. Faecal sample was homogenized using a stomacher before proceeding to fermentation.

***In vitro* Colon Model Fermentation**
Fermentation was conducted using a colon model to mimic the human colon environment *(Sarbini et al., 2013)*. Generally, sterile basal nutrient media (BNM, 40 mL) was added into the colon model vessel (Figure 1) and purged overnight with nitrogen gas (15.0 mL/min) to eliminate presence of oxygen. Digested sample (0.5 g) was then added into vessel before the addition of human faecal slurry (10 mL). Fermentation was performed anaerobically at 37°C and pH 6.8 for 24 hours. Sampling was performed at 0, 6, 12, and 24 hours for analyses.

Prebiotic Evaluation
Human Colonic Microbial Enumeration
Fluorescent *in situ* hybridization (FISH) technique was used to enumerate colonic microbial changes *(Rawi et al., 2021)*. Enumeration was conducted using synthetic oligosaccharide probe with specific targeted region of 16s rRNA molecule labelled with fluorescent Cy3 dye. Specific probes used include Bif164, Lab158, Chis150, and Bac303 for the enumeration of *Bifidobacteria*, *Lactobacilli*, *Clostridium Histolyticum*, and *Bacteroides* group respectively. Enumeration of the stained bacterial was conducted under a epifluorescence microscope (*Olympus CX3*).

Metabolites Production by Colonic Microbiome
Short-chain fatty acids (SCFA) quantification was performed using High-Performance Liquid Chromatography (HPLC) equipped with C12 silica column (*Phenomenex*) heated to 40°C, UV detector with wavelength at 210 nm, 0.25 mM sulphuric acid as mobile phase. Sample (20 uL) was introduced into the HPLC at a flow rate of 0.5 mL/min for the detection of acetic acid, butyric acid, and propionic acid.

RESULTS AND DISCUSSION

Table 1 showed the population changes of human faecal microbiota from the *in vitro* colonic fermentation. It is revealed that EBN and hydrolysate positively stimulates the growth of human gastrointestinal microbe. Particularly the probiotic strains, both double-boiled EBN and hydrolysate has increased their growth similar to those in positive control (FOS). This is the contribution of the oligosaccharides in carbohydrate-glycan part of EBN. Low molecular weight (MW) oligosaccharides are preferable for *Bifidobacteria* (Gibson, 2004). Foucaud *et al.*(2001) reported that these bacteria relied on exogenous nitrogen source such as proteins, peptides and amino acids for growth. Hebert *et al.*(2000) also stated the utilization of protein/peptide source preferred a low MW. Therefore, EBN hydrolysate is a more suitable substrate for *Lactobacillus*. Garrett and Onderdonk (2015) reviewed that *Bacteroides* are symbiotic in the colon which benefit the host metabolism and immunity. They are mainly saccharolytic metabolism which promote

their growth in Table 1. The findings also showed a suppressing effect on the growth of *Clostridium histolyticum* group in the fermentation of EBN and hydrolysate. Toxigenicity study of *Clostridium histolyticum* has reported that sugar is one of the growth inhibiting factor (Nishida and Imaizumi, 1966). Thus, *Clostridium* growth is inhibited by the carbohydrate-glycan as well as the presence of protein and peptide as major nutrient in EBN. The antimicrobial metabolites produced by *Bifidobacterium* and *Lactobacillus* also provide an inhibition to the growth of *Clostridium* (Collado *et al.*, 2005). These antimicrobial metabolites include short-chain fatty acids (SCFA) and antimicrobial peptides named bacteriocin and pediocin.

Table 1. Population of human faecal microbiota from the *in vitro* fermentation of EBN and hydrolysate.

Sample	Time (h)	Population of Specific Bacterial Group (Log 10 cells/mL)			
		***Bifidobacterium* spp.**	***Lactobacillus-Enterococcus***	***Clostridium histolyticum* group**	***Bacteroidaceae spp., Prevotellaceae spp.* and *Porhyromonadaceae spp.***
FOS	0	7.92 ± 0.04	7.30 ± 0.07	7.56 ± 0.05	8.04 ± 0.04
	6	8.32 ± 0.03 ^{abc*}	7.93 ± 0.08 ^{ab*}	7.77 ± 0.03 ^{a*}	8.33 ± 0.04 ^{b*}
	12	8.39 ± 0.03 ^{a*}	8.00 ± 0.07 ^{a*}	7.71 ± 0.05 ^{b*}	8.32 ± 0.05 ^{c*}
	24	8.43 ± 0.02 ^{a*}	8.10 ± 0.03 ^{a*}	7.64 ± 0.07 ^{b}	8.42 ± 0.03 ^{abc*}
NEG	0	7.92 ± 0.04	7.30 ± 0.07	7.56 ± 0.05	8.04 ± 0.04
	6	8.27 ± 0.03 ^{c*}	7.83 ± 0.01 ^{d*}	7.75 ± 0.03 ^{a*}	8.38 ± 0.03 ^{a*}
	12	8.20 ± 0.05 ^{d*}	7.61 ± 0.14 ^{c*}	7.85 ± 0.05 ^{a*}	8.44 ± 0.03 ^{a*}
	24	8.17 ± 0.03 ^{c*}	7.60 ± 0.10 ^{d*}	7.91 ± 0.02 ^{a*}	8.48 ± 0.05 ^{ab*}
ER	0	7.92 ± 0.04	7.30 ± 0.07	7.56 ± 0.05	8.04 ± 0.04
	6	8.30 ± 0.03 ^{abc*}	7.85 ± 0.07 ^{cd*}	7.62 ± 0.03 ^{b}	8.32 ± 0.06 ^{bc*}
	12	8.33 ± 0.01 ^{bc*}	7.90 ± 0.10 ^{ab*}	7.57 ± 0.08 ^{c}	8.36 ± 0.05 ^{bc*}
	24	8.39 ± 0.05 ^{a*}	8.01 ± 0.01 ^{b*}	7.46 ± 0.08 ^{c*}	8.41 ± 0.03 ^{bc*}
EH-1	0	7.93 ± 0.07	7.38 ± 0.06	7.53 ± 0.05	8.02 ± 0.03
	6	8.32 ± 0.05 ^{ab*}	7.87 ± 0.02 ^{bcd*}	7.62 ± 0.02 ^{b}	8.28 ± 0.02 ^{c*}
	12	8.35 ± 0.04 ^{ab*}	7.93 ± 0.03 ^{ab*}	7.40 ± 0.09 ^{d*}	8.36 ± 0.01 ^{bc*}
	24	8.40 ± 0.03 ^{a*}	8.00 ± 0.07 ^{b*}	7.48 ± 0.04 ^{c*}	8.44 ± 0.05 ^{abc*}

$^{a, b, c, d}$ Superscript with unlike letters shows significantly higher/lower in comparing among samples in the same fermentation period (t) at confidence level of 95%. * Means that shows significantly difference compared with 0 hour within the same sample from 0 to 24 hours of fermentation period (t) at confidence level of 95%. (n=3). (Note: FOS represents fructo-oligosaccharide as positive control, NEG represents treatment without any substrate as negative control, EBN represents edible bird's nest, ER represents double-boiled raw EBN and; EH-1 represents EBN hydrolysates with 1 hour hydrolysis)

Table 2. Short-chained fatty acids (SCFA) production from the *in vitro* fermentation of EBN and hydrolysate.

Sample	Time (h)	Acetic Acid (mM)	Propionic Acid (mM)	Butyric Acid (mM)
FOS (+)	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	6	154.52 ± 81.44 ^{ab*}	46.55 ± 18.71 ^{a*}	55.84 ± 10.79 ^{b*}
	12	112.91 ± 22.48 ^{ab*}	43.02 ± 16.45 ^{ab*}	34.31 ± 11.00 ^{b*}
	24	141.11 ± 21.51 ^{ab*}	49.10 ± 17.98 ^{a*}	39.87 ± 7.51 ^{b*}
NEG (-)	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	6	54.10 ± 6.73 ^{b*}	26.61 ± 3.27 ^{a*}	159.70 ± 58.11 ^{a*}
	12	60.56 ± 28.18 ^{b*}	31.72 ± 3.76 ^{abc*}	130.59 ± 99.17 ^{a*}
	24	67.71 ± 14.23 ^{b*}	37.37 ± 8.94 ^{a*}	138.07 ± 107.38 ^{a*}
ER	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	6	127.16 ± 36.33 ^{ab*}	47.23 ± 28.77 ^{a*}	99.07 ± 39.54 ^{b*}
	12	201.50 ± 137.38 ^{ab*}	52.22 ± 14.63 ^{a*}	56.80 ± 39.52 ^{ab}
	24	201.44 ± 97.89 ^{ab*}	50.71 ± 39.72 ^{a*}	21.24 ± 17.81 ^{b}
EH-1	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	6	189.89 ± 118.27 ^{ab*}	33.97 ± 7.78 ^{a*}	85.37 ± 51.11 ^{b*}
	12	291.56 ± 132.19 ^{a*}	35.01 ± 1.65 ^{abc*}	55.16 ± 38.44 ^{ab}
	24	310.44 ± 186.47 ^{a*}	28.31 ± 18.22 ^{a*}	13.42 ± 6.71 ^{b}

$^{a, b, c, d}$ Superscript letters shows significantly higher/lower concentration (mM) in comparing among samples in the same fermentation period (t) at confidence level of 95%. * Means that shows significantly difference compared with 0 hour within the same sample from 0 to 24 hours of fermentation period (t) at confidence level of 95%. (n=3) (Note: FOS represents fructo-oligosaccharide as positive control, NEG represents treatment without any substrate as negative control, EBN represents edible bird's nest, ER represents double-boiled raw EBN and; EH-1 represents EBN hydrolysates with 1 hour hydrolysis)

Table 2 showed the organic acids production from the *in vitro* colonic fermentation of EBN and hydrolysates. Results showed that EBN and hydrolysate stimulates the SCFA production better than those in FOS. In which, SCFA production in EBN and hydrolysate has a regular increasing trend with higher concentration when comapred to those in FOS. Table 2 showed that acetic acid is the major metabolites derived from the fermentation of EBN and hydrolysates. This is result from the conversion of lactate through cross feeding among various colonic bacteria. In which, Louis *et al.* (2007) stated that anaerobic fermentation of carbohydrate by intestinal microbes produces acetic acid in abundant amount. This is mostly the contribution of the lactic acid bacteria (LAB) such as *Bifidobacteria* as

well as proteobacteria (Flint *et al.*, 2015; Ríos-Covián *et al.*, 2016). Garrett and Onderdonk (2015) also reported that *Bacteroidaceae* spp., *Prevotellaceae* spp. and *Porphyromonadaceae* spp. ferment carbohydrate and derives metabolites such as acetic acid. In terms of propionic acid production, this is related to the significant high population of *Bacteroides* group. It is also reported that main producers of propionic acid is mainly the *Bacteroides-Prevotella* group and *Clostridium* in the colonic bacteria (Olano-Martin et al., 2000). The fermentation of EBN and hydrolysate provide sufficient substrates for *Bacteroides* growth due to their capability in utilizing both carbohydrate-glycan and protein sources. The main butyrogenic bacteria belongs to the Firmicutes phylum, specifically in the *Clostridial* group (Vital et al., 2014). Therefore, the suppressed of *Clostridial* group in fermentation of EBN and hydrolysates has decreased the butyrate production.

CONCLUSIONS

Conclusively, edible bird's nest (EBN) and hydrolysate has positive prebiotic responses as a functional food for the well-being of human gastrointestinal health. In which, EBN promotes the growth of colonic strains, particularly the probiotics; suppressed growth of pathogenic *Clostridium* group; and aids in production of beneficial metabolites, i.e., the short-chain fatty acids.

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THE LOCAL INDIGENOUS FOOD OF SARAWAK AS POTENTIAL FUNCTIONAL FOOD

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Abstract: Sarawak has a distinctive flora and fauna. Until today, the potential of the local Sarawak food ingredient is very much unexploited. The local people of Sarawak usually made unique dishes out from these unique food resources. One of the main produces of Sarawak is the starch from *Metroxylon sagu*. Other than positive bioactivities such as anti-oxidative, our studies also finds that sago starch has a prebiotic response. In which, it promotes probiotic growth, suppress on pathogen, and production of health beneficial short-chained fatty acids. Other studies also reported on indigenous fruits and vegetables as potential functional food. Our work on the edible fern (*midin*), edible palm hearts (*umbut*), spices such as turmeric and pepper from Sarawak also revealed a positive functionality in terms of prebiotic potential, anti-oxidative, anti-microbial, and others. It is summarised the potential functional food from the local Sarawak market. This may contribute to not only value-added the rare local food resources, it also increases the production and economic of the local market by introducing these valuable foods to a wider market.
***Keywords*:** Borneo food, Prebiotic, Spices, Wild crops, Malaysian Food

INTRODUCTION

By having large sources of fruits and vegetables, Sarawak, Malaysia has a remarkable diversity of flora. These precious resources have been an important food resources for the local Sarawakian ever since. These wild crops therefore has become unique dishes and even significance symbols in their culture. One of the signiture food in Sarawak is the Sago starch, whereby sago pearls are widely distribute in many places and served as a dessert. For instances, the *Sagu Gula Melaka* (Sago pearls in coconute syrup). In Sarawak, sago starch is served not only as a dessert, but also as the main dish in the daily meals of Sarwakian. To go with these main dishes, vegetables such as the edible fern known as *midin*, the core of palm tree as edible palm hearts, the sour eggplant named *terung asam*, spices like black pepper, white pepper, and many rare and wild flavouring plants were involved in their daily meals. Not to mentioned the unqie local fruits, namely *Dabai*, the Sarawak "Olive", the native *Terap* fruit that looks like a jackfruit, the nipa palm sugar (*gula apong*), *Engkalak*, the Sarawak "Avocado" and *Embang*, the local wild mango. Not to forget, one of the most high priced product of Sarawak, the edible bird's nest (EBN), which is the "caviar of the East" and Sarawak is the third largest producer throughout the world. These indigenous foods and crops are rare, yet commonly utilised by the local people due to the beliefs of its high valued nutritional fucntionalities.However, studies regarding the nutritional value and health beenficial functionalities are scarce. This review thus increased the awareness of researchers in this related field. This then contribute to the broaden the market value of these indigenous food with great nutritional functionalities.

THE SAGO STARCH

Malaysia is currently the largest producer of sago starch, particularly Sarawak of East Malaysia. Sago starch derived from the pith of a sago palm tree (*Metroxylon sagu*) using wet milling process (Zailani et al., 2022). Whereby, the tree trunk of the sago palm tree is break into pieces, grinded, filtered, and left for sedimentation to obtain the sago starch, the locally known *Lemantak* (Arshad et al., 2018). Sago starch is usually consumed in the form of sago pearls, cookies, also in the form of glue-like starch paste called *linut* in Sarawak. Studies have reported that consumption of sago starch is beneficial to human health. In which, it helps to manage body weight, reduce risk for heart diseases, control blood pressure, and improve circulation. This is due to the high resistant starch (RS) content of up to 69% which made the sago starch itself to be low digestible and thus, an alternative for starch with low glycaemic index and prebiotic effects (Thompson et al., 2023).

MODIFICATION OF RESISTANT STARCH FROM SAGO

Food products with a high content of low digestible starch will have a low glycaemic index which is beneficial to diabetic patients (Zailani et al., 2022). Modification of starch was conducted on various starches which include corn, cassava, rice, potato, and lotus starch. The modification of starches involves three main treatment techniques which are the physical, chemical, or enzymatic treatments. The combination of either two or all three types of methods was also investigated. The chemical and enzymatic treatment had displayed promising improvement in the functionality and benefit of starches such as better emulsifying properties and higher resistant starch content.

THE SARAWAK BLACK AND WHITE PEPPER

Black and white pepper of the species Piper nigrum L. is regarded as the king of spices, and Malaysia is the second largest producer of peppercorns, after Indonesia. This spice contains abundant bioactive compounds that are capable of enhancing human health. This spice contains high bioactive compounds in enhancing human health and contains up to 33% dietary fibre. Other than that, the presence of alkaloids, polyphenols and flavonoids also increases its ability to act as a prebiotic. Some studies conducted proved that these secondary metabolites might contribute to improving intestinal health by maintaining the microbial environment in the gut through stimulation of *Lactobacilli* and *Bifidobacteria*, and inhibiting the pathogenic bacteria population in the human gut, which exerting the prebiotic-like effects (Dreger et al., 2014). *Piper nigrum* L. also showed promising result and its effectiveness as prebiotic ingredient as food ingredient (Nashri et al., 2023). Also, in livestock production, it has a positive effect towards the appetite also act as antibiotic substitute.

THE FAMOUS EDIBLE FERN: *MIDIN*

Stenochlaena palustris or *midin* is an indigenous edible fern found in southeast Asia. This red young fern is commonly consumed among local people as a vegetable dish, juice, and traditional medicine. Traditionally, *midin* is widely consumed among the local people as stir-fried dish mixed with garlic, dry shrimps and shrimp paste (Dash, 2016). When cooked, *midin* has a crunchy-succulent texture. *Midin* is also served as salad with dressing of vinegar, dried shrimps or anchovies, locally it is known as '*ulam*' in Malay language (Chai, 2016; Dash, 2016). Besides that, the studies also mentioned that *midin* is consumed as juice in the old days. In which, it is believed that juice of *midin* is used to treat fever (Ponnusamy et al., 2013). The unique component of *midin* is the abundant mucilage, a sticky, gluey substance within the plant which may contribute to the pharmacology properties of *midin*. Studies validated these health beneficial beliefs of *midin* such as antimicrobial, antifungal and anti-oxidative. Today, our study also revealed that *midin* has a positive prebiotic response. In which, *in vitro* study of *midin* stimulates the growth of probiotic strains like *Lactobacillus spp. a*nd *Bifidobacteria spp.* (Hui Yan et al., 2022).

THE LOCAL OLIVE: *DABAI* FRUIT

One of the underutilised local indigenous fruits in Sarawak that come from the Burseraceae family is Dabai (*Canarium odontophyllum*). Dabai is highly demanded due to its natural delicious creamy and 'fatty' taste almost similar to avocado, being exotic and unique to Sarawak, and also its rich in nutritional properties such as protein, fat, energy and carbohydrate (Mundi et al., 2022). Hence, the oil extraction of nutritive *dabai* has become one of the interests of the recent studies. The chemical characteristics of the extracted oils from both the *dabai* flesh and the kernel were discussed. A total of approximately 75 recognised species have been found mainly in Asia, the Pacific and tropical Africa. Later led to further investigation of the possible cholesterol-lowering agent due to its high antioxidant ability. The fruit is slightly triangular in the cross-section, ovoid to ellipsoid. It is also rich in phenolic compounds and vitamin E, such as γ-tocopherol. In addition to the nutritional value of dabai fruit, it has been proven that extract from dabai is able to lower plasma cholesterol (Mokiran et al., 2014). It is also reported that *dabai* has a potential for anti-acetylcholinesterase activity from the extract of the flesh and seed of dabai (Ali-Hassan et al., 2013). In which, acetylcholinesterase is responsible to breakdown of acetylcholine, which act as a neurotransmitter that prevent the loss of cholinergic neurons that causes Alzheimer's disease.

GULA APONG (NIPA PALM SUGAR), THE MUST HAVE INGREDEINT FOR SARAWAK ICE-CREAM

Nipa sap or locally known as *air sadap* or *air nira* is a traditional beverage consumed by people in Sarawak. While, *gula apong* is the nipa palm sugar (*Nypa fructicans*) is made by boiling to concentrate the sap of nipa palm tree. It has a golden caramel colour and unique fragrance sweet taste (Jaraee et al., 2023). It is commonly used as an alternative sweetener in cakes/*kuih*, desserts and beverages in Sarawak. Whereby, it contains high amounts of vitamins and minerals and hence its popularity increased as a healthier alternative of white and brown sugar.

THE NATIVE FRUIT, *TERAP*

In Sarawak, *Terap* or *Artocarpus odoratissimus* is famous for the unique fragrance, taste, and crispy texture. In which, *Terap* has been gaining visibility in the local industries in recent years. However, studies on the nutritional health benefits of *Terap* is scarce. It is reported that *Terap* contains high carbohydrate content, Vitamin B1, and potassium is the major mineral component found in *Terap* (Ismail et al., 2021). Recent studies on the seed and oil of *Terap* also reported with several functional properties (Ismail et al., 2022). In which, they are fried and consumed as snacks with fatty flavour similar to hazelnuts.

EMBANG, THE WILD MANGO OF SARAWAK

Embang is also commonly known as *bambangan* (*Mangifera pajang*) (Jahurul et al., 2018). In fact, it is a ovoid shaped wild mango in Anacardiaceae family, Sapindales order, and Magnoliopsida class. It has a bright yellow flesh with sweet and sour taste. Meanwhile, the kernel of *embang* is high in carbohydrate and plant fats. It is rich in source of fibre, vitamin C, carotenoid, anti-oxidative compounds, and phenolic contents. Particularly the total phenolic content (TPC), studies on the fats in the kernal of *embang* is economically important for production of ducntional food, pharmaceutical and nutraceutical products instead of discard as waste.

ENGKALAK, THE LOCAL "AVOCADO"

Engkalak or scientifically known as *Litsea garciae* is the local "avocado" of Sarawak. It has a white flesh with a creamy sweet taste (Mahlan, 2022) . The fruit is usually soaked in hot water until soften before consumption. It can be eaten raw or with rice and other spices and flavoring. It is reported the *engkalak* has an energy content less than avocado but higher than those in banana. In which, it has a higher fat content (6.8%) than its protein content (1.4%). It also has a high saturated fatty acid (76.94%) with lauric acid as the main component (40.73%). Other than that, it also contains phenolic contents, flavonoids which lead to high antioxidative properties. (Amit & Zinyin, 2021). The study also reported a positive response in terms of antibacterial, antifungal, anticancer, and anti-inflammatory activities. In which, these nutritional functionalities have made *engkalak* a potential functional food beneficial for human health.

EDIBLE PALM HEARTS (*UMBUT)*

Edible palm hearts (EPH), known as palmito, chonta or swamp cabbage in America or umbut in Malaysia, is a type of vegetable harvested from palm tree species. Edible palm hearts (EPH) are the edible cores from palm tree stems, and are also an alternative source of carbohydrates and/or dietary fibre (Lee-Ling et al., 2022). Its crisp texture makes EPH an ideal addition in dishes such as salads and stir-fry meals. The EPH is firm and smooth and described as having a flavour resembling artichoke. Therefore, our previous work on the nutritional and prebiotic potential of EPH as a food ingredient disclosed that EPH contain abundant carbohydrates, insoluble fibre as well as essential macro-minerals such as potassium, magnesium, phosphorus, and calcium. In which, these has supported the growth of *Lactobacillus*, the probiotic strains which made EPH a potential prebiotic ingredient. It also helps in inhibiting the growth of colonic pathogen and being selectively fermented by the colon microbiota.

TERUNG ASAM SARAWAK, THE SOUR EGGPLANT

The sour eggplant or *Terung Asam* Sarawak (TAS) or scientifically known as *Solanum lasiocarpum Dunal* is one of the indigenous crops in Sarawak (Soon & Ding, 2021). Traditionally, it is commonly served in the form of soup cooked with seafood, or any meat. It is also a choice of the locals to cook with fish due to their ability to reduce the fishy odour and fattiness of pork. In terms of nutritional value, it is high in Vitamin C, also calcium, fibre, phosphorus, potassium and also other minerals. Other than that, the TAS fruit contains phenolic compounds and flavonoids with powerful anti-oxidative activities (Ab Rahman et al., 2019).

THE PRECIOUS EDIBLE BIRD'S NEST

Edible swiftlet's nest (ESN) is dried gelatinized saliva secreted by swiftlets during the breeding season. The ESN has been widely consumed as a food and medicine since the ancient dynasty of China, particularly in the practice of Traditional Chinese Medicine (TCM). As a food with health-promoting effects, this made ESN a potential functional food. Whereby, functional food is food that can be consumed in the daily diet which then enhanced human health through nutritional aspect, but not as the cure of a disease. Scientific evidence has proven that ESN consists of the unique glycoprotein of great value which provides high nutritional and functional properties for human health benefits. These include anti-ageing, anti-hypertension, immunity and neurological enhancement contributed by not only the unique glycoprotein but also sialic acid, epidermal growth factor (EGF) and other bioactive compounds (Hui Yan et al., 2021). ESN also can be incorporated further as a complete functional food by fortification of vitamins, high fibre

diets and prebiotic ingredients which contribute in promoting probiotic growth, suppressing the growth of pathogen and promote the production of beneficial organic acids in the human body.

OTHERS

Other indigenous food in Sarawak includes the *kelulut* honey or stingless bee honey, which contains higher protein content and phenolic compounds that lead to high anti-oxidative activities and anti-bacterial capacity (Tuksitha et al., 2018). Also, indigenous durians namely *Durio graveolens* and *Durio oxleyanus* with extraordinary flavour and aroma (Sujang et al., 2022). There are also a few indigenous food flavouring plants that were used by the locals like *Pangium edule* (daun kepayang), *Premna serratifolia* (daun singkil), *Pycnarrhena tumefacta* (daun tubu), *Scorodocarpus borneensis* (daun kesinduk), and *Syzygium polyanthum* (daun bungkang) as flavour enhancer or modifier in their cooking (Yusli et al., 2022). In which, these wild herbs are high in anti-oxidative activities for its phenolic, flavonoid, and other biocompounds.

CONCLUSIONS

There are various of ethnic foods in Sarawak of Malaysia, in which these foods are commonly consumed by the locals in various ways. The indigenous foods and crops contain nutrients which then promote human health in many ways as a food that can be consumed daily. However, information on these wild crops and foods are scarce and required further studies by researchers. Thus, contribute in broaden the local market of Sarawak.

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QUALITY EVALUATION OF MUFFINS INCORPORATED WITH OKARA FLOUR

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Abstract: The present study was carried out to develop an optimum formulation of baked muffins incorporated with Okara flour. The experiment was conducted in an Extreme Vertices Design and generated formulations with different levels of critical components including wheat flour, okara flour, and refined granulated sugar. The oral texture using QDA, physicochemical using Textural analysis by TPA and internal structure, ink imprint test, and physical characteristics (pH and Water Activity) were evaluated for all formulated baked muffins and control samples. The results revealed that the optimum formulation of incorporation was F6 which comprised 26% okara flour, 37% wheat flour, and 37% refined granulated sugar and the optimum control formulation was C3 with 63% wheat flour and 37% refined granulated sugar. Microbiological and textural qualities (springiness) of the optimum baked muffin formulation incorporated with Okara flour and control were analyzed. As the incorporation of okara flour is added, the cohesiveness and sweetness of the muffins decreased and the baking loss rate, pH, and water activity lowered. In conclusion, Okara flour when incorporated into muffins has indeed an influence on its physical, physico-chemical, sensory, and textural properties, and the developed optimum formulation was F6. To further discover the potential of this product as an additional healthy option in line with baked goods, it is recommended to conduct proximate and dietary fiber content analyses. It is also suggested to conduct cost analysis and shelf-life testing to determine its marketability

***Keywords*:** Okara flour

INTRODUCTION

Processing a kilogram of soybean produces a 1.2 kg fresh soybean by-product called Okara. It has a short shelf life, an unpleasant odor, and is unappetizing hence, leading to its disposal, thereby contributing to food waste and environmental problems. However, Okara is a potentially useful functional ingredient with health-promoting effects. Thus, in response, Okara, being a water-insoluble product, can be dried, repurposed as flour, and incorporated into different food products to increase the foods' nutritional value. Furthermore, Muffins is one of the most consumed baked products in the world, and their consumption is expected to grow globally. From 8 million dollars in 2021, the global muffin market size would reach 9 million dollars by the end of 2028 as reported. However, nutritionally, muffins are normally characterized as low in dietary fiber and protein contents which is not in line with the expectation of consumers for healthy foods. When noncommunicable diseases (i.e. COVID-19) have become a primary concern, a lot of people are attempting to eat a healthier diet. Health awareness may increase demand for healthier baked products. Knowing this, Okara flour can partially replace the base flour of muffins, and study its potential to increase the dietary fiber and protein contents–providing another healthy choice for the consumers. Thus, incorporation of Okara flour will not only enrich the quality of the product, but also the problem of waste disposal regarding Okara will be resolved. This also opens the potential to reduce food loss and waste by creating value-added food in connection with the 2030 Agenda for Sustainable Development Goals (SDG target 12.3 on food loss and waste). With these, the main aim of this study is to develop an optimum formulation of baked muffins incorporated with Okara flour. Specifically, it ought to characterize the physical, physico-chemical, and sensory properties of all baked muffins, then identify the optimum sample and further characterize its texture profile using TPA (Texture Profile Analysis), and microbiological quality.

MATERIALS AND METHODS

The proponents conducted experiments to compare various muffin formulations, analyzing their sensory, physical, textural, proximate composition, total dietary fiber content, and physicochemical, and microbiological properties. The experimental group comprised muffins made with Okara flour (OF), while the control group utilized the original muffin recipe. The objective was to establish the feasibility of incorporating Okara flour in muffin production, enhance understanding of utilizing soybean by-products in the food industry, and determine the ideal quantity of okara flour for enhancing muffin quality. The study utilized a Mixture Design of Experiment (DOE), specifically the Extreme Vertices Design, to create muffin formulations with Okara flour. Constraints were applied to ensure the percentage of the three critical components fell within the range of 0% to 100% and summed up to 100%. The three critical

components considered were Okara flour, All-purpose flour, and Granulated sugar. Previous studies on incorporating Okara flour in quick bread were consulted due to the limited research on Okara flour in muffins.

RESULTS AND DISCUSSION

The findings of this study anchor to support the data gathered from the methods of analysis for muffins and quick bread. This study aimed to develop an optimum formulation of baked muffins incorporated with Okara flour (OF). Table 2 shows the baking loss rate, ink imprint test (identifying cell count and size), and physicochemical properties.

Table 2

Physical and Physico-chemical Properties of All Baked Muffins Incorporated with Okara Flour (and varying levels of Okara Flour, All Purpose Flour & Granulated Sugar

Formulation (OF: APF: GS) (%)	Physical Properties			Physico-chemical Properties		
	Baking Loss (%)	Cell Characteristics		Aw	Moisture	pH
		Cell Count	Average Cell Size (in mm^2)			
C1 (0:67:33)	8.65 (0.17)	762.33 (401.34)	42.37 (12.80)	0.87 (0.00)	24.22 (0.16)	6.59 (0.04)
C2 (0:59:41)	8.88 (0.14)	876.33 (237.53)	29.05 (1.64)	0.85 (0.00)	23.17 (0.07)	6.54 (0.01)
C3 (0:63:37)	6.79 (0.10)	1025.67 (65.43)	37.97 (5.25)	0.86 (0.00)	23.09 (0.20)	6.52 (0.02)
F1 (52:15:33)	2.52 (0.56)	616.00 (145.77)	37.24 (9.32)	0.84 (0.00)	24.36 (0.02)	5.67 (0.02)
F2 (52:7:41)	4.50 (0.32)	766.33 (62.58)	32.55 (1.83)	0.83 (0.00)	23.66 (0.28)	5.65 (0.01)
F3 (52:11:37)	10.28 (0.06)	734.00 (82.40)	41.08 (6.75)	0.81 (0.00)	20.66 (0.13)	5.77 (0.03)
F4 (26:41:33)	9.13 (0.19)	1138.33 (311.51)	36.04 (7.78)	0.84 (0.00)	22.10 (0.08)	6.00 (0.02)
F5 (26:33:41)	7.09 (0.34)	1374.00 (257.11)	33.78 (2.85)	0.84 (0.00)	23.71 (0.16)	6.01 (0.01)
F6 (26:37:37)	8.52 (0.06)	1220.33 (647.02)	31.16 (6.79)	0.85 (0.00)	24.57 (0.09)	6.06 (0.01)
F-statistic	258.6	2.127	1.211	702.6	206.6	917.7
df	(8,18)	(8,18)	(8,18)	(8,18)	(8,18)	(8,18)
p-value	< 0.0001*	0.0876	0.3470	< 0.0001*	< 0.0001*	< 0.0001*

Note: The values inside the parenthesis indicates standard deviations.
**Denotes significance at a 5% alpha.*

Regarding the baking loss rate, F3 has the highest average baking loss at 10.28% (SD = 0.06%) while F1 has the lowest mean baking loss at 2.52% (SD = 0.56%). It can be observed that the formulation of muffins with the lowest average baking loss are the ones with the highest amount of okara flour. In determining the optimal formulation, it is ideal to have a low baking loss rate since a high loss of moisture during the baking process can lead to the product's weight. In BLR, the optimal formulation is C1 at 8.65% which has the lowest among the 3 control formulations. Regarding cell count, F5 has the highest average cell count at 1374.0 (SD = 257.11). Observe that formulations that have the lowest average cell count have 52% okara flour content while formulations that have the highest average cell count have 26% okara flour content. In terms of average cell size, C1 has the highest mean for the average cell size at 42.37 mm2 (SD = 12.80 mm2). It can be observed that all calculated p-values are more significant than 0.05, which means that there are no significant differences in both the cell count and average cell size of the muffins. The study shows that cell count increases with lower levels of Okara as noted above while greater muffins incorporated with Okara flour show lower cell count.

The formulations with the lowest average Aw are the muffins that have the highest amount of okara flour (F3). Meanwhile, the C1 is the highest average Aw which has the highest percentage content. Regarding moisture, F6 has the highest average moisture for a muffin at 24.57% (SD = 0.09%), and the lowest average moisture is F3 at 20.66% (SD = 0.13%), it can be observed that the formulations with the lowest amount of okara flour are the highest. The best optimum sample for physico-chemical was provided by Formulation 6 (F6) having a 6.06 pH level closer to the approximate pH, which is 6.53 for bread based on the USDA 21 CFR 114.90. (Gunasekara et al., 2021), and Lostie et al., (2015) have stated that a typical cake has a moisture content between 15 and 30%, and favoring the incorporation of 26% okara flour since the ideal percentage range of okara flour in a quick bread (butter cake) contains 20% of GSBO (Green Soybean Okara) flour (Nguyen Doan Mai, et al., 2021).

Table 3 presents the median and interquartile range of the panel's response during the first trial of QDA of the okara muffins. The different formulations were assessed by the 10 panelists based on four parameters: springiness, moisture absorption, the cohesiveness of mass, and sweetness. For the springiness parameter, results have shown that the commercial muffin (CC1) has the highest median among the control and overall formulations which is 6.10 and has an interquartile range of 3.10. This may imply that for the first trial, more than 50% of the raters think that the commercial-controlled muffin is the springiest among the ten muffins.

Qualitative Descriptive Analysis of Baked Muffin Incorporated with Okara Flour

Table 3

Summary Statistics of the Panel's Response on the First Trial of QDA of Okara Muffins

Formulation % (OF: APF: S)	Springiness	Moisture Absorption	Cohesiveness of Mass	Sweetness
C1 (0:67:33)	5.00 (2.40)	11.65 (1.88)	12.40 (4.93)	5.50 (1.75)
C2 (0:59:41)	4.40 (2.75)	11.65 (1.40)	9.85 (5.65)	6.50 (2.38)
C3 (0:63:37)	5.45 (3.06)	12.60 (0.65)	10.75 (3.53)	6.75 (4.50)
CC1	6.10 (3.10)	12.75 (1.75)	11.75 (2.98)	8.00 (5.00)
F1 (52:15:33)	3.00 (2.28)	11.30 (2.80)	4.00 (7.65)	3.25 (4.00)
F2 (52:7:41)	3.00 (1.00)	12.85 (2.80)	3.50 (3.30)	1.25 (3.25)
F3 (52:11:37)	1.25 (3.38)	10.50 (5.75)	4.13 (9.00)	3.50 (5.25)
F4 (26:41:33)	3.00 (0.75)	12.10 (3.70)	7.00 (2.35)	1.50 (6.08)
F5 (26:33:41)	3.75 (2.00)	12.50 (3.25)	8.50 (6.75)	4.65 (2.93)
F6 (26:37:37)	4.00 (2.60)	11.90 (3.23)	7.00 (6.35)	3.95 (3.75)
Friedman test statistic	30.582	9.825	31.441	43.841
p-value	0.0003	0.3648	0.0002	< 0.0001

For moisture absorption, it can be observed that the median values for all formulations are relatively higher than the other attributes for both trial 1 and trial 2, as seen in Tables 2 and 3 respectively. It can be seen that the second formulation of muffin (F2) has the highest median. This result may indicate that among the ten formulations, more than 50% of the raters in the first trial consider the F2 muffin, which has the highest sugar and lowest wheat flour content among the three formulations, as having the largest amount of moisture absorption while the F3 muffin has the least amount. Regarding the cohesiveness of mass, results have shown that the first controlled muffin (C1) has the highest median which is 12.40, with an interquartile range of 4.93. This may imply that during the first trial, 50% of the raters think that the C1 muffin has the tightest mass among the muffins, while the F2 muffin has the loosest mass. Notice that the formulations with the high okara flour content (52%) have the lowest median score for cohesiveness of mass and those that are controlled have the highest median. For the sweetness parameter, the commercial muffin (CC1) has the highest median among the ten muffins which is 8.00, and an interquartile range of 5.00. The fifth muffin formulation (F5) has the highest median of 4.65 with an interquartile range of 2.93 among the muffin formulations with okara flour. The formulations F2 and F4 have different okara flour content, thus this suggests that the sweetness score is also due to the amount of the other ingredients in the muffin such as wheat flour and refined granulated sugar.

On the identification of the optimum muffin incorporated with Okara flour, the results in the QDA suggest that F5 is the optimum sample because it has the highest median in all texture parameters, including Springiness, Moisture Absorption, and cohesiveness of Mass. This indicates that it has the highest texture values among all the formulations with Okara Flour. However, it can also be observed that F6 can be the optimum considering other analyses. Although F5 has higher median texture values than F6, it does not mean that it is the most acceptable among the panels. This is supported by the results in the study of Doan Mai (2021), which states that quick bread (green soybean Okara flour cake) incorporated with 20% Okara flour has lower springiness value, yet has the highest acceptability. Thus, muffins incorporated with 26% Okara flour could be candidates for optimum, and these are F4, F5, and F6. To decide on the optimum, the results of other tests shall be considered next. Results in the physical analysis state that F1 is the optimum however it is supported by an outdated related literature, while results in the physico-chemical analysis states that F6 is the optimum because its values were closer to the standard. Thus, F6 is the optimum formulation of baked muffin with Okara flour.

Based on the results, there are significant differences between the mean hardness 1 and 2, and chewiness of the F6 and C3 however, there are no significant differences between the mean cohesiveness in which the desegregation effect

of the fiber is reflected in the lower requirement of energy when TPA performs a second compression, mean springiness— wherein, the deformation rate between the first compression and the second compression are reflected, and mean adhesiveness of F6 and C3 muffins. The springiness and adhesiveness of muffins with added OF were significantly lower than in the controls. While, hardness, cohesiveness, chewiness, and adhesiveness with the addition of okara flour increased. The results of the textural instrumental technique in the present study were similar to those found in the study of Heo et al. (2019).

CONCLUSIONS

In conclusion, Okara flour when incorporated into muffins has indeed an influence on its physical, physico-chemical, sensory, and textural properties. Incorporation of a high amount of Okara flour results in baked muffins with heavier mass, denser, have low water activity values, and low pH values than the control. Meanwhile, muffins incorporated with a low amount of Okara flour have higher moisture content values than the control. Furthermore, the developed optimum formulation was F6. It consists of 26% Okara flour, 37% all-purpose (wheat) flour, and 37% granulated sugar. It was less springy however it does not mean that is not acceptable—as supported by Doan (2021). Importantly, it is the baked muffin incorporated with Okara flour that was most compliant with the USDA standards in terms of its physicochemical properties, specifically on its pH value. On its texture profile, the control sample C3 was more springy and chewier than the optimum sample. Lastly, the optimum sample baked in this study was compliant with the national microbiological standards, thus it is safe for consumption. Moreover, since the mixture design had created three control formulations, this study was also able to discover an optimum formulation for control along the experimentation process—although it is not in the objectives. The control formulation C3 which has 0% OF, 63% APF, and 37% GS, has closer textural sensory characteristics to the commercial sample used as reference in this study, thus it was identified as the control sample.

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Department of Health - Food and Drug Administration. Revised Guidelines for the Assessment of Microbiological Quality

The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
Borneo Convention Centre, Kuching, Sarawak

EFFECTS OF MIXED EMULSIFIER (TWEEN 20 AND SPAN 20) ON THE PHYSICOCHEMICAL PROPERTIES AND THE STABILITY OF MULBERRY LEAF EXTRACT – RED PALM OLEIN (MLE-RPO) EMULSION

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Abstract: Mulberry leaves exhibited prominent antioxidant, anti-inflammatory, and anti-diabetic properties, whereas red palm olein (RPO) is high in antioxidant activity due to its high level of carotenoid and vitamin E. Therefore, this study aimed to investigate the effects of the mixed emulsifiers Tween 20 and Span 20 on the stability of emulsions prepared using mulberry leaf extract (MLE) and RPO. Initially, MLE was prepared through cellulase pretreated-ultrasonic water extraction, then homogenized with RPO at a ratio of 25:75 (MLE:RPO) for water-in-oil (W/O) and 75:25 for oil-in-water (O/W) emulsions at 10,000 rpm for 10 min, followed by ultrasonication for 10min. The stability of the emulsions using different ratios of Tween 20-to-Span 20 (10:0, 8:2, 6:4, 4:6, 2:8, and 0:10) were assessed against storage time, temperature, and ionic strength (using NaCl and $CaCl_2$) based on creaming stability (CI), viscosity, droplet size distribution, zeta-potential, and colour. The results showed that MLE-RPO W/O emulsions containing Tween20: Span20 in the ratios of 10:0 and 4:6 were the most stable at ambient storage temperature. Tween 20 alone (10:0) was sufficient to stabilize the W/O emulsion with the lowest creaming at 6% after 30 days of storage at 45ºC. However, increasing ionic strength significantly increased the CI, particle size, and zeta potential of all emulsions. Compared to W/O emulsion, O/W emulsion demonstrated a lower stability. This study provides insight into potential applications of MLE-RPO emulsion in functional food development.
Keywords: Mulberry leaf extract, red palm olein, emulsion, stability, emulsifier.

INTRODUCTION

Mulberry leaves have long been recognized for its antioxidant, antihypertensive, hypoglycemic, and anti-inflammatory properties, and for preventing atherosclerosis. Among the bioactive compounds, 1-deoxynojirimycin, an alkaloid renowned for its therapeutic effects on various diseases, particularly type II diabetes, is the most notable phytochemical in mulberry leaves as described by Tomczyk et al. (2019). Presently, mulberry leaf extract (MLE) is widely prepared through water-based extraction as described by (Tomczyk et al., 2019). Red palm olein (RPO), on the other hand, is well known for its high carotenoids and vitamin E content in the forms of tocopherols and tocotrienols. RPO was proven to exhibit prominent antioxidant properties by Sari et al., (2018). With the health benefits of both MLE and RPO, the emulsion formed by combining MLE and RPO is hypothesized to display enhanced nutraceutical benefits, which potentially offer greater preventive measures against diseases such as diabetes, cancers, and heart disease through diet. However, due to the immiscibility of MLE and RPO, emulsifiers are crucial in producing a stable emulsion of MLE-RPO. Besides, various studies have also been proved that emulsion offers a wide array of advantages across various industries and applications, among which the excel of emulsion in enhancing bioavailability and extending the storage life of encapsulated active ingredients stand out as a prominent example (Sari et al., 2018).

In an emulsion stabilized by non-ionic emulsifiers, the stability of the interface is dependent on the amount of the emulsifier molecules attached to the surface of the emulsion droplets. The addition of another emulsifier with different hydrophilic-lipophilic balance (HLB) value in the emulsion potentially enhances the stability of the emulsion by reducing the interfacial tension (McClements & Jafari, 2018). To effectively incorporate an emulsion into a food matrix, the stability and shelf life of the emulsion play a critical function. Therefore, this study was conducted to formulate MLE-RPO emulsions by using different mixed emulsifiers composed of Tween 20 and Span 20. The shelf life of the MLE-RPO emulsions was evaluated based on its storage stability. Furthermore, the effects of storage temperature and ionic strength on the stability of the emulsions were also investigated. At the end of this study, a MLE-RPO emulsion which is expected to be stable in the food matrix is produced.

MATERIALS AND METHODS

The freshly collected mulberry leaves (voucher number: ID010/2021) were air-dried and ground into ≤150 µm before subjected to cellulase pretreated-ultrasonic ultrasonic extraction to prepare the aqueous extract of mulberry leaf (MLE) according to the preliminary study. To prepare MLE, about 8% w/v of mulberry leaf powder in 0.1 M citrate buffer (pH 4.8) was added with 2 % w/v cellulase enzyme (9.81 U/g). The mixture was incubated at 50ºC with continuous stirring (100 RPM) for 18.5hrs, and followed by ultrasonic extraction at 50ºC for 1.5 hrs. MLE was obtained by separating the leaf residues from the extract through filtration using 11µm pore size filter paper.

In this study, oil-in-water (O/W) and water-in-oil (W/O) emulsions were prepared by homogenizing MLE and RPO at the ratios of 75:25 and 25:75 (MLE: RPO), respectively. Span 20 was dissolved in the RPO, whereas Tween 20 was dissolved in the MLE separately, with the oil-to-surfactant ratio of 8:2. The ratios of Tween 20-to-Span 20 were tested at 10:0, 8:2, 6:4, 4:6, 2:8, and 0:10, with the HLB value of 16.70, 15.11, 13.82, 12.26, 10.41, and 8.6 respectively. Different combinations of emulsifiers were tested on both W/O and O/W emulsions. Both MLE and RPO were stirred until homogeneous before the aqueous phase (MLE) was added into the oil phase (RPO). The mixture was homogenized at 10,000 RPM for 10 min using a high speed homogenizer, and then further stabilized by ultrasound using an ultrasonic probe set at 20 kHz (100 % amplitude) for 10 min. The emulsions formed were rested at room temperature for 24 hrs before proceeding to the subsequent analysis. The first sample screening was carried out based on the creaming index (CI). Only emulsions which are stable with no or minimum creaming were selected to proceed with storage stability, viscosity, droplet size distribution, zeta-potential, color, and ionic stability analysis.

Creaming stability of the emulsions was determined by transferring each emulsion into two 15 mL sealed cylindrical tubes separately. Then, one of each pair was stored at ambient temperature, whereas another was stored at 45ºC for 30 days. The microstructure of the emulsions was observed at room temperature under a light microscope at 40× magnification power, and the microscopy images were captured using a camera. The emulsion's color was determined based on the L^*, a^*, b^* color system by using a Minolta Colorimeter CR-300. Delta E was calculated by comparing the color of the emulsion from day 1 to day 30 of storage. Viscosity was determined using IKA Rotavisc equipped with VOLS-1 at 150 RPM. The droplet size distribution and zeta (ζ)-potential of the emulsions were measured using Anton Paar Litesizer 500. Prior to the analysis, the emulsions were diluted to 0.0625 % w/v droplet concentration to prevent multiple light scattering. For the zeta-potential measurement, 1 mL of the diluted sample was transferred into the Univette for reading. To determine the ionic stability of the emulsions, 50 mM of NaCl and 5 mM of $CaCl_2$ salt solution were added into the emulsion at 50 % v/v, respectively. The stability of the emulsions was evaluated based on CI. A sample blank was prepared by diluting the emulsion with distilled water, instead of salt solution at 50 % v/v.

All data was presented in mean ± standard deviation. SPSS software version 27 was used to carry out Student t-test to compare the means. A p-value below 0.05 is considered there is a significant difference between the means.

RESULTS AND DISCUSSION

Based on Figure 1, the W/O emulsions containing Tween 20 and Span 20 emulsifiers at the ratios of 10:0, 8:2, 6:4, 4:6, and O/W emulsions containing Tween 20 and Span 20 emulsifiers at the ratios 10:0, 8:2, 6:4, 2:8 remained stable without separation after 1 day of storage (CI % = 0). This indicates that the emulsions were formed at an acceptable stability using these mixed emulsifiers. Among the emulsions, W/O emulsion stabilized by Tween 20 alone (10:0) and 4:6 (Tween 20: Span 20) mixed emulsifiers were the most stable at ambient temperature storage, even up to 30 days. Compared to W/O emulsion containing Tween 20 alone, emulsion containing 4:6 (Tween 20: Span 20) mixed emulsifiers was more stable at 45°C storage. After 30 days of storage at 45ºC, the emulsion demonstrated the highest stability at 2% CI. On the other hand, obvious separations were observed in all O/W emulsions using different combinations of Tween 20 and Span 20 emulsifiers under both ambient and 45°C storage conditions. However, among the O/W emulsions, the one containing 8:2 (Tween 20: Span 20) mixed emulsifier demonstrated the lowest CI (18%) after 30 days of storage at ambient temperature. However, the CI was increased drastically to 40% when the emulsion was stored at 45ºC for 30 days. Notably, when the storage temperature for the emulsion increases to 45ºC, CI of all emulsions increased. Temperature increase results in a greater thermal energy in the emulsions, hence, promotes droplets movement and coalescence, eventually destabilizing the emulsion at a faster rate (Badolato et al., 2008). Based on results in figure 1, W/O emulsions stabilized by Tween 20 (10:0) (labelled as A) and 4: 6 (Tween 20: Span 20) mixed emulsifier (labelled as B), and O/W emulsion stabilized by 8:2 (Tween 20: Span 20) mixed emulsifier (labelled as C) were selected to proceed in the subsequent study.

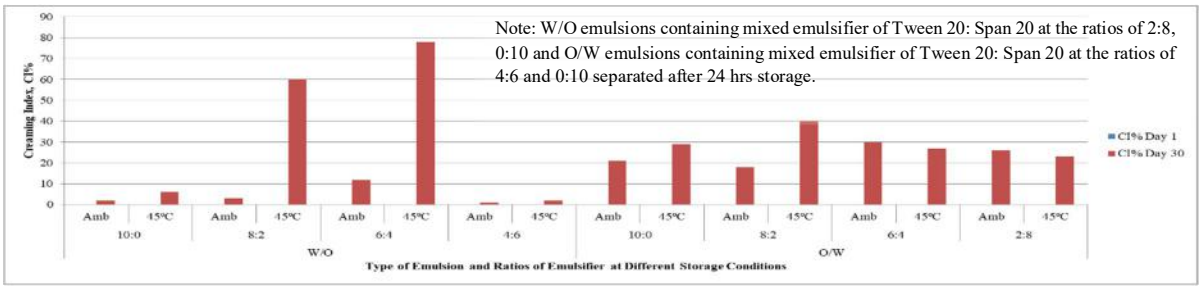


Figure 1. Creaming index (CI, %) of W/O and O/W emulsions stabilized by different emulsifier ratios, storage temperature (ambient and 45ºC) and time (day 1 and day 30).

Based on Table 1, the droplet size of all the selected emulsions became larger after 30 days storage, notably obvious in sample A and C. It is possible that prolonged storage time and temperature weaken the repulsive forces between the droplets and promote coalescence, eventually leading to the increase of droplet size and phase separation (Badolato et al., 2008). Table 2 presents the color difference, viscosity, particle size distribution, zeta-potential, and ionic strength stability of the selected emulsions.

Table 1. Microscopic observation at 40× magnification of the selected MLE-RPO emulsions at day 1 and day 30.

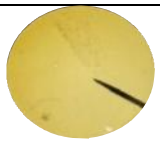
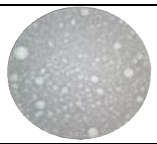
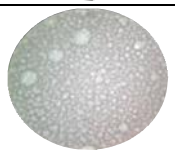
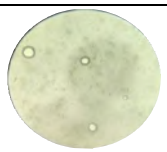
Sample / Day	A	B	C
Day 1			
Day 30			

Table 2. Comparison of color difference, viscosity, particle size distribution, zeta-potential, and ionic strength stability of sample A, B, and C at day 1 and day 30 storage.

Test	Sample	A		B		C	
		Day 1	Day 30	Day 1	Day 30	Day 1	Day 30
Color difference, ΔE	Ambient	17.48 ± 0.13^{a}		4.90 ± 1.01^{a}		8.93 ± 2.52^{b}	
	45ºC	12.59 ± 0.11^{b}		6.77 ± 0.83^{a}		49.26 ± 0.40^{a}	
Viscosity, cP	Ambient	109.73 ± 0.24^{c}	109.40 ± <0.00^{c}	109.77 ± 0.09^{c}	109.90 ± <0.00^{c}	4.34 ± 0.01^{c}	2.99 ± <0.00^{d}
	45ºC	109.90 ± <0.00^{c}	109.40 ± <0.00^{c}	109.90 ± <0.00^{c}	109.83 ± 0.09^{c}	4.27 ± <0.00^{d}	2.70 ± <0.10^{c}
Average particle size, nm	Ambient	6.60 ± 0.20^{d}	40.78 ± 0.77^{c}	77.10 ± 8.45^{d}	145.31 ± 30.98^{c}	7.92 ± 0.50^{d}	609.90 ± 73.62^{c}
	45ºC	6.43 ± 0.18^{d}	13.06 ± 0.82^{c}	79.56 ± 18.17^{d}	208.93 ± 113.69^{c}	7.87 ± 1.14^{d}	104.22 ± 18.58^{c}
Zeta-potential, mV	Ambient	-44.31 ± 0.75^{c}	-38.12 ± 0.84^{d}	-37.78 ± 3.66^{c}	-37.48 ± 3.68^{d}	-16.2 ± 0.72^{c}	-22.37 ± 1.45^{d}
	45ºC	-38.05 ± 0.27^{c}	-32.37 ± 0.86^{d}	-38.95 ± 2.68^{d}	-57.45 ± 2.59^{c}	-16.23 ± 0.35^{c}	-18.6 ± 1.71^{c}
Ionic stability, CI %	Water	0.0	1.1	0.0	60.0	0.0	2.0
	NaCl	0.0	3.2	0.0	52.0	0.0	5.0
	$CaCl_2$	0.0	3.0	0.0	42.0	0.0	15.0

Note:
a-b: Different alphabet indicates there is significant difference between means at different storage temperature ($p<0.05$).
c-d: Different alphabets indicates there is significant difference between means at different storage time ($p<0.05$)

Results in table 2 suggest that color change in B at ambient temperature storage was the smallest, however color change of C increased significantly by 5 folds after 30 days of storage at 45°C. The lowest ΔE in sample B could be explained by its emulsion stability at ambient temperature storage. Phase separation can gradually revert an emulsion to its heterogeneous phase, causing the emulsion's color to shift back to its individual components. Stable emulsions, however, can resist phase separation and maintain their color throughout storage without significant changes (Chantrapornchai et al., 2008). This also explains why C, the least stable emulsion among the three selected samples exhibited the highest ΔE. In terms of viscosity, table 2 highlights that W/O emulsions (A and B) were more viscous than O/W emulsion (C). This is because RPO is more viscous than MLE, thus causing the viscosity of the W/O emulsion to be dependent on the oil phase, which makes up 75% of the system (Badolato et al., 2008). Besides, table 2 also shows that the viscosity of A and B remained constant throughout storage, but not C. Viscosity of C significantly reduced upon 30 days storage at ambient temperature and 45ºC. The migration of oil droplets into the creaming layer, leading to reduced droplet interactions, which consequently reduces the viscosity due to phase separation (McClements & Jafari, 2018). In addition, the average particle size of all emulsions increased over the storage period at both ambient temperature and 45°C. Among the samples, average particle size change over 30 days storage in A was the smallest at both ambient temperature and 45°C. Average particle size of A stored at 45ºC for 30 days was the smallest. This might be due to the migration of large droplets to the cream layer, left behind the small droplets in the emulsion system (Badolato et al., 2008). This result is in accordance with the result in table 1 that suggests the occurrence of coalescence in the emulsion. In addition, the ζ-potential of A and B were also found to be higher than C. This result further proved that A and B were more stable than C. However, ζ-potential of A significantly reduced at both ambient temperature and 45ºC, whereas ζ-potential of B stored at 45ºC and C stored at ambient temperature increased after 30 days of storage. The decrease of ζ-potential indicates reduced emulsion stability. However, the increase of ζ-potential may not always assure the emulsion stability. Redistribution of emulsifiers in the emulsion system due to the decrease of the effective surface area caused by Ostwald ripening, coalescence, and creaming processes might also lead to the increase of ζ-potential (Shao et al. 2017). High ζ-potential of A and B (≥30 mV) signifies that the emulsions were still stable after 30 days storage. CI after 30 days storage of A and B at ambient temperature was about 2 %. In terms of ionic stability in the presence of NaCl and $CaCl_2$ salts, A and C were destabilized by salts, however B was stabilized by the salt, especially $CaCl_2$. These results indicate that NaCl and $CaCl_2$ significantly influenced the emulsion stability, however the exact mechanisms were unknown. According to Shao et al. (2017), the influence of ions on the stability of an emulsion is varied and distinct. Collectively, A was identified as the most stable emulsion among the selected samples.

CONCLUSION AND FUTURE RECOMMENDATION

Although MLE-RPO W/O emulsion stabilized by Tween 20 alone (10: 0) demonstrated the highest creaming stability, ionic stability and smallest average particle size, this emulsion was less stable than W/O emulsion stabilized by 4: 6 (Tween 20: Span 20) mixed emulsifier when stored at 45ºC. Even though the particle size of the latter was larger, its ζ-potential was higher than the former. Compared to W/O emulsion, O/W emulsion was the least stable. This study provides a preliminary overview on the stability and physicochemical properties of MLE-RPO emulsion for potential functional food applications in the near future. However, in-depth exploration of the chemical and bioactive characteristics of MLE-RPO emulsion is necessary to gain a comprehensive understanding of the emulsion.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
Borneo Convention Centre, Kuching, Sarawak

EXPLORING THE PRESERVATION AND MODENISATION OF CHILLED PRE-PACKED UMAI: SARAWAK TRADITIONAL RAW FISH DELICACY

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Abstract: Umai is a popular traditional raw fish delicacy in Sarawak, consisting of thinly sliced raw fish cured in sour fruit juice mixed with other spices. Chilled pre-packed umai holds the potential to become a convenient delicacy, as it allows for direct mixing of sliced fish with the marinating sauce upon serving. This study aimed to modernize the presentation of pre-packaged chilled red tilapia umai, which includes fish cutlets and marinade sauce, for market convenience. A study was conducted to extend the chill-storage life of sliced fish (Red Tilapia cutlets) through a dipping treatment in an organic acid solution. The results indicated that sliced fish frozen at -23°C for 7 days and treated with a 1.5% citric acid dipping solution exhibited the best storage quality, as evidenced by lower total aerobe and total coliform counts, as well as enhanced colour intensity. Fish muscle tissue often undergoes acid-induced denaturation within the pH range of 2 to 5. Therefore, the umai sauce was formulated to have a fixed pH value of 3.3, which met the denaturation process and sensory attributes, as well as the FDA requirements for limiting the growth of bacterial pathogens that are of greatest concern in seafood processing. Compared to commercial chili sauce, umai sauce has significantly lower calorie and carbohydrate content, with 49.7 kcal and 8.7%, respectively, compared to 113 kcal and 24.5%.
Keywords: pre-packed umai, sliced fish, marinating sauce, convenient delicacy, chill-storage life

INTRODUCTION

Umai, also known as Sarawak raw fish salad **(Figure 1)**, is a popular traditional dish originating from the Melanau community in Sarawak's Mukah region. It consists of thinly sliced raw fish cured in sour fruit juice, mixed with onions, chillies, and shallots, and seasoned with salt and spices. Comparable to Japan's sashimi and Latin America's ceviche, umai distinguishes itself through its unique preparation and serving. However, its short shelf life limits broader distribution and commercial viability. To address this issue, a method for extending umai's storage life in chilled pre-packed form **(Figure 2)** is crucial for maintaining quality, safety, and minimizing wastage (Chua, 2021).

The growing demand for chilled and frozen foods presents an opportunity for umai to emerge as a convenient delicacy, meeting consumers' desires for quick and easy preparation (Chua et al., 2021). Yet, concerns about the safety of raw seafood arise alongside this trend. Uncooked seafood, including umai, can pose health risks due to parasites like nematodes (*Anisakis* sp.) and trematodes (*Heterophyes* sp.) (Golden et al., 2022). Therefore, ensuring freshness and proper pre-treatments for fish is essential. While safety guidelines from the U.S. Food and Drug Administration (FDA, 2022) recommend blast-freezing to at least –35 ºC for 15 hours; or regular freezing to at least –23 ºC for 7 days. However, this practice has not been adopted in traditional umai preparation due to the belief that it may cause undesirable changes in the eating quality of the product.

This study aimed to create a modern presentation for chilled pre-packed umai using red tilapia, featuring umai cutlets (sliced fish) and marinating sauce, catering to market convenience. The work involved extending umai cutlets' chill-storage life through an organic acid solution dipping treatment and developing the umai marinating sauce. Comprehensive assessments covered physical, chemical, microbiological, and sensory aspects.

Figure 1. Umai; Sarawak raw fish salad

Figure 2. Chilled pre-packed umai

MATERIALS AND METHODS

Dipping Treatment of Umai Cutlets

Live red tilapia *(Oreochromis niloticus)* were deboned and cut into fillets, which were then soaked in a 2% sodium tripolyphosphate (STPP) solution for 2 minutes to reduce rancidity and dripping. These fillets were then packed into low-density polyethylene (LDPE) bags and frozen at -23°C for 7 days, following FDA guidelines (2023).

Three organic acid dipping solutions (citric acid 1.5%, lactic acid 2.0%, acetic acid 3.0%) were prepared by dissolving them in deionized distilled water. The solution pH of 4.00 ± 0.20 was determined by the organic acid's strength. The pH value of 4 was chosen to preserve taste, texture, and colour based on preliminary tests, avoiding taste alterations at lower pH levels, and lacking shelf-life extension at higher pH levels.

Umai cutlets, measuring approximately 5 cm x 1 cm x 3 mm, were sliced from thawed fillets. 100 g duplicate samples were immersed in a 300 mL dipping solution, agitated for 2 minutes, drained for 5 minutes, then packed in polypropylene (PP) containers. After a 10-minute chill at -18 ± 1°C, cutlets were stored at 2 ± 2°C, with untreated cutlets as the control.

The study spanned a predetermined 8-day storage period, during which cutlet samples were intermittently assessed. Evaluations covered physical attributes (pH, color intensity), chemical analysis (total volatile base nitrogen), microbiological testing (total aerobic bacteria, coliform count), and sensory evaluation to determine the dipping treatment's impact on cutlet quality.

Development of Umai Marinating Sauce

Umai marinating sauce was processed using a sensory-developed formulation from preliminary studies. Fresh ingredients **(Table 1)** were processed following the outlined steps in **Figure 3**. As a shelf-stable sauce, preservatives such as sodium benzoate may be added at a dosage of not exceeding 750 mg/kg. Nonetheless, the use of preservatives are not required for short-term storage in chilled condition, owing to the low pH condition of the sauce.

The sauce's moisture, protein, fat, crude fiber, and ash content were analyzed in duplicate using the AOAC standard method (2023). Additional evaluations encompassed pH, water activity, percent titratable acid, soluble solids, viscosity, and color expressed in the L*, a*, b* notation.

Table 1. Umai marinating sauce formulation

Ingredients	(%)(w/w)
Onion	24
Chilli	14
Shallot	14
Ginger	8
Lime peel	1.2
Lime juice	17.6
Acetic acid 4%	17.6
Salt	2.7
Monosodium glutamate	0.9

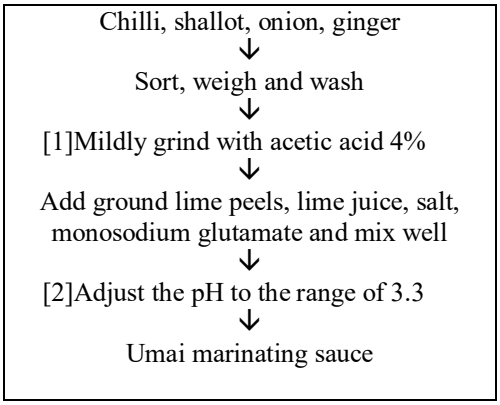

Figure 3. Processing of umai marinating sauce

Sensory Evaluation

All sensory evaluations were carried out after the results of the microbiological evaluations obtained showed that the products were safe for consumption. Chilled cutlet samples were assessed, then transformed into umai by mixing with the umai marinating sauce. A 30-person taste panel compared these umai samples with freshly prepared samples (control) using a 9-point hedonic scale, rating from 1 (dislike extremely) to 9 (like extremely) (Poste et al., 1991).

RESUITS AND DISCUSSION

Preparation of Umai Cutlets

In this experiment, organic acids were added as bacteriostatic agents to deter spoilage and pathogens. The result of the physical, chemical, microbiological analysis and sensory evaluation of organic acid-treated umai cutlets on the 8th day of storage at 2 ± 2 °C are shown in **Table 2**.

Fish muscle tissue contains around 70-84% water and 15-24% protein, primarily myosin. Proteins are intricate molecules composed of amino acid chains that fold into three-dimensional structures. Heating causes denaturation, a process where proteins lose their natural structure due to broken hydrogen bonds (Strzelczak et al. 2021). Acid also denatures proteins similarly. In umai preparation, acidic marinating sauce denatures fish muscle proteins, resulting in a thick, dense, opaque appearance and firm texture, akin to cooking. However, this change is purely structural, not involving the chemical processes of heat-induced cooking (Logren et al., 2022).

Table 2. Mean value of Least Significant Difference Test of organic acid solutions treated umai cutlets on the 8th day of storage at 2 ± 2 °C

Parameters		Dipping Treatments (pH 4.00 ± 0.20)			
		Control	Citric acid 1.5%	Lactic acid 2%	Acetic acid 3%
pH		7.05 a	5.11 b	5.14 b	5.18 b
TVBN (mg N/100 g)		39.8 a	24.6 b	27.1b	24.5 b
Total aerobes log cfu/g		6.3 a	3.5 b	4.1 bc	4.7 c
Total coliform log cfu/g		3.3 a	2.4 c	2.9 b	2.2 c
Sensory scores		5.2 a	7.3 bc	6.9 c	7.4 bc
Colour	L	62.58 a	50.26 bc	51.36 c	57.43 d
	a	1.69 a	1.86 a	1.66 a	1.74 a
	b	4.29 a	4.33 a	4.21 c	4.05 b

** Mean in the same row with the same letter are not significantly different (p >0.05)*

Umai cutlets' pH consistently rose over the 8-day storage period, potentially due to spoilage bacteria generating alkaline compounds such as amines and ammonia (Metin et al., 2001). The limit of acceptability is usually 6.8-7.0, which is lower in acid-treated samples. Control samples began at a pH of 6.13, reaching 7.05 by day 8, while acid-treated samples saw smaller increases from an initial pH of 4.74 to a maximum of 5.18. However, differences in pH between these treated samples were found to be statistically insignificant ($p > 0.05$). For most organic acids to act as effective anti-microbial agents, a condition of low pH values around 5.5 is required (Papadochristopoulos et al., 2021).

The total volatile base nitrogen (TVBN), a result of microbial spoilage and endogenous enzymes, is controlled in fish products with an acceptability limit of 35 mg TVBN/100 g (Metin et al., 2001). During storage, all treated samples remained below 28 mg/100 g. By the 8th day, samples treated with an organic acid solution had significantly lower TVBN values ($p < 0.05$) than the controls. Acid-treated samples exhibited reduced microbial loads and slower spoilage, resulting in consistently lower TVBN levels. Control samples on the 8th day, recorded a TVBN value of 39.8 mg/100 g; surpassing or nearing the 35 mg/100 g limit, which is considered less acceptable according to sensory evaluation.

Microbiological analysis revealed increasing total aerobes and coliform counts over time in all umai samples. Initially, control samples had 3.8 log cfu/g total aerobes and 1.3 log cfu/g coliforms, rising to 6.3 and 3.3 log cfu/g, respectively, by day 8. Statistical analysis showed significant differences ($p < 0.05$) in total aerobes and coliform counts between control and organic acid-treated samples. Acid-treated samples had aerobes counts below 6.0 log cfu/g, indicating organic acid treatment inhibited mesophilic aerobic bacteria growth. Citric acid 1.5% dipping solution exhibited the lowest total aerobes count on day 8. All organic acid-treated samples displayed significantly lower total coliform counts, with acetic acid 3% or citric acid 1.5% dipping solutions showing the lowest counts by day 8.

Umai cutlets were deemed acceptable if their odour, appearance, and texture remained unchanged, with a score above 6. Sensory evaluation indicated significant differences ($p < 0.05$) between control and treated samples after 8

days. Panellists preferred treated samples over control, with treated samples consistently earning higher scores until the 6th day of storage. An acidified dipping solution at pH 4 had no adverse effects on taste and texture.

While umai cutlets have various quality attributes, colour stands out as a crucial consumer criterion. Colour measurements revealed a significant difference ($p < 0.05$) between control and treated samples after 8 days. In control samples, umai cutlet lightness (L*) increased from 46.94 to 62.58 initially, and redness (a*) decreased from +2.04 to +1.69 over the 8-day storage. A 1.5% citric acid dipping solution exhibited superior colour preservation.

Development of umai marinating sauce

The physical and chemical characteristics of the ready-to-use umai marinating sauce are displayed in **Table 3.** Fish muscle tissue denaturation through acid induction frequently occurs within the pH range of 2 to 5. The umai marinating sauce, formulated with a consistent pH value of 3.3, met the denaturation process and sensory attribute, as well as the requirements for limiting growth of bacterial pathogens that are of greatest concern in seafood processing (FDA, 2022).

The comparison of proximate composition between umai marinating sauce and commercial chili sauce showed that umai marinating sauce exhibits notably lower calorie and carbohydrate levels than its commercial chili counterpart, specifically, 49.7 kcal compared to 113 kcal and 8.7% versus 24.5%, respectively.

Table 3. Physical and chemical characteristics of umai marinating sauce

Parameter		Value
pH		3.37
Water activity (a_w)		0.98
Titratable acidity (%, as acetic acid)		1.83
Total soluble solids (ºBrix, 30ºC)		11.5
Viscosity (cps)		1.84×10^4
Colour	L	41.54
	a	+15.27
	b	+16.45

CONCLUSION AND RECOMMENDATIONS

This study successfully explored the preservation and modernization of chilled pre-packed umai using red tilapia. It addressed the challenge of extending the storage life of umai cutlets through an organic acid dipping treatment, resulting in improved quality attributes such as pH stability, reduced total volatile base nitrogen, controlled microbial growth, and enhanced sensory appeal. The development of a pH-adjusted umai marinating sauce further complemented the preservation efforts, meeting denaturation requirements and safety standards. These advancements pave the way for the commercial viability of pre-packed umai, offering consumers a convenient and safe culinary experience. While the processing technique of pre-packed umai is relatively simple, strict control is needed along the processing line to maintain the product's quality.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
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RESIDENTIAL ROLES AND LEVELS OF PRACTICE IN FOOD WASTE MANAGEMENT COMPONENTS

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***Abstract*:** The bulk of household food waste is still mixed with other general waste, being burnt or dumped in landfills. It is difficult to lessen the reliance on landfills and its related negative impacts since sustainable practices like reusing leftovers, separating waste, and food composting is generally not performed massively by the residents. There is a lack of unified information regarding food waste management and food-related behaviour, and yet to determine whether a lack of engagement is the true root cause of the problem. A questionnaire survey was used to assess the residents' present practises or approaches to managing food waste at home. In measuring the level of practices, analysis using the scoring method was applied. Regular meals prepared at home result in leftovers typically entails discarding and simply throwing them away. Each of the components has a different level of practises. For food composting, poor practises are more noticeable and has the weakest link of participation. The other two components of reusing leftovers and separating waste were found to have a more encouraging level of practise. This research contributes by providing evidence for residents' current routine, constituting a significant opportunity to create awareness, drive intention and influence change towards sustainable management and reduction of food waste from the source.

INTRODUCTION

Malaysia produces 44.5% of food waste, enough to feed 2.9 million people three meals a day. Acknowledging the call for a circular economy, several countries have implemented practises known as sustainable food waste management (SFWM) as a response to weak economic and social conditions and environmental degradation. Government policies and organisational initiatives have long promoted the idea of reducing, reusing, and recycling. In addition, as part of the efforts, the campaign for the separation of waste has been encouraged, along with other practises such as the reuse of food leftovers and the composting of household food waste. These are seen as a viable solution to future issues including food security, population increase, and climate change, which all contribute to the current waste management and landfill crisis.

Households have been identified as the primary source of food waste, particularly in urban residential areas. Given the high amount of food waste occurring at the household stage, strategies to increase the practises of sustainable food waste management in the final stage of the supply chain are of utmost importance. However, it is difficult to design strategies to lessen the reliance on landfills and its related negative impacts when there is insufficient information pertaining to the current level of practices. A recent systematic literature review by Munir (2022) highlights the need to explore practices, propelling this study to be carried out to provide insights on households' level of practising reusing leftovers, household waste separation, and food composting. This is to ensure data availability as a foundation for identifying areas that require attention and improvement measures.

MATERIALS AND METHODS

Questionnaires were distributed online to assess the households' present practises or approaches to managing food waste at home. Several ways to measure the level of practises are mentioned in the literature. Abdu (2016) employed a method that measured the level of practises by computing the sum of scores for each outcome to 'yes' or 'no' statements. This was done in order to determine the overall degree of practises. It was determined that having good practises of the behaviour at hand was equivalent to obtaining a high score that was above the cut-off mark. According to Mulat et al. (2014), the classification was divided based on percentages, with scores below 65% representing poor practises and scores between 70% and 100% representing excellent practises. Using a survey methodology, Limon

and Villarino (2020) evaluated the practises for managing food waste in rural areas. Respondents gave ratings on a five-point Likert scale from strongly disagree to strongly agree. The collected data was then evaluated using factor analysis. The measurement conducted by Memon et al. (2015) also incorporated a scalar scoring method. Accordingly, a score of one point was awarded for each accurate response, and a score of zero point was awarded for responses that were incorrect or ambiguous. Due to the variations in the questionnaire style and the responses given to the statements describing the practises, there can be a small variance in the measurement of the degree of practises compared to earlier studies. Nonetheless, there are similarities in the scoring system and classification of practise levels. Therefore, analysis using the scoring method continues to be the most popular way for gauging the degree of practises in empirical studies, thus employed in the current study.

RESULTS AND DISCUSSION

The study's setting, the Klang Valley, is made up of ten major local authorities with a population of 2.25 million households. The number of respondents participated in this study was 520 representing Malaysia's urban households and encompassing all ten areas; Ampang Jaya, Kajang, Klang, Kuala Lumpur, Petaling Jaya, Putrajaya, Selayang, Sepang, Shah Alam, Subang Jaya. There were 16.6% more men than women participating overall. Most respondents (32.6%) were between the ages of 40 and 49. Malays (77.1%) make up the majority of responses. According to their marital status, 84.6% are married. Most of the people who answered have at least a Bachelor's degree (58.3%). In terms of occupation, 34.8% of respondents are employed in the private sector, while 22.1% are employed by the government. Over half of respondents are in the B40 group, which has a monthly household income of less than RM4,850. Households in the middle-income bracket, also known as the M40 group, earn between RM4,851 and RM10,970 per month (27.7%), whereas those in the T20 or upper-class household income bracket earn more than RM10,970 per month (12.3%). Malaysian urban dwellers who live in landed housing area account for 55.4% of the study sample. On the other hand, 44.6% of them reside in high-rise structures. Analysing the number of people and children living in each family, 42.3% have two adults and 36.3% have no children. Families with children range from those with one child (17.9%) to those with six or more children (1.7%). The overall profile provided a reasonably fair description and overview of the structure of urban households in Malaysia.

Current practises in residential areas

The vast majority of respondents eat meals prepared at home on a daily basis. This finding is in relation to the current norms in Malaysia. Regular meals prepared at home result in leftovers or food waste that must be managed. Managing food waste or food surplus typically entails discarding and simply tossing away food (62.1%), as determined by an analysis of responses from households. Waste sorting (34.4%) and reuse leftovers (52.8%) are practised, although lesser as compared to discarding food. Composting, feeding animals as feed, sharing food with others, recycling food, and burying food waste are all much less typical practises, as shown in Figure 1.

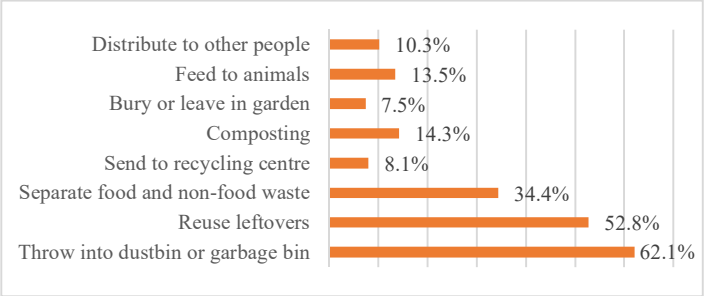

Figure 1. Common Household Food Waste and Food Surplus Management Practises
Source: Field survey data (2021)

There are six distinct types of food waste generated in individual homes (Figure 2). Participants were asked to rate how often each kind of food waste occurred in their homes. Each category's average percentage was determined and ranked accordingly. Eggshells, fruit and vegetable peel, and stems seem to create the most food waste, up to 29 percent. Meal leftovers (21%), bones and scraps (20%), and expired food (13%) follow closely after. The last two categories of uneaten fruits and vegetables received 9% and 8%, respectively. Slorach et al. (2020) distinguished between "avoidable" and "unavoidable" food waste in the home. Unconsumed food items go under the category of avoidable waste, whilst inedible items like bones and peels fall under the category of unavoidable waste.

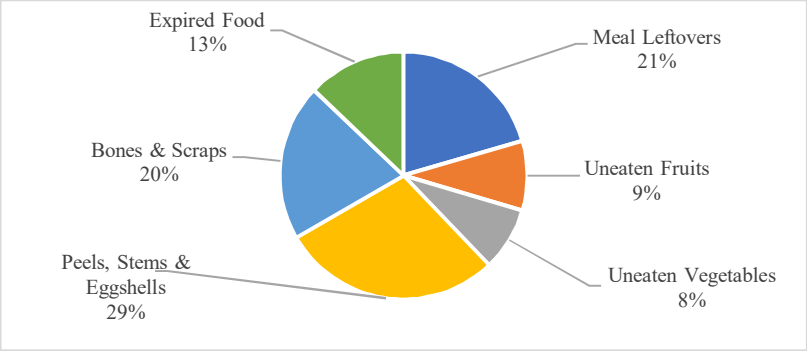

Figure 2. Categories of Food Waste at the Household Level
Source: Field survey data (2021)

In assessing the households' present practises or approaches to managing food waste at home, responses to 16 specific questions were analyzed. The analysis was carried out using a scalar scoring approach. The score was recorded according to their original responses, ranging from 1 to 7, because all of the original questionnaire items were positively worded. Those who scored the items between 1 and 5 are judged to be engaging in a low level of SFWM, while those who scored between 6 and 7 are doing so at a good level. The item-level analysis was scored using the overall score. Table 1 presents the results in response to the respondents' answers to the questions on their own actual practises for managing their household food waste.

Table 1. The Ratings of Respondents Practising SFWM

Statement	1	2	3	4	5	Low (%)	6	7	High (%)
Leftovers are eaten in the same form or reheated for reuse.	5	7	12	41	91	156 (30.0)	127	237	364 (70.0)
Leftover food will usually be used and transformed into a different food.	29	26	32	54	93	234 (45.0)	126	160	286 (55.0)
In the case of leftover fried chickens, they will be cut and transformed into other meals.	11	16	19	43	64	153 (29.42)	125	242	367 (70.58)
Adjust meal plan to make use of leftovers.	11	8	14	42	74	149 (28.65)	127	244	371 (71.35)
Separate food waste from other household waste on a regular basis.	11	12	22	83	106	234 (45)	126	160	286 (55)
Positively engage in food waste separation.	7	19	27	89	100	242 (46.54)	118	160	278 (53.46)
Participate in waste separation at home.	7	13	22	81	102	225 (43.27)	125	170	295 (56.73)
Usually dispose all food waste at home separately from other household waste.	10	19	26	69	104	228 (43.85)	118	174	292 (56.15)
Involved in waste separation activities.	13	23	22	89	108	255 (49.04)	103	162	265 (50.96)
Compost food waste at home.	102	53	63	84	71	373 (71.73)	63	84	147 (28.27)
Positively engage in food composting.	95	53	59	85	77	369 (70.96)	65	86	151 (29.04)
Separate household waste for composting purposes.	95	53	60	86	74	368 (70.77)	59	93	152 (29.23)
Attempt to separate household waste for composting purposes.	92	43	63	93	66	357 (68.65)	66	97	163 (31.35)
Involved in home composting activities.	101	51	64	82	68	366 (70.38)	61	93	154 (29.62)
Purchase composting bins for household use.	131	71	69	79	58	408 (78.46)	39	73	112 (21.54)
Use available tools or technology to practise home composting.	129	58	59	82	63	391 (75.19)	51	78	129 (24.81)

Source: Field survey data (2021)

The following pattern of behaviour can be observed in urban Malaysian households. Each of the three sustainable food waste management components has a different level of practises (Table 2). The first aspect, which involves reusing food leftovers, represents a level that is encouraging. It is evident that 68 percent (n=354) of urban households utilize their food leftovers in beneficial ways and have modified their meal plans to accommodate leftovers. They reported being able to put their leftovers to good use at home. This means that the goal of not throwing away food makes people change their habits so that they use leftovers from meals as much as possible (Talwar et al., 2022). The second component, which entails the separation of waste, appears to have varying degrees of implementation across urban households. 52% of urban residents have a decent level of waste separation practises, separating their waste and disposing of all food waste separately from other types of household waste. Meanwhile, another 48 percent (n=251) practise inadequate waste separation at home. More observation and focus on this is needed from the relevant authorities. The majority of households in the survey (75% or n=388) reported poor practises when it pertains to the food composting component, whereas only a quarter of respondents (n=132) indicated good practises. This shows that while waste separation may occur, it is not done for composting as the majority of people do not routinely compost

their unavoidable food waste. Furthermore, no attempts were made to assure household composting through the purchase of composting bins or the use of any available techniques or technologies.

Table 2. Components under Sustainable Food Waste Management: Level of Practises

Component	Poor practises	Good practises
Reusing food leftovers	166 (32%)	354 (68%)
Separating household waste	251 (48%)	269 (52%)
Composting	388 (75%)	132 (25%)

Source: Field survey data (2021)

CONCLUSION AND RECOMMENDATIONS

Two components of food waste management were found to have quite an encouraging level of practise. First, there are good habits in homes when it comes to using food leftovers and making changes to a meal plan so that leftovers can be used. Second, urban households participate in waste separation and demonstrate attempts to implement waste separation for recycling purposes, such as sending used cooking oils to recycling centres. The results of this study support the assertion that more families are starting to embrace waste separation (Radhi, 2020). The findings demonstrate that in the food composting component, poor practises are more noticeable. Even though recyclables and organic waste from the home were sorted, they were not meant to be composted. Very few people made an effort to compost at home, even though it is fairly easy to do with the right tools or technology or by buying composting bins. It appears critical to ease the role of managing food waste for urban residents in order to boost their confidence, potentially by constructing a simplified and affordable version of food composting devices that are economical and easy to use. Creators of new technologies may work with entrepreneurs or government bodies to develop a unified and globally accepted technology. Building specialised facilities for the treatment of food waste could make it simpler for residents living in high-rise buildings with limited space to apply food waste separation and composting practises.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
Borneo Convention Centre, Kuching, Sarawak

QUALITY AND STORAGE PROPERTIES OF FISH SAUSAGE WITH REDUCED-SODIUM SAUCE

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Abstract: Fish sausage is a processed product manufactured by mixing fish meat and starch. It is often sold as a snack with sauce included sodium and can lead to increased sodium consumption. Therefore, this study was performed to evaluate the quality and storage properties of fish sausage with reduced-sodium sauce. Reduced-sodium (25% and 50%) sauce for fish sausage was prepared, and the chromaticity, pH, total acidity, and texture were analyzed at 15-day intervals during 75 days of storage at 4°C, 25°C, and 40°C. There were no significant differences in fish sausage chromaticity, pH, total acidity, or texture according to sodium level or storage temperature. The quality changed only according to storage period. These findings confirm that the quality of fish sausage with sauce can be maintained even with a reduction of the sodium level.
Keywords: quality, storage, reduced sodium, sauce, fish sausage

INTRODUCTION

A plethora of research has been conducted worldwide on the relationship between sodium intake and various diseases. As many countries recognize the effects of excessive sodium intake, they have implemented policies to reduce it (1). Korean adults, on average, consume 3,889 mg of sodium daily, which is approximately 2.5 times higher than the World Health Organization's recommended intake of 2,000 mg (2). While sodium is essential for sustaining life, its excessive intake is problematic due to its association with hypertension and cardiovascular diseases, particularly coronary artery disease and strokes. The impact of higher sodium intake on blood pressure is substantial (3). In addition to hypertension, which is directly caused by sodium, excessive sodium intake has also been associated with the other three major diseases in adults: kidney disease, diabetes, and eye disease (4).

Fish sausage, which is commonly classified as fish cake, is produced by adding an appropriate amount of salt to fish meat to extract salt-soluble proteins. Spices and various proteins like ISP (Isolated Soy Protein) and gelatin are added to retain moisture, and the mixture is then heated (5). The preparation process involves combining fish meat and starch as the main ingredients, and seasoning the mixture with sauces like salt, red pepper, garlic, egg white, and sugar as sub-ingredients. Typically, salt is added at a concentration of 2.5% to 3.5% (6). It's important to note that consuming fish sausages frequently as snacks can lead to a relatively high intake of salt, which may become problematic.

Thus, we prepared a reduced-sodium sauce for fish sausages to lower the sodium content in the final product. To assess the effectiveness of the reduced-sodium sauce, we compared the quality and storage properties of fish sausages with the reduced-sodium sauce to those made with a commercial sauce. The aim in this study was to confirm the potential for sodium reduction in fish sausages using the newly developed sauce.

MATERIALS AND METHODS

Materials

0.1N-NaOH was purchased from OCI company (Seoul, Korea). And commercial fish sausages and fish sausages made with reduced-sodium sauce were provided from CHEILJEDANG Company.

Preparation of sodium-reduced fish sausages

In the present study, we reduced the sodium content in commercially available fish sausage sauce by 25% and 50% compared to the original formulation. The modified sausages prepared with reduced sodium sauce were then subjected to storage at three different temperatures: 4°C, 25°C, and 40°C. Over a period of 75 days, we analyzed and compared the quality changes of the fish sausages at 15-day intervals.

Chromaticity

The chromaticity of fish sausages was measured using a colorimeter (CR-300, Minolta, Osaka, Japan) for the Hunter L* (lightness), a* (redness), and b* (yellowness) values. All samples were measured 3 times using a white board as the reference color (L*=93.5, a*=0.31, and b*=0.32).

pH and total acidity

Five grams of sample transferred into 50 mL conical tube, and then 25 mL of distilled water added. And sample was homogenized for 5 min using a homogenizer (Vortex Mixer, JEIO TECH, Korea), and then centrifuged at 12,000×g for 5 min, the supernatant was filtered through a filter paper (110mm, Whatman Co., Little Chalfont, Buckinghamshire, USA). The filtered sample was measured using a pH meter (Thermo Electron Corp., USA) at room temperature (25℃). Total acidity of sample was calculated by below equation after titrating with 0.1 N-NaOH until pH 8.3 at room temperature (25℃), and was calculated as percentage of acetic acid on a weight basis

$$\text{Total acidity (AA eq \%)} = \frac{0.006 \times 0.1N\ NaOH\ consumption\ of\ sample \times D \times F}{Weight\ of\ sample} \times 100$$

F: Factor of 0.1N-NaOH, D: Dilution ratio of sample, 0.006: Equivalent weight of acetic acid(g)

Texture

Hardness and Resilience of fish sausages using texture profile analysis (TPA) was performed using a texture analyzer (TA-XT2, Stable Micro System Ltd., England). Samples were cut into cubes with the 1×1×1 cm^3, and then the hardness and resilience were measured at the center of sausage slices. The conditions of TPA were performed as the pretest speed was 2 mm/s, the test speed was 2 mm/s, the post-test speed was 2 mm/s, the distance was 4 mm (40% compression), and the trigger load was 25 g.

Statistical analysis

Results are reported as mean±standard deviations (n=3). The significance of differences among treatment means was determined using the one-way analysis of variance (ANOVA) and correlation calculated by SAS version 9.3(Statistical Analysiss System, SAS Institute Inc., Cary, NC, USA) with a significance level of $p < 0.05$ by Duncan multiple range's test.

RESULTS AND DISCUSSION

Comparison of quality characteristics between commercially available fish sausages and fish sausages prepared with a sodium-reduced sauce

In this study, the quality characteristics of fish sausages were analyzed and compared based on different levels of sodium reduction. The parameters assessed included color, pH, total acid, and texture (hardness and resilience). The results, as shown in Table 1, indicated that there were no significant differences in the quality characteristics of fish sausages with varying levels of sodium reduction. This suggests that reducing the sodium content in the sausages did not have a substantial impact on their color, pH, total acid, or texture (hardness and resilience). Hence, it can be inferred that the overall quality of fish sausages remained consistent regardless of the degree of sodium reduction implemented in the sausage preparation.

Table 1. Comparison of physicochemical qualities of fish sausages with sauce reduced sodium content.

Variable		Control	R25	R50
Color	L	99.10±0.40	98.90±0.18	100.10±0.10
	a	1.67±0.06	1.83±0.16	1.38±0.09
	b	-8.19±0.43	-8.21±0.71	-6.48±0.56
pH		6.55±0.01	6.60±0.01	6.56±0.01
Total Acidity (%)		1.80±0.1	1.32±0.1	1.62±0.1
Resilience		0.43±0.03	0.44±0.03	0.43±0.03
Hardness(g)		2207.2±118.6	2089.4±286.3	2041.9±291.9

[1)] Mean ± SD

R25: Sausage prepared with 25% Sodium reduced sauce

R50: Sausage prepared with 50% Sodium reduced sauce

Comparison of change of chromaticity between commercially available fish sausages and fish sausages prepared with a sodium-reduced sauce

As a result of analyzing the quality change of fish sausages according to sodium reduction while storing at 4, 25, and 40 ℃, the L*-value did not show any change according to the storage temperature of each sodium-reduced fish sausage (Figure 1). The a*-value also showed no change according to the storage temperature and period, but the b*-value increased rapidly with the storage period regardless of the temperature and then showed a tendency to become constant (Figure 2 and 3). Therefore, the study found that the storage period had a greater impact on the color change of fish sausages than the sodium concentration contained in the product. The L*-value and a*-value remained relatively stable, while the b*-value exhibited a notable change during the storage period.

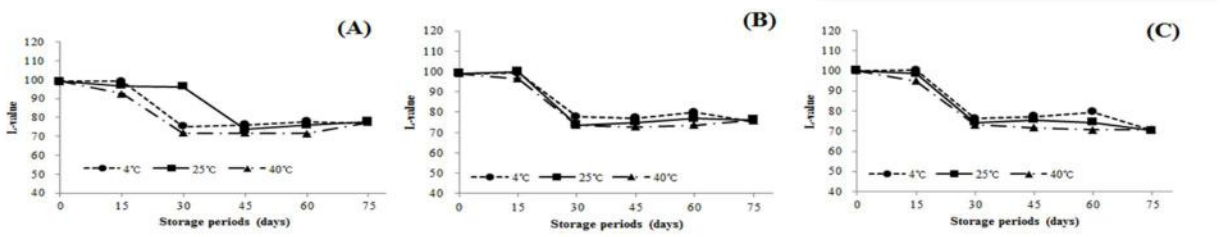

Figure 1. Changes of L*-value for fish sausage with (A) control, (B) R25 and (C) R50 during storage at 4, 25, and 40℃ for 75 days.

R25: Sausage prepared with 25% Sodium reduced sauce, R50: Sausage prepared with 50% Sodium reduced sauce

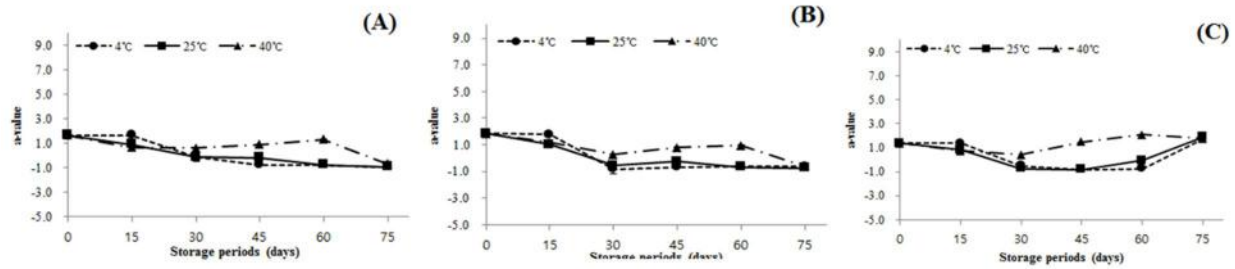

Figure 2. Changes of a*-value for fish sausage with (A) control, (B) R25 and (C) R50 during storage at 4, 25, and 40℃ for 75 days.
R25: Sausage prepared with 25% Sodium reduced sauce, R50: Sausage prepared with 50% Sodium reduced sauce

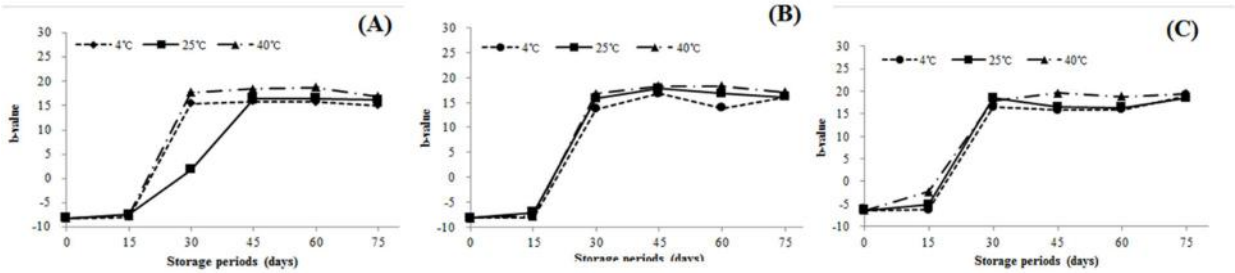

Figure 3. Changes of b*-value for fish sausage with (A) control, (B) R25 and (C) R50 during storage at 4, 25, and 40℃ for 75 days.
R25: Sausage prepared with 25% Sodium reduced sauce, R50: Sausage prepared with 50% Sodium reduced sauce

Comparison of change of pH and total acidity between commercially available fish sausages and fish sausages prepared with a sodium-reduced sauce

As the storage period increased, the total acid in the fish sausages slightly increased at 4℃, 25℃, and 40℃, but it remained within an almost constant range (as shown in Figure 4). Additionally, the pH of all the reduced fish sausages, regardless of the reduction rate, was consistently maintained within a certain range at all storage temperatures (4℃, 25℃, and 40℃) (as depicted in Figure 5). This indicates that both the total acid and pH of the fish sausages are kept stable during storage because there is no significant sodium reduction in the final sterilized and commercialized fish sausages, and any acid production in the fish meat that might occur is effectively supported during storage at various temperatures.

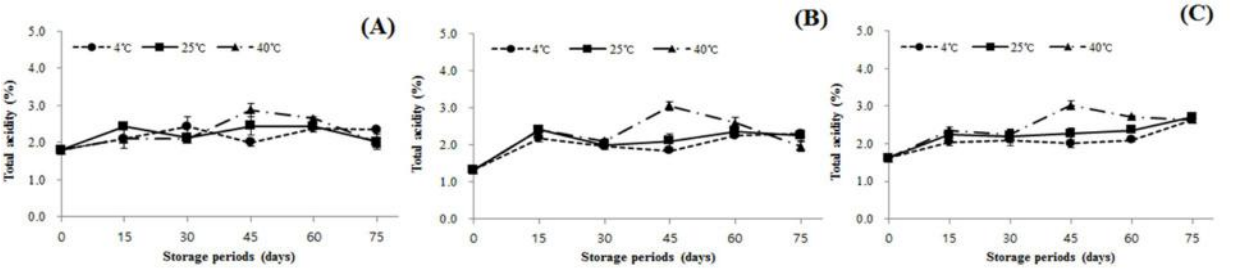

Figure 4. Changes of total acidity for fish sausage with (A) control, (B) R25 and (C) R50 during storage at 4, 25, and 40℃ for 75 days.
R25: Sausage prepared with 25% Sodium reduced sauce, R50: Sausage prepared with 50% Sodium reduced sauce

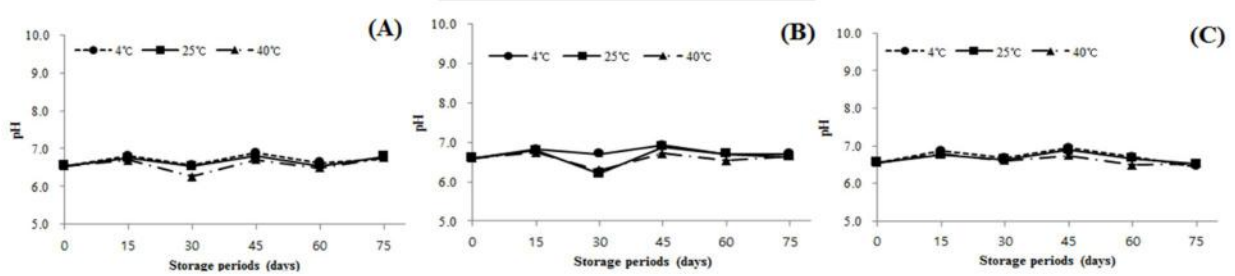

Figure 5. Changes of pH for fish sausage with (A) control, (B) R25 and (C) R50 during storage at 4, 25, and 40℃ for 75 days.
R25: Sausage prepared with 25% Sodium reduced sauce, R50: Sausage prepared with 50% Sodium reduced sauce

Comparison of change of hardness and resilience between commercially available fish sausages and fish sausages prepared with a sodium-reduced sauce

In the compression hardness and elasticity tests, the hardness of fish sausages showed a tendency to increase and then decrease with the reduction in sodium, depending on the storage period. However, the elasticity remained constant throughout the storage

period. Furthermore, no significant difference was observed in the changes during storage at 4°C, 25°C, and 40°C, and there was also no difference based on the degree of sodium reduction (as depicted in Figure 6 and 7). Based on these findings, it can be concluded that even with sodium reduction in the manufacturing of fish sausages, there is little effect on the overall quality, including texture, during the storage of fish sausages. The hardness and elasticity of the fish sausages remain relatively stable, indicating that the product maintains its desired textural attributes throughout the storage period.

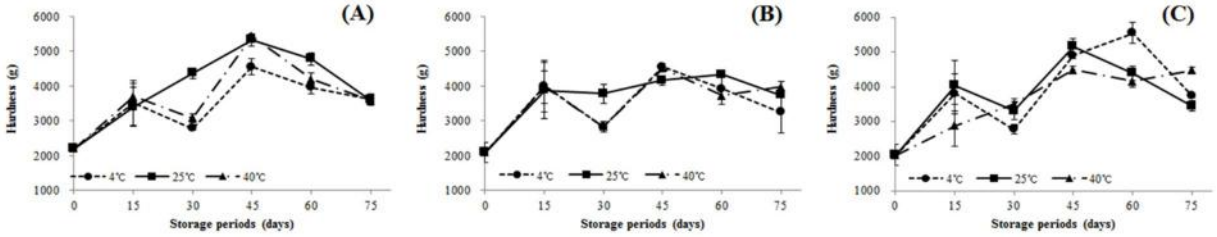

Figure 6. Changes of hardness for fish sausage with (A) control, (B) R25 and (C) R50 during storage at 4, 25, and 40°C for 75 days.
R25: Sausage prepared with 25% Sodium reduced sauce, R50: Sausage prepared with 50% Sodium reduced sauce

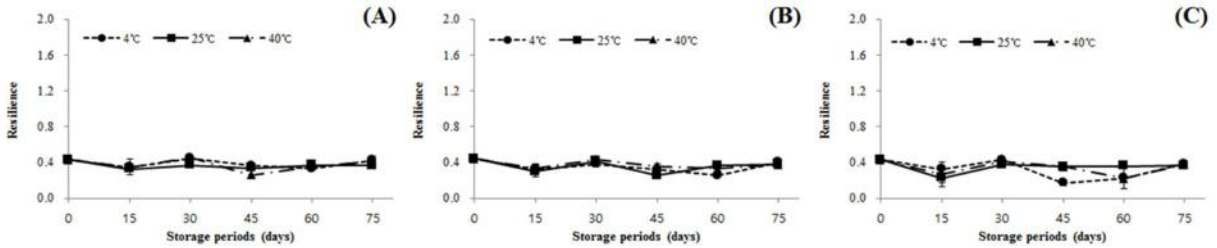

Figure 7. Changes of resilience for fish sausage with (A) control, (B) R25 and (C) R50 during storage at 4, 25, and 40°C for 75 days.
R25: Sausage prepared with 25% Sodium reduced sauce, R50: Sausage prepared with 50% Sodium reduced sauce

CONCLUSION

The results of the study indicate that there were no significant differences in fish sausage chromaticity, pH, total acidity, or texture based on the sodium level or storage temperature. The only factor that influenced the quality of the fish sausages was the storage period. Therefore, these findings suggest that reducing the sodium level in the fish sausage sauce does not have a substantial impact on its overall quality. Despite the sodium reduction, the quality of the fish sausages remained consistent throughout the storage period. This suggests that it is possible to maintain the quality of fish sausages with sauce even with a reduction in sodium content.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
Borneo Convention Centre, Kuching, Sarawak

ANALYSIS OF RETINOL CONTENT IN COMMONLY CONSUMED FOODS IN KOREA FOR NATIONAL NUTRIENT DATABASE

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Abstract: Food composition data are essential for calculating nutrient intake based on food consumption statistics. In the Korean food composition database, reliable analytical data for retinol is necessary. Therefore, this study aimed to provide information on retinol contents in commonly consumed foods in Korea. Retinol contents were determined using saponification extraction method and HPLC with a fluorescence detector. A total number of 12 eggs, 5 dairy products, 13 meats, 16 seafoods, 22 seasonings, and 8 sugar products were analyzed. The retinol contents were relatively higher in eggs ranged from 30.5 to 730.7 μg/100 g followed by dairy products and meats. Seafoods, seasonings, and sugars products were ranged from 1.9 to 32.5, 8.8 to 11.2, 1.6 to 40.9 μg/100 g, respectively. The analytical method validation parameters such as accuracy, precision, limit of detection, limit of quantification, and linearity were calculated to ensure the method's validity. Overall recovery was higher than 90% and precision was less than 5%. This study provides reliable retinol data in commonly consumed foods in Korea for the development of the Korea nutrient database.
***Keywords*:** retinol, analysis, food composition database, method validation

INTRODUCTION

Retinol is one of the fat-soluble vitamins, which is an essential nutrient important for maintaining healthy skin, vision, and immune function (D'Ambrosio et al., 2011). Retinol is found in various foods, primarily in animal sources such as liver, eggs, dairy products, and fish. Retinol is absorbed in the form of retinyl ester from animal foods, hydrolyzed in the intestine, and absorbed through the epithelial cell membrane of the small intestine (Harrison, 2012). Vitamin A deficiency can cause diseases such as dry eye syndrome, night blindness, and impaired immune cell function, and fat-soluble vitamins can accumulate in the body and cause toxicity when consumed excessively (Sathe & Patel, 2010). Establishing a retinol database in food could have several potential advantages. The retinol database would provide valuable information to consumers, healthcare professionals, and researchers (Elmadfa & Meyer, 2010). For individuals who need to monitor their vitamin A intake, such as pregnant women or those with certain health conditions, a retinol database can aid in planning balanced and adequate diets (Strobel et al., 2007). Food manufacturers and regulatory agencies could use the database to ensure accurate labeling of vitamin A content on food products, contributing to transparent and accurate nutritional information for consumers. Ultimately, the establishment of a retinol database in foods could provide a valuable resource for promoting better nutrition and health. This study was conducted to provide information on retinol contents in various food groups consumed in Korea.

MATERIALS AND METHODS

The analytical samples were obtained from the Rural Development Administration (RDA) in the Republic of Korea between 2018 and 2021. The pretreatment for retinol analysis followed the saponification method outlined by Slavin and Yu (2012). Samples were ground and combined with an ethanol solution containing 6% pyrogallol and 60% potassium hydroxide. The mixture was heated under reflux conditions at 75°C for 50 min, then extracted using a solvent containing 2% sodium chloride and hexane:ethyl acetate (85:15, v/v). The organic phases were collected and made up to 50 mL and the resulting solution, containing retinol, was evaporated under nitrogen gas. The residues were redissolved in hexane and filtered through a 0.45 μm polytetrafluoroethylene (PTFE) membrane filter. Analysis was conducted using an HPLC system with fluorescence detection (excitation wavelength of 326 nm and an emission wavelength of 470 nm). Analysis of retinol was performed on a LiChrosphere×Diol 100 column (250 × 4 mm, i.d., 5 μm) using a mobile phase of hexane/isopropanol (95:5, v/v) at a flow rate of 1.0 mL/min. To verify the analytical

method, the Standard Reference Materials (SRM) provided by the National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA) were analyzed 3-4 times a year, and the recovery (%) was obtained from the certified value and the analytical value of this study. The infant formula was used as an in-house control material and quality control charts ensured consistent analysis quality and supported the reliability of the analysis method.

RESULTS AND DISCUSSION

Retinol contents in eggs

Table 1 shows the retinol contents of the food groups. In the egg group, retinol was not detected in the egg whites of all samples, but retinol was detected in the egg yolks. Bertechini and Mazzuco (2013) reported that retinol exists only in egg yolk due to its lipophilic nature. Egg yolks contain a higher amount of fat compared to egg whites, which are mostly composed of protein and water. Retinol, being a fat-soluble vitamin, has an affinity for lipids and tends to associate with fats rather than water. This is why retinol concentrates in the fatty portion of foods, like egg yolks, where it can dissolve and be stored more effectively. Among eggs, quail egg yolk showed the highest content at 730.72 μg/100 g, and duck egg yolk showed the lowest content at 33.91 μg/100 g. In addition, when comparing raw and boiled eggs, the content of retinol was lower in boiled eggs. According to a report by Sungpuag et al. (1999), 11% of retinol was lost when eggs were boiled, because retinol is heat labile. Therefore, it is thought that retinol was lost in the boiling process of eggs in this study.

Retinol contents in sugar products

Retinol was not detected in jellies (gummy candy and royal jelly), and it is thought that retinol was detected in chocolate-containing foods because chocolate is prepared by adding milk. The retinol content of chocolate products was 1.68~40.99 μg/100 g. White chocolate had the highest retinol content, and chocolate syrup had the lowest. White chocolate is thought to have a high content of retinol because it contains a lot of milk when it is manufactured (Bianchi et al., 2021).

Retinol contents in milk and dairy products

The retinol content of heavy cream was 237.68 μg/100 g, and the retinol content of animal whipped cream was 244.01 μg/100 g, but retinol was not detected in vegetable whipped cream. Since, animal whipped cream is made from dairy-based ingredients like heavy cream, while vegetable whipped cream is made using plant-based fats such as coconut or soybean oil. Shredded mozzarella and fresh mozzarella are two different types of cheese that vary in terms of texture, moisture content, and culinary uses. Shredded mozzarella cheese was 116.94 μg/100 g, and fresh mozzarella cheese was 135.19 μg/100 g.

Retinol contents in meats

Retinol was detected at 6.99-7.01 μg/100 g in ham and 1.97~3.90 μg/100 g in bacon and boiled or baked bacon tended to decrease retinol. Lešková et al. (2006) studied the loss rate of vitamins in response to heat treatment and found that up to 43% of retinol was lost during heat treatment. Therefore, bacon is thought to lose retinol during the boiling or baking process. The range of retinol contents in rabbit meat and pork rind were 22.34~22.73 and 1.68~3.01 μg/100 g, respectively. In bovine by-products, retinol was detected in a range of 4.93 to 181.85 μg/100 g, and the highest retinol content was 181.85 μg/100 g in the small intestine.

Retinol contents in seafoods

The study assessed retinol content in various seafood types. Retinol was absent in certain items, such as salted clams, flying squid, fish sauces, imitation crab sticks, and dried pollack. The presence of retinol in seafoods is generally linked to the intestines or liver, and gutted or liver-removed items might lack detectable retinol. Pollock, shrimp, mackerel, saury, and salmon exhibited varying levels of retinol depending on the products. Loss of retinol during cooking, washing, or storage might explain discrepancies between observed and expected retinol content. Seasoned squid strips demonstrated higher retinol than raw squid, potentially due to seasoning effects. Smoked chum salmon had 13.14 μg/100 g of retinol, despite raw salmon being higher in retinol content according to RDA data (18 μg/100 g), suggesting heat sensitivity could lead to retinol loss.

Retinol contents in seasonings

Mayonnaise had 8.93 μg/100 g of retinol, with low-calorie mayonnaise at 9.07 μg/100 g, yielding similar results to USDA data. Barbecue sauce contained 9.81 μg/100 g of retinol, likely due to additives like caramel, mustard, and anchovies. Thousand island dressing showed 11.28 μg/100 g of retinol, associated with mayonnaise addition. The retinol content of 8.85 μg/100 g in caramel sauce could be a result of milk being added during the process of making the sauce.

Table 1. Retinol contents of food groups

Food groups	Sample	Retinol (μg/100 g)	Food groups	Sample	Retinol (μg/100 g)
Eggs	Egg, white, raw	ND	**Seafoods**	Pollock guts, salt-fermented	30.37 ± 0.35
	Egg, white, boiled	ND		Alaska pollock (*Theragra chalcogramma*) Roe	32.52 ± 1.20

	Egg, yolk, raw	425.93 ± 8.81
	Egg, yolk, boiled	262.60 ± 12.26
	Quail, white, raw	ND
	Quail, white, boiled	ND
	Quail, yolk, raw	730.72 ± 16.38
	Quail, yolk, boiled	701.86 ± 2.25
	Duck, white, raw	ND
	Duck, white, boiled	ND
	Duck, yolk, raw	33.91 ± 3.16
	Duck, yolk, boiled	30.52 ± 2.35
Sugar products	Jelly, gummy candy	ND
	Royal jelly	ND
	Chocolate bar, peanut and caramel	25.14 ± 0.36
	Chocolate bar, cookie and caramel	24.09 ± 0.13
	Chocolate syrup	1.68 ± 0.26
	Chocolates, white	40.99 ± 7.92
	Chocolate pie, cocoa cream	24.11 ± 0.94
	Chocolate pie, fresh cream	12.20 ± 0.27
Milk & Dairy products	Cream, heavy cream	237.68 ± 18.24
	Cream, whipping, milk fat	244.01 ± 0.72
	Cream, whipping, vegetable fat	ND
	Cheese, mozzarella, shredded	116.94 ± 3.44
	Cheese, mozzarella, fresh	135.19 ± 2.30
Meats	Ham, quadrangle, raw	6.99 ± 1.00
	Ham, sliced, raw	7.01 ± 0.09
	Bacon, raw	3.90 ± 0.18
	Bacon, blanched	2.51 ± 0.20
	Bacon, roasted	1.97 ± 0.12
	Rabbit meat, raw	22.34 ± 1.37
	Rabbit meat, boiled	22.73 ± 0.96
	Pork rind, raw	2.27 ± 0.20
	Pork rind, blanched	1.68 ± 0.02
	Pork rind, roasted	3.01 ± 0.02
	Beef edible offal, small intestine, pan-broiled, freezed	181.85 ± 0.78
	Beef edible offal, large intestine, pan-broiled, freezed	12.29 ± 2.45
	Beef edible offal, abomasum, pan-broiled, freezed	4.93 ± 0.52
	Beef edible offal, large intestine, Pan-broiled, freezed	12.29 ± 2.45
	Clam, salt-fermented	ND
	Japanese flying squid (*Todarodes pacificus*)	ND
	Shrimp, salt-fermented	28.08 ± 1.27
	Sandlance (*Ammodytes personatus*), fish sauce	ND
	Anchovy (*Engraulis japonicus*), fish sauce	ND
	Mackerel (*Scomber japonicus*), whole, canned	20.11 ± 0.81
	Pacific saury (*Cololabis saira*), whole, canned	8.53 ± 0.75
	Chum salmon (*Oncorhynchus keta*), whole, canned	1.92 ± 0.00
	Dried shredded squid	10.80 ± 0.21
	Imitation crab stick, raw	ND
	Imitation crab stick, blanched	ND
	Dried pollock, raw	ND
	Chum salmon (*Oncorhynchus keta*), smoked	13.14 ± 1.18
	Seasoning, anchovy flavored, powder	ND
Seasonings	Mayonnaise	8.93 ± 0.13
	Mayonnaise, low-calorie	9.07 ± 0.07
	Garlic, powder	ND
	Cooking alcohol, mirim	ND
	Seasoning sauce, barbecue sauce, soy sauce based	9.81 ± 0.80
	Vinegar, Balsamic	ND
	Salad dressing, Thousand Island	11.28 ± 0.35
	Salad dressing, Italian	ND
	Ginger, powder	ND
	Vinegar, persimmon	ND
	Curry, powder	ND
	Hot sauce	ND
	Pepper (*Piper nigrum* L.)	ND
	Jajang sauce (black bean sauce), powder	ND
	Vinegar, brown rice	ND
	Doenjang, Korean traditional type	ND
	Soy sauce, Korean traditional type	ND
	Gochujang, Korean traditional type	ND
	Caramel sauce	8.85 ± 0.11
	Sauce, bulgogi sauce	ND
	Seasoning, beef-flavored, powder	ND
	Ramyeon, instant, seasoning	ND

ND, Not detected.

Method validation

Table 2 presents the results of analyzing SRM over a 4-year period (2018~2021), indicating that all values were within the NIST reference range. The recovery rates, calculated from analytical and reference values, were 81.36% to 104.63%. Comparing these rates to Association of Official Agricultural Chemists (AOAC) guidelines, which vary based on sample concentrations, the retinol analytical values for all standard reference materials met the acceptance range, highlighting high accuracy. The study also used an infant formula as an in-house control material over a 4-year period (Figure 1). The quality control charts ensured consistent analysis quality and supported the reliability of the analysis method.

Table 2. Accuracy of retinol analysis

Year	SRM[1)]	Retinol (μg/100 g)		Recovery (%)[4)]
		Certified value[2)]	Analytical value[3)]	
2018	SRM 1845a	78 ± 18	67.54 ± 0.97	86.59
	ERM-BD600	380 ± 60	397.63 ± 14.65	104.64
	SRM 3252	2380 ± 150	2400.12 ± 97.89	100.84
2019	SRM 1845a	78 ± 18	63.46 ± 0.20	81.36
	SRM 3280	44400 ± 4600	45847 ± 974.72	103.26
	ERM-BD600	380 ± 60	382.59 ± 2.56	100.68
	SRM 3252	2380 ± 150	2333.19 ± 182.44	98.03
2020	SRM 3280	44400 ± 4600	43243.72 ± 4242.05	97.40
	SRM 3290	248 ± 40	240.51 ± 5.02	96.98
	SRM 3252	2380 ± 150	2374.82 ± 69.29	99.78
	SRM 3235	66.2 ± 7.9	64.45 ± 5.65	97.36
2021	SRM 3280	44400 ± 4600	41140.58 ± 934.13	92.66
	SRM 3290	248 ± 40	237.51 ± 2.63	95.77
	SRM 3252	2380 ± 150	2388.16 ± 31.20	100.34
	SRM 3235	66.2 ± 7.9	60.04 ± 1.45	90.69

[1)] SRM, standard reference material

[2)] The certified value for the contents of corresponding analytes in SRM provided by NIST.

[3)] The analytical value obtained in this study.

[4)] Recovery (%) = analytical value / certified value × 100

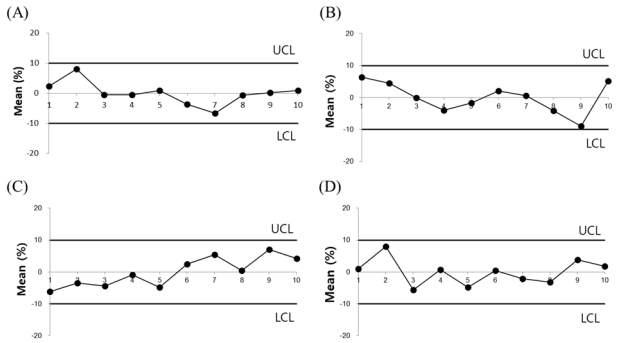

Figure 1. Quality control chart for monitoring the contents of retinol in infant formula in (A) 2018, (B) 2019, (C) 2020, and (D) 2021. UCL: upper control line (+10% of mean); LCL: lower control line (-10% of mean).

CONCLUSIONS

This study aims to establish a database of retinol contents in foods commonly consumed in Korea. In the egg group, quail yolk exhibited the highest retinol content (730.72 μg/100 g). Heat treatment including boiling decreased retinol content in eggs. Some sugar products including jelly were not detected retinol, while chocolate products contained retinol (1.68~40.99 μg/100 g), with white chocolate having the highest due to added milk. Milk and dairy products containing milk had retinol, but vegetable whipped cream didn't. Meats showed varying retinol levels, with the small intestine having the highest at 181.85 μg/100 g. In the seafood group, retinol contents of post-cooking seafoods were generally lower than in fresh seafoods. Seasonings like mayonnaise, barbecue sauce, Thousand Island, and caramel sauce contain retinol, which may be added through eggs, milk, or meats. Method validation ensured accuracy, with recovery rates meeting the AOAC guideline. A quality control chart using infant formula supported analysis reliability. In conclusion, this study provides reliable retinol analytical data in commonly consumed foods in Korea for the development of the Korean nutrient database.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
Borneo Convention Centre, Kuching, Sarawak

ANTIOXIDANT AND CYTOPROTECTIVE ACTIVITIES OF DIFFERENT SOLVENT EXTRACTS OF JUJUBE (*Ziziphus jujuba* Mill.).

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Abstract: Jujube (*Ziziphus jujuba* Mill.) belongs to the *Rhamnaceae* family and is largely used in traditional Asian medicine due to high nutritional composition. Flavonoids, phenolic acids, amino acids, triterpenic acids, saponins, and polysaccharides have been reported as the major phytoconstituents in jujube fruits. The phytoconstituents play important roles in antioxidant, anti-inflammatory, anti-obesity, anti-diabetic, and hepatoprotective activities. This study examined the antioxidant and cytoprotective activities of various solvent (water, methanol, ethanol, ethylacetate, chloroform, hexane) extracts of jujube. Extracts of jujube were investigated for their antioxidant properties using ABTS and DPPH radical scavenging capacity assays, reducing power, and the Folin-Ciocalteu method. The cytoprotective activities of jujube extracts against UVB-induced skin photo-aging in human skin fibroblast (Hs68 cells) and *tert*-butyl hydroperoxide(TBHP)-induced oxidative stress in human hepatocytes (HepG2 cells) were investigated. Among the six different solvents, methanol and water extracts showed significantly higher antioxidant activities based on DPPH and ABTS radical scavenging activities and reducing power. In addition, methanol and water extracts showed higher total phenolic contents (478.0±4.0, 354.8±1.9 mg gallic acid equivalent/100 g, respectively). The highest cytoprotective activities in Hs68 and HepG2 cells were observed in water extract followed by methanol extract. Taken together, water is the most efficient extracting solvent for the antioxidant and cytoprotective activities of jujube. Therefore, further experiments are progressing to elucidate the anti-photoaging and hepatoprotective mechanism of jujube water extracts.
***Keywords*:** jujube, antioxidant, cytoprotective activity, skin fibroblasts, hepatocytes

INTRODUCTION

Oxidative stress generated by reactive oxygen species, such as hydroxyl radicals and hydrogen peroxide, is thought to be implicated in pathological conditions such as inflammation and cancer (Gordon, 1996). Oxidative stress can induce cell damages, which potentially impairs the functions of target organs, including the skin and liver (Pizzino et al., 2017). Fruits and vegetables serve as rich sources of antioxidants, such as vitamin C, tocopherol, and phenolic acids, which contribute to their capacity for antioxidant and free radical scavenging effects (Scalbert et al., 2005). Dietary antioxidants play a role in stimulating cellular defenses and aiding in the protection of cellular components from oxidative damage (Evans & Halliwell, 2001). Jujube (*Ziziphus jujuba* Mill.) contains plenty of nutrients like minerals, vitamins, and polyphenols. It also has been used as a traditional medicine for the treatment of various diseases (Wang et al., 2010). Jujube is rich in polyphenolic compounds exhibiting admirable antioxidant properties (Du et al., 2013). Recently, numerous studies have suggested that jujube exhibits various bioactivities including antioxidant, anti-inflammatory, and anti-cancer effects. For this reason, jujube may be considered a functional food, with both nutritional and medicinal uses (Gao et al., 2012). In the present study, we investigated the antioxidant activities of six different solvent extracts of jujube and their cytoprotective properties in fibroblasts and hepatocytes exposed to UVB and TBHP-induced oxidative stress respectively.

MATERIALS AND METHODS

Chemicals

Dimethyl sulphoxide (DMSO), 2,2-diphenyl-l-picrylhydrazyl (DPPH), 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), potassium ferricyanide, trichloroacetic acid, ferric chloride, Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), gallic acid, *tert*-butyl hydroperoxide (TBHP), 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), ascorbic acid, and quercetin were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Sample preparation
Jujube was purchased from a farm located in Korea. Jujube (20 g) was extracted with 400 mL of water, methanol, ethanol, ethyl acetate, chloroform, and hexane by shaking for 24 h at room temperature. Extracts were filtered using a Whatman No. 2 filter paper and then concentrated by freeze-drying or evaporating under a vacuum. The dried residues were dissolved in distilled water or DMSO, and stored at -20°C until use.

Determination of antioxidant activities
DPPH radical scavenging activity was determined according to the method by Kim et al. (2002) with some modifications. Briefly, 0.2 mM solution of DPPH radical solution in methanol was prepared and then 1 mL of this solution was mixed with 50 μL of sample solution, after standing in the dark for 30 min, the absorbance was measured at 520 nm against blank samples. A decrease in absorbance indicates DPPH free radical scavenging activity. Results were expressed as Trolox equivalent antioxidant activity.
ABTS assay was performed according to the method reported by Re et al. (1999) with some modifications. Briefly, ABTS was dissolved in 10 mL water to a 7.4 mM concentration. The ABTS radical cation was generated by adding 7mM ABTS to 2.45mM potassium persulfate solution, and the mixture was left to stand overnight in the dark at room temperature. The ABTS solution (1 mL) was added to 50 μL of sample solution. Absorbance was measured at 735 nm after 30 min. Results were expressed as Trolox equivalent antioxidant capacity.
The reducing power was estimated using the method of Oyaizu (1986). Briefly, extracts were mixed with a 0.2 M phosphate buffer at a pH of 6.6 and 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min and mixed with 10% trichloroacetic acid. The mixture was centrifugated at 10,000 rpm (9,800 ×g) for 5 min. The supernatant was mixed with distilled water and 0.1% ferric chloride. The absorbance was read at 700 nm on a spectrophotometer. The reducing power was expressed as Trolox equivalent antioxidant capacity.

Determination of total phenolic content
Total phenolic content (TPC) was measured by the Folin-Ciocalteu colorimetric method. A 100 μL of the extracts were mixed with the solution of 2 mL of 2% Na_2CO_3 solution and 100 μL of 50% (v/v) Folin-Ciocalteu reagent solution. After incubation for 5 min at room temperature, the absorbance was read at 750 nm on a spectrophotometer. Polyphenol content was calculated using a gallic acid standard curve.

Cell culture and cytoprotective effects
Hs68 cells were obtained from the American Type Culture Collection (ATCC; VA, USA) and cultured in DMEM supplemented with 10% FBS. The cells were incubated in a humidified atmosphere of 5% CO_2 at 37°C. Hs68 cells were seeded in 96-well plates at a density of 1×10^4 cells/well. After incubation for 24 h, the cells were pre-treated with the samples in serum-free medium for 24 h and then irradiated with UVB (30 mJ/cm^2) using a UVB lamp (Sankyo Denki Lamps, GL20SE, Marine, Japan). After irradiation, cells were reprocessed with the samples for additional 24 h. Cell viability was determined by MTT assay. All experiments were performed in triplicate. Ascorbic acid (100 μM) was used as a positive control.
HepG2 cells were purchased from the Korean Collection for Type Cultures (KCTC; Daejeon, Korea) and cultured in DMEM supplemented with 10% FBS. The cells were incubated in a humidified atmosphere of 5% CO_2 at 37°C. HepG2 cells were seeded in 96-well plates at a density of 1×10^5 cells/well. After incubation for 24 h, the cells were treated with the samples for 12 h, and then the culture medium was removed. Oxidative stress was induced by treatment with 500 μM TBHP for 3 h. Cell viability was determined by MTT assay. All experiments were performed in triplicate. Quercetin (10 μM) was used as a positive control.

Statistical analysis
The results were expressed as mean ± standard error (SE) and were representative of at least 3 independent experiments. Data were analyzed by one-way ANOVA followed by Tukey's post hoc test using GraphPad Prism software (GraphPad Software Inc., La Jolla, CA, USA).

RESULTS AND DISCUSSION

Determination of antioxidant activities and TPC in jujube extracts.
Oxidative stress arises from an imbalance between the production of free radicals and the body's antioxidant defense mechanisms. Bioactive compounds, such as polyphenols, flavonoids, and vitamins, have been reported to neutralize excess free radicals and maintain intracellular balance in the body, thereby exerting protective effects against oxidative stress (Lobo et al., 2010). In this study, we analyzed ABTS and DPPH radical scavenging activities, reducing power, and TPC to assess the antioxidant capacities of various jujube extracts. The ABTS and DPPH methods are widely

used for evaluating the antioxidant activity of both purified phenolic compounds and natural plant extracts. Figure 1 presents a comparison of the DPPH, ABTS, reducing power, and TPC values of six different solvent extracts of jujube. The water and methanol extracts showed high antioxidant activities, followed by ethanol, ethyl acetate, chloroform, and hexane extracts. Furthermore, the water and methanol extracts showed higher TPC compared to other solvent extracts. Phenolic compounds are well known to have high antioxidant activities. Caffeic acid, *p*-hydroxybenzoic acid, ferulic acid, and *p*-coumaric acid are the most abundant phenolics reported in *Ziziphus* (Muchuweti et al., 2005), contributing to its substantial antioxidant activity, reducing power, and free radical scavenging effect (Kamiloglu et al., 2009). The higher antioxidant activities in methanol and water extracts might have been attributed to the higher levels of phenolic compounds in the extracts.

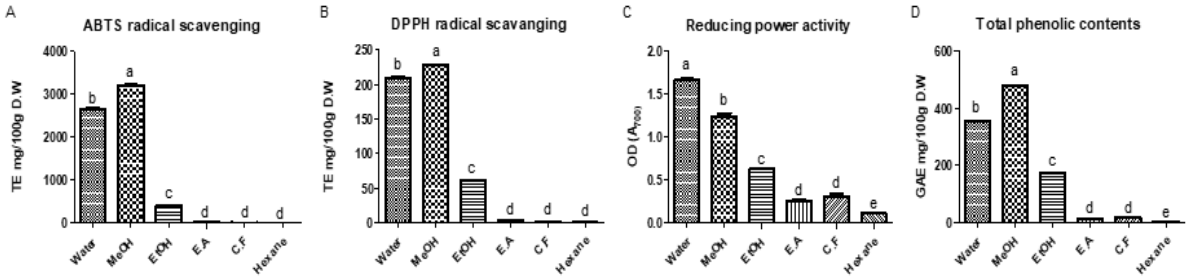

Figure 1. Determination of antioxidant activities and TPC in jujube extracts. ABTS (A) and DPPH (B) radical scavenging activities, reducing power (C), and TPC (D). Values in the same column followed by the same upper case letter are not significantly different by Duncan's multiple range test ($p<0.05$).

Cytoprotective activities of jujube extracts in Hs68 and HepG2

In the present study, UVB-induced Hs68 cells and TBHP-induced HepG2 cells were used to investigate the protective effects of jujube extracts against oxidative cell damages. Hs68 cells have been widely used in many studies as a human dermal fibroblast model to evaluate the anti-photoaging activity of dietary compounds (Han et al., 2019). UVB irradiation modulates diverse signal transduction systems and induces DNA damage in skin cells (Cadet et al., 2005). To examine the protective effects of jujube extracts against UVB-induced skin damage, Hs68 cells were irradiated with UVB and then incubated with extracts of various concentrations (12.5-50 μg/mL) for 24 h. As shown in Figure 2A, UVB exposure led to a decrease in cell viability to approximately 65%, whereas treatment with water, methanol, and ethanol extracts recovered cell viability.

HepG2 cells have been widely used as a human hepatic model in numerous studies to evaluate the biological activities of dietary compounds (Noh et al., 2018). To examine the protective effects of jujube extracts in HepG2 cells, we treated the cells with TBHP and varying concentrations of jujube extracts (12.5-50 μg/mL). As shown in Figure 2B, TBHP treatment markedly decreased cell viability compared to the control. However, pretreating HepG2 cells with jujube extracts before adding TBHP significantly increased cell viability, particularly notable in the case of jujube water extract at 50 μg/mL. Taken together, these results suggest that jujube water extract significantly enhances cytoprotective effects in both Hs68 and HepG2 cells.

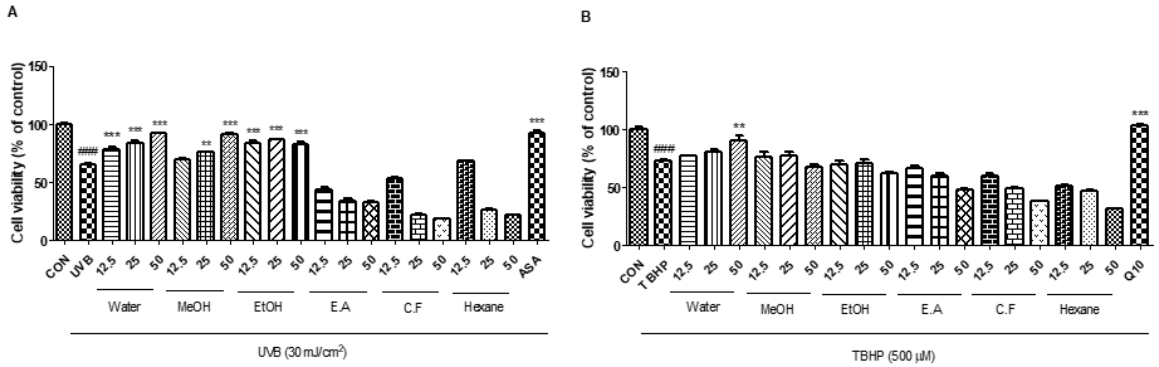

Figure 2. Effects of jujube extracts on protective effects against UVB-induced Hs68 cells (A), and TBHP-induced HepG2 cells (B). Each value was expressed as the mean ± SE (n=3). Significance was tested using Tukey's test. ###$p<0.001$ versus the non-treated control. **$p<0.01$ and ***$p<0.001$ versus the UVB or TBHP-treated control.

CONCLUSIONS

In conclusion, among the six different jujube extracts, both methanol and water extracts showed high TPC levels. Furthermore, notable antioxidant activities were observed in the methanol and water extracts, whereas the hexane extract displayed the lowest antioxidant properties. Based on cytoprotective activities, the water extract of jujube showed relatively higher protective effects against UVB-induced cell damage in Hs68 cells and TBHP-induced oxidative stress in HepG2 cells. These findings suggest that water is the optimal extraction solvent for biological activities from jujube. Collectively, our data conclude that jujube may have the potential as a functional food ingredient for the development of nutraceutical foods to increase antioxidant and cytoprotective activities. Therefore, further studies investigating anti-photoaging and hepatoprotective mechanisms and the corresponding phytochemicals for jujube water extracts are required.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
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DETERMINATION OF VITAMIN E FOR KOREAN FOOD COMPOSITION DATABASE

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Abstract: Vitamin E is a generic term for tocopherol and tocotrienol homologs. Analytical data of the vitamin E contents is lacking in Korean food composition database. The purpose of this study was to analyze the vitamin E contents in commonly consumed foods in Korea. The samples included 13 nuts, 26 cereals, 12 eggs, 19 legumes, 9 oils, and 31 vegetables. The vitamin E contents were analyzed by saponification extraction followed by normal phase liquid chromatography. The results were expressed as α-tocopherol equivalent (α-TE). The α-TE values of nuts, cereals, eggs, legumes, oils, and vegetables ranged from 0.27 to 9.05, 0.00 to 5.15, 0.18 to 2.03, 0.26 to 29.40, and 0.11 to 4.39 α-TE/100 g, respectively. Pine nut (9.05 α-TE/100 g), millet (1.55 α-TE/100 g), egg yolk (5.15 α-TE/100 g), small black soybean (2.03 α-TE/100 g), sunflower seed oil (29.40 α-TE/100 g), steamed hot pepper (4.39 α-TE/100 g) showed the highest α-TE values among nuts, cereals, eggs, legumes, oils, and vegetables, respectively. The vitamin E contents of vegetables increased by heat treatment. The results from method validation procedures showed good accuracy (82.5-108.6%) and precision (less than 5%). This study provides reliable analytical data for development of Korean food composition database.

***Keywords*:** vitamin E, tocopherol, tocotrienol, method validation, food composition database

INTRODUCTION

Vitamin E is classified into four tocopherols and four tocotrienols (α-, β-, γ-, and δ-) based on the presence of double bonds in the side chains and the number of attached methyl groups. (Knecht et al., 2015). These isomers are known to differ in their contents in foods and physiological activities (Park et al., 2016; Kamal-Eldin & Appelqvist, 1996). Therefore, it is necessary to analyze each of the eight isomers individually to accurately measure their physiological activities. Vitamin E functions as an antioxidant in the body, scavenging free radicals and reducing lipid oxidation (Fanali et al., 2017). It has also been reported to prevent chronic diseases such as cardiovascular diseases and cancer, as well as lower blood cholesterol levels (Hill, 1998). Vitamin E deficiency can lead to conditions including peripheral neuropathy, myopathy, and anemia, particularly in children and the elderly (Dror & Allen, 2011). The prevailing method for analyzing vitamin E is high-performance liquid chromatography (HPLC), which allows for the comprehensive analysis of all eight vitamin E isomers (Piironen et al., 1984). The most commonly used extraction method is saponification through alkaline hydrolysis, which facilitates the extraction of vitamin E by breaking the ester bonds of triglycerides, phospholipids, and sterols (Lee et al., 1999). The current Korean food composition table (RDA (rural development administration), 2021) includes nutritional analysis data for 3,113 foods. Out of these, the vitamin E content is specified for 1,641 foods, representing only 52.7%. In addition, the content is expressed as mg/100 g of the sample, which complicates its use in situations requiring precise measurement of physiological activity. Therefore, in this study, we analyzed all of the vitamin E isomers in 13 nuts, 26 cereals, 12 eggs, 19 legumes, 9 oils, and 31 vegetables and the results were expressed as α-tocopherol equivalent (α-TE).

MATERIALS AND METHODS

1) Materials and Reagents

A total of 13 nuts, 26 cereals, 12 eggs, 19 legumes, 9 oils, and 31 vegetables were provided by the RDA. All samples were stored at -18 °C. Tocopherol and tocotrienol were purchased from Merck (Darmstadt, Germany).

2) Saponification method and HPLC analysis

Approximately 3-4 g of the samples were weighed and saponified at 75 °C in a water bath for 50 minutes after adding 6% pyrogallol in ethanol (20 mL) and 60% KOH (8 mL). The suspension was cooled and then mixed with 20 mL of a 2% NaCl solution. The saponified sample was extracted three times with 20 mL of an extraction solvent (hexane:ethyl acetate = 85:15, v/v, containing 0.01% BHT). The extract was dehydrated using anhydrous $MgSO_4$, and the volume was adjusted to 50 mL with the extraction solvent. A 2 mL aliquot of the final solution was taken. After evaporating the solvent under nitrogen, the residue was dissolved in 1 mL of hexane and then filtered through a 0.45 μm PTFE membrane filter (Whatman, Clifton, NJ, USA) (Lee et al., 1999).
For the HPLC system, a solvent delivery pump M930 (Young Lin Instrument Inc, Korea) and a fluorescence detector (Model LC305, Thermo Separation Products Inc, CA, USA) were used. The analytical column was a LiChrosphere® Diol 100 column (250×4 mm, i.d. 5 μm, Hibar Fertigsaube RT, Darmstadt, Germany)

3) Calculation of α -tocopherol equivalents
The equation used to calculate the α-tocopherol equivalent (α-TE), which represents the in vivo activity of vitamin E, is as follows, and γ-tocotrienol and δ-tocotrienol were not included in the equation because their physiological activity has not yet been established (Park et al., 2016). T refers to tocopherol, and T3 represents tocotrienol.
α-TE = (α-T × 1.0) + (β-T × 0.5) + (γ-T × 0.1) + (δ-T × 0.01) + (α-T3 × 0.3) + (β-T3 × 0.05)

RESULTS AND DISCUSSION

The vitamin E content of nuts, grains, and eggs is presented in Table 1. The α-TE values of nuts ranged from 0.27-9.05 α-TE/100 g, with pine nuts exhibiting the highest value. The α-TE values of cereals ranged from 0.29-1.69 α-TE/100 g, with corn, millet, and brown rice exhibiting higher values. Brown rice exhibited relatively higher tocotrienol content. According to Qureshi et al. (1991), tocotrienols have been reported to have effects such as reducing cholesterol, regulating blood pressure, and providing neuroprotection.The α-TE values of eggs ranged from 0.00-5.15 α-TE/100 g. Vitamin E was only found in the egg yolk and was not detected in the egg white. This result is consistent with reports from the USDA NRS national nutrient database (USDA, 2019), which also states that vitamin E is absent in the egg whites of chicken, quail, and duck eggs. The vitamin E content of legumes, oils, and vegetables is presented in Table 2. The α-TE values of legumes ranged from 0.18-2.03 α-TE/100 g and the total vitamin E content in legumes decreased after cooking, except for adzuki beans. This decrease could be attributed to non-enzymatic reactions accelerated by heat and the activity of tocopherol oxidase during the initial stages of cooking. These reactions occur when tissue damage happens and enzymes are not completely heat-inactivated (Knecht et al., 2015). The α-TE values of oils ranged from 0.26-29.40 α-TE/100 g, with sunflower seed oil exhibiting the highest value. Oils exhibited high levels of γ-tocopherol content, and γ-tocopherol is known to be associated with the oxidative stability of oils (Marmesat et al., 2008; Warner & Moser, 2009). The α-TE value of vegetables ranged from 0.11-4.39 mg/100 g and the vitamin E contents of vegetables increased by heat treatment. This increase may be due to the inhibition of tocopherol oxidase activity and the increase in extraction yield caused by the softening of tissues during the heating process.

Table 1. The contents of tocopherols and tocotrienols in nuts, cereals and eggs

Foods	Tocopherol and tocotrienol contents (mg/100 g)									
	α-T	β-T	γ-T	δ-T	α-T3	β-T3	γ-T3	δ-T3	Total	α-TE[1)]
Nuts										
Almond, raw	4.26±0.13	0.07±0.01	0.34±0.02	-	0.23±0.01	-	-	-	4.90±0.16	4.40±0.13
Almond, seasoned	4.13±0.03	0.08±0.00	0.45±0.01	-	0.17±0.02	-	-	-	4.83±0.07	4.27±0.04
Brazil nut, seasoned	2.15±0.12	-	5.10±0.27	-	-	-	-	-	7.25±0.39	2.66±0.15
Cashew nut, raw	0.29±0.01	-	4.91±0.30	-	-	-	-	-	5.20±0.30	0.78±0.04
Cashew nut, seasoned	0.27±0.02	-	3.85±0.27	-	-	-	-	-	4.11±0.29	0.65±0.05
Peanut, raw	2.18±0.08	0.06±0.00	2.95±0.16	-	0.02±0.00	-	-	-	5.21±0.24	2.51±0.01
Peanut, boiled	2.95±0.01	0.10±0.01	5.10±0.39	-	-	-	-	-	8.16±0.39	3.51±0.03
Peanut, roasted	1.81±0.02	0.07±0.00	2.27±0.00	-	0.03±0.01	-	-	-	4.19±0.01	2.09±0.02
Peanut, seasoned	3.49±0.14	0.15±0.01	3.37±0.05	-	-	-	-	-	7.01±0.20	3.90±0.15
Pecan, raw	0.88±0.09	0.16±0.00	8.49±0.20	-	-	-	-	-	9.53±0.30	1.81±0.11
Pecan, seasoned	0.61±0.03	0.13±0.02	7.65±0.70	-	-	-	-	-	8.39±0.69	1.44±0.05
Pine nut, raw	8.27±0.41	-	7.84±0.43	-	-	-	0.40±0.02	-	16.50±0.86	9.05±0.45
Pine nut, gruel	0.26±0.02	-	0.07±0.01	-	-	-	-	-	0.34±0.01	0.27±0.02
Cereals										
Corn, Andaok, dried	1.28±0.05	-	0.77±0.02	-	0.26±0.01	-	0.21±0.00	-	2.52±0.07	1.43±0.05
Corn, Changdaok, dried	1.17±0.02	-	0.40±0.01	-	0.28±0.00	-	0.11±0.00	-	1.95±0.01	1.29±0.02
Corn, Cheonganok, dried	1.32±0.03	-	0.68±0.01	-	0.22±0.00	-	0.12±0.00	-	2.34±0.04	1.46±0.03
Corn, Dapyeongok, dried	1.05±0.00	-	0.60±0.00	-	0.21±0.00	-	0.17±0.00	-	2.10±0.00	1.21±0.00
Corn, Hwangdaok, dried	1.05±0.03	-	0.77±0.03	-	0.20±0.00	-	0.17±0.00	-	2.20±0.05	1.19±0.03
Corn, Jangdaok, dried	0.99±0.00	-	1.25±0.04	-	0.21±0.00	-	0.18±0.00	-	2.62±0.05	1.18±001
Corn, Pyeonggangok, dried	1.37±0.06	-	0.97±0.06	-	0.26±0.01	-	0.19±0.01	-	2.80±0.13	1.55±0.07
Corn, Pyeongangok, dried	0.94±0.01	-	1.30±0.03	-	0.31±0.01	-	0.48±0.01	-	3.04±0.06	1.17±0.02
Corn, Yanganok, dried	0.89±0.05	-	1.52±0.01	-	0.20±0.00	-	0.12±0.00	-	2.72±0.07	1.10±0.06
Millet, Dahwangme, raw	0.71±0.06	-	2.45±0.20	-	0.06±0.00	-	0.08±0.00	-	3.30±0.27	0.97±0.08
Millet, Daname, raw	1.40±0.02	-	1.35±0.02	-	0.05±0.00	-	0.04±0.00	-	2.83±0.01	1.55±0.02
Millet, Gyeonggwan1ho, raw	1.12±0.14	-	2.52±0.29	-	0.09±0.00	-	0.07±0.00	-	3.81±0.42	1.40±0.16

Millet, Gyeonggwan2ho, raw	1.24±0.01	-	2.45±0.03	-	0.05±0.00	-	0.07±0.00	-	3.82±0.04	1.51±0.01
Millet, Hwangmichal, raw	0.87±0.11	-	2.57±0.23	-	0.06±0.01	-	0.13±0.01	-	3.63±0.34	1.14±0.13
Millet, Johwangme, raw	0.73±0.08	-	2.57±0.26	-	0.06±0.00	-	0.13±0.01	-	3.49±0.36	1.00±0.11
Millet, Samdame, raw	0.70±0.02	-	2.28±0.09	-	0.05±0.00	-	0.09±0.00	-	3.12±0.11	0.94±0.03
Millet, Samdachal, raw	0.79±0.12	-	2.75±0.40	-	0.06±0.01	-	0.10±0.01	-	3.71±0.54	1.09±0.16
Prosomillet, Hallachal, raw	0.14±0.02	0.09±0.02	1.43±0.20	-	-	-	-	-	1.66±0.23	0.33±0.05
Prosomillet, Hwangsilchal, raw	0.12±0.00	0.09±0.00	1.29±0.07	-	-	-	-	-	1.50±0.07	0.29±0.01
Prosomillet, Ibaekchal, raw	0.12±0.00	0.10±0.00	1.87±0.06	-	-	-	-	-	2.09±±0.06	0.36±0.01
Rice, brown, Goami2, raw	1.31±0.15	0.09±0.00	0.07±0.00	-	1.09±0.11	-	0.83±0.09	0.17±0.02	3.56±0.37	1.69±0.19
Rice, brown, Haiami, raw	0.94±0.00	0.04±0.00	0.12±0.00	-	0.65±0.01	-	0.79±0.01	0.08±0.00	2.62±0.00	1.17±0.00
Rice, brown, Ilpumbyeo, raw	0.77±0.04	0.04±0.00	0.09±0.01	-	0.72±0.02	-	0.72±0.02	0.11±0.01	2.46±0.10	1.02±0.05
Sorghum, Donganme, raw	0.39±0.01	-	0.62±0.00	-	-	-	-	-	1.01±0.01	0.45±0.01
Sorghum, Nampungchal, raw	0.31±0.00	-	1.04±0.01	-	-	-	-	-	1.36±0.01	0.42±0.01
Sorghum, Sodamchal, raw	0.36±0.01	-	1.06±0.00	-	-	-	-	-	1.42±0.01	0.47±0.01
Eggs										
Egg, duck, white, raw	-	-	-	-	-	-	-	-	-	-
Egg, duck, white, boiled	-	-	-	-	-	-	-	-	-	-
Egg, duck, yolk, raw	4.66±0.09	-	1.38±0.16	-	0.22±0.01	-	-	-	6.07±0.25	4.67±0.11
Egg, duck, yolk, boiled	4.45±0.05	-	1.53±0.01	-	0.23±0.01	-	-	-	6.40±0.06	4.87±0.05
Egg, quail, white, raw	-	-	-	-	-	-	-	-	-	-
Egg, quail, white, boiled	-	-	-	-	-	-	-	-	-	-
Egg, quail, yolk, raw	1.10±0.11	-	0.89±0.06	-	0.16±0.01	-	0.09±0.00	-	2.25±0.18	1.24±0.12
Egg, quail, yolk, boiled	1.19±0.15	-	0.94±0.07	-	0.17±0.01	-	0.09±0.00	-	2.39±0.22	1.33±0.16
Egg, white, raw	-	-	-	-	-	-	-	-	-	-
Egg, white, boiled	-	-	-	-	-	-	-	-	-	-
Egg, yolk, raw	4.84±0.05	-	2.22±0.01	-	0.28±0.00	-	-	-	7.34±0.06	5.15±0.05
Egg, yolk, boiled	3.35±0.32	-	1.72±0.06	-	0.26±0.01	-	0.11±0.01	-	5.44±0.36	3.60±0.32

[1)] : α-Tocopherol equivalent. - : Not detected.

Table 2. The contents of tocopherols and tocotrienols in legumes, oils and vegetables

Foods	Tocopherol and tocotrienol contents (mg/100 g)									
	α-T	β-T	γ-T	δ-T	α-T3	β-T3	γ-T3	δ-T3	Total	α-TE[1)]
Legumes										
Adzuki bean, black, raw	0.18±0.00	-	1.61±0.01	-	-	-	-	5.63±0.05	7.42±0.04	0.34±0.01
Adzuki bean, black, boiled	0.13±0.02	-	0.79±0.08	-	-	-	-	3.31±0.24	4.23±0.34	0.21±0.03
Adzuki bean, red, raw	0.11±0.00	-	1.71±0.02	5.61±0.03	-	-	-	-	7.44±0.06	0.34±0.01
Adzuki bean, red, boiled	0.13±0.00	-	0.76±0.01	-	-	-	-	2.41±0.01	3.29±0.02	0.20±0.00
Cowpea, raw	0.01±0.00	-	1.69±0.02	-	-	-	2.59±0.00	-	4.28±0.02	0.18±0.00
Cowpea, boiled	0.01±0.00	-	1.99±0.13	-	-	-	2.61±0.25	-	4.61±0.38	0.21±0.02
Soybean, raw	1.00±0.04	0.16±0.01	6.98±0.08	-	-	-	-	-	8.14±0.13	1.78±0.05
Soybean, boiled	0.61±0.06	0.09±0.00	5.29±0.09	-	-	-	-	-	5.98±0.15	1.18±0.07
Black soybean, Seoritae, raw	0.92±0.04	0.17±0.02	7.24±0.22	3.43±0.22	-	-	-	-	11.77±0.50	1.76±0.07
Black soybean, Seoritae, boiled	0.53±0.02	0.10±0.00	5.61±0.27	2.45±0.09	-	-	-	-	8.70±0.39	1.17±0.05
Black soybean, Heuktae, raw	0.85±0.08	0.20±0.02	7.32±0.26	4.97±0.24	-	-	-	-	13.34±0.60	1.73±0.12
Black soybean, Heuktae, boiled	0.40±0.01	0.10±0.01	4.65±0.45	2.61±0.16	-	-	-	-	7.76±0.28	0.94±0.03
Jwinunikong (small black soybean), raw	1.23±0.12	0.21±0.02	6.65±0.18	3.12±0.30	-	-	-	-	11.21±0.26	2.03±0.12
Jwinunikong (small black soybean), boiled	0.62±0.11	0.10±0.00	4.24±0.14	1.74±0.06	-	-	-	-	6.71±0.18	1.11±0.12
Mung bean, without skin, raw	0.07±0.00	-	6.48±0.15	0.48±0.02	-	-	-	-	7.04±0.17	0.73±0.01
Mung bean, without skin, boiled	0.04±0.00	-	2.35±0.07	0.15±0.00	-	-	-	-	2.54±0.08	0.28±0.01
Mung bean, without skin, steamed	0.04±0.00	-	3.47±0.09	0.23±0.00	-	-	-	-	3.75±0.09	0.39±0.01
Mung bean, with skin, raw	0.17±0.00	-	6.53±0.12	0.42±0.03	-	-	-	-	7.11±0.15	0.82±0.02
Mung bean, with skin, boiled	0.09±0.00	-	3.08±0.08	0.17±0.00	-	-	-	-	3.34±0.08	0.40±0.01
Oils										
Avocado oil	15.17±0.12	2.15±0.02	2.15±0.02	-	-	-	-	-	19.47±0.17	16.46±0.13
Coconut oil	0.13±0.01	-	-	-	0.41±0.01	0.05±0.00	0.11±0.00	-	0.71±0.03	0.26±0.01
Lard	1.87±0.08	-	-	0.21±0.00	0.41±0.00	-	0.49±0.00	-	2.98±0.08	2.00±0.08
Olive oil	4.17±0.22	0.24±0.02	8.37±0.38	0.88±0.01	-	-	-	-	13.67±0.19	5.14±0.17
Palm oil	10.38±0.21	0.48±0.02	0.48±0.02	1.09±0.02	2.77±0.27	-	4.26±0.36	-	19.46±0.90	11.51±0.13
Rice bran oil	7.18±0.08	-	0.28±0.01	0.27±0.02	0.83±0.00	1.89±0.05	8.27±0.25	0.39±0.03	19.11±0.28	7.56±0.08
Shortening	6.56±0.38	0.19±0.01	0.19±0.01	0.20±0.01	1.82±0.06	-	2.03±0.09	0.32±0.00	11.31±0.55	7.22±0.41
Sunflower seed oil	29.40±1.86	-	-	-	-	-	-	-	29.40±1.86	29.40±1.86
Walnut oil	1.30±0.03	12.64±0.22	12.64±0.22	-	-	-	-	-	26.58±0.46	8.88±0.16
Vegetables										
Asparagus, raw	0.58±0.01	-	0.24±0.00	-	-	-	-	-	0.82±0.01	0.60±0.01
Asparagus, blanched	0.66±0.01	-	0.24±0.00	-	-	-	-	-	0.90±0.01	0.68±0.01
Basil, leaves, raw	0.65±0.05	-	0.28±0.01	-	0.10±0.01	-	-	-	1.04±0.04	0.71±0.05
Basil, leaves, blanched	1.07±0.05	-	0.48±0.01	-	0.06±0.00	-	-	-	1.61±0.04	1.14±0.05
Broccoli, raw	0.21±0.03	-	0.08±0.01	-	-	-	-	-	0.29±0.04	0.22±0.03
Broccoli, blanched	0.80±0.07	-	0.22±0.03	-	-	-	-	-	1.02±0.09	0.82±0.07
Broccoli, steamed	0.63±0.10	-	0.17±0.01	-	-	-	-	-	0.80±0.11	0.64±0.10
Burdock, raw	0.11±0.01	-	-	-	-	-	-	-	0.11±0.01	0.11±0.01
Burdock, blanched	0.21±0.04	-	-	-	-	-	-	-	0.21±0.04	0.21±0.04
Carrot, raw	0.08±0.01	-	-	-	-	-	-	-	0.08±0.01	0.08±0.01
Carrot, blanched	0.16±0.03	-	-	-	-	-	-	-	0.16±0.03	0.16±0.03
Carrot, steamed	0.15±0.00	-	-	-	-	-	-	-	0.15±0.00	0.15±0.00
Garlic, raw	0.29±0.00	-	-	-	-	-	-	-	0.29±0.00	0.29±0.00
Garlic, blanched	0.27±0.00	-	-	-	-	-	-	-	0.27±0.00	0.27±0.00
Green garlic, raw	0.43±0.01	-	-	-	-	-	-	-	0.43±0.01	0.43±0.01
Green garlic, blanched	0.42±0.04	-	-	-	-	-	-	-	0.42±0.04	0.42±0.04
Hot pepper, red, raw	3.87±0.06	0.10±0.00	0.15±0.00	-	0.02±0.00	-	-	-	4.13±0.07	3.94±0.06
Hot pepper, red, boiled	4.22±0.16	0.12±0.01	0.20±0.02	-	0.02±0.00	-	-	-	4.56±0.20	4.31±0.17
Hot pepper, red, steamed	4.31±0.17	0.11±0.00	0.20±0.02	-	0.02±0.00	-	-	-	4.64±0.18	4.39±0.18
Kale, raw	0.29±0.01	-	-	-	-	-	-	-	0.29±0.09	0.29±0.01
Kale, boiled	0.83±0.06	-	-	-	-	-	-	-	0.83±0.17	0.83±0.06
Paprika, red, raw	1.72±0.09	0.05±0.00	-	-	-	-	-	-	1.77±0.08	1.75±0.09
Paprika, red, boiled	2.09±0.16	0.06±0.01	-	-	-	-	-	-	2.14±0.06	2.11±0.17

Paprika, red, steamed	1.13±0.07	0.04±0.00	-	-	-	-	-	-	1.18±0.08	1.16±0.07
Perilla leaves, raw	0.34±0.06	0.05±0.00	-	-	-	-	-	-	0.38±0.06	0.36±0.06
Perilla leaves, blanched	0.78±0.01	0.06±0.00	-	-	-	-	-	-	0.84±0.02	0.81±0.02
Perilla leaves, steamed	0.56±0.02	-	-	-	-	-	-	-	0.56±0.02	0.56±0.02
Tomato, raw	0.30±0.01	-	0.13±0.00	-	-	-	-	-	0.44±0.01	0.32±0.01
Tomato, blanched	0.40±0.04	-	0.15±0.01	-	-	-	-	-	0.55±0.06	0.41±0.04
Tomato, cherry tomato, raw	0.42±0.02	-	0.24±0.02	-	-	-	-	-	0.67±0.04	0.45±0.02
Tomato, cherry tomato, blanched	0.51±0.03	-	0.28±0.02	-	-	-	-	-	0.79±0.04	0.54±0.03

[1)] : α-Tocopherol equivalent. - : Not detected.

CONCLUSIONS

In this study, we analyzed the vitamin E content of commonly consumed foods in Korea. The highest α-TE values among nuts, cereals, eggs, legumes, oils, and vegetables were pine nut (9.05 α-TE/100 g), millet (1.55 α-TE/100 g), egg yolk (5.15 α-TE/100 g), small black soybean (2.03 α-TE/100 g), sunflower seed oil (29.40 α-TE/100 g), and steamed hot pepper (4.39 α-TE/100 g), respectively. The vitamin E content varied depending on the variety. Oil generally has a high vitamin E content, and vegetables show an increase in vitamin E content with heat treatment. This data could provide analytical information for the development of a Korean food composition database.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
Borneo Convention Centre, Kuching, Sarawak

OPTIMIZATION OF EXTRACTION CONDITION OF PHYTOSTEROLS IN QUINOA BY RESPONSE SURFACE METHODOLOGY

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Abstract: Phytosterols have received extensive attention owing to their excellent cholesterol-lowering activity and the role in cardiovascular diseases prevention. Cereals and vegetable oils are the two major dietary sources of phytosterols. In this study, response surface methodology (RSM) was applied to optimize the extraction procedures for the determination of phytosterols in quinoa sample. The effects of varying the amount of 60% potassium hydroxide (KOH), saponification time at 75°C, and final ethanol concentration (EtOH) on the phytosterol contents were evaluated to optimize extraction and saponification steps by RSM. The optimized parameters were obtained by ridge analysis. Based on the ridge analysis, optimum conditions found were: ml 60% KOH, 5.0; saponification time (min), 33.4 at 75°C; and %EtOH, 34.8. Under the optimized conditions, the experimental values agreed with values predicted by ridge analysis.
Keywords: phytosterols, optimization, response surface methodology, saponification, quinoa

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is a healthy grain marketed as a superfood with great nutritional value. Many of its nutritional qualities are significantly higher than the daily consumption crops such as wheat, rice and corn etc. (Chen et al., 2023). Diverse studies have revealed that quinoa contains bioactive compounds including proteins, polysaccharides, saponins, flavonoids and phytosterols. Therefore, these bioactive compounds have antioxidants (Ren et al., 2023), anti-diabetes (Mudgil et al., 2020; Tan et al., 2020), anti-inflammatory (Yao et al., 2015), and anti-cancer (Mohamed et al., 2019; Stikić et al., 2020). The main phytosterols found in food are β-sitosterol, campesterol, and stigmasterol, which exist in free form or in ester and glycoside binding form (Lagarda et al., 2006). It is essential to hydrolyze the combined sterol into a free form using saponification before quantification (Islam et al., 2017). Such saponification is a common step in hydrolyzing ester bonds to analyze total phytosterol (Feng et al., 2022). Extraction using saponification is affected by conditions such as solvent, KOH concentration, saponification time, saponification temperature, and derivatization time (Garcia-Llatas et al., 2021). Therefore, in this study, we tried to optimize the extraction conditions of campesterol, stigmasterol, and β-sitosterol, which are major phytosterols from quinoa using response surface methodology (RSM).

MATERIALS AND METHODS

Materials

Campesterol, stigmasterol, β-sitosterol, 5α-cholestane standards and N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) were purchased from Sigma-Aldrich, and pyridine was purchased from Junsei, KOH and NaCl was purchased from Samchun.

Experimental design

According to the Box-Behnken design (BBD), three independent variables (X1: 60% KOH amount, X2: saponification time at 75°C, and X3: final ethanol concentration) were set. The ranges of three independent variables were set and encoded in three stages: -1, 0, and 1. An extraction experiment was conducted by setting a total of 15 sections according to BBD (Table 1).

Phytosterol extraction

2 g of quinoa was taken 20 mL of ethanol containing 6% pyrogallol was added, and a certain amount of 60% KOH was added according to the experimental design, and then saponified at 75°C water bath. After cooling at room

temperature, distilled water containing 2% NaCl was added to adjust the ethanol concentration, and the ethanol concentration was extracted three times with a 20 mL extraction solvent, and moisture of the extract was removed through $MgSO_4$ and purified to 50 mL.

Analysis of phytosterol components using GC-FID

The Phytosterol analysis was performed by concentrating 5 mL of quinoa extract purified at 50 mL with nitrogen, re-dissolving 1 mL of chloroform containing 5α-cholestane at a concentration of 101.16 μg/mL. The derivatization was carried out at 60°C for 20 minutes by adding 50 μL of MSTFA and pyridine respectively. The reaction solution after derivatization was completed was filtered with 0.45 mm PTFE membrane filter and analyzed with GC-FID.

RESULTS AND DISCUSSION

Assessment of the model suitability

In order to obtain efficient extraction conditions of phytosterol from quinoa using the RSM, the amount of 60% KOH (5-15 mL), the saponification time (15-55 min), and the final ethanol concentration (25-45%) were set as independent variables. The dependent variables were set to the main phytosterol, campesterol, stigmasterol, and β-sitosterol. Table 1 shows the results of the dependent variable obtained under the extraction conditions of 15 sections designed by BBD. The content of phytosterol according to each condition was 0.71-1.68 mg/100g of campesterol, 1.43~2.69 mg/100g of stigmasterol, and 14.01~17.50 mg/100g of β-sitosterol, under the condition that 5 mL of 60% KOH was added, saponified at 75°C for 35 min and the final ethanol concentration was extracted at 35%, all three phytosterols showed high values. Based on the results of the regression analysis, the suitability of the dependent variables campesterol, stigmasterol, and β-sitosterol models was evaluated (Table 2). In all three dependent variables, the R^2 value of the regression model was 0.91 or more, and suitability was recognized at a confidence level of 91% or more. The lack of fit test was not significant at P>0.1 for the three phytosterol contents, confirming that the response surface model was suitable for explaining changes in their campesterol, stigmasterol, and β-sitosterol contents.

Table 1. Box-Behnken design (BBD) matrices and responses for experimental values

Run	Variables			Phytosterol contents of quinoa		
	Amount of 60% KOH (mL), X_1	Saponification time[1] (min), X_2	Final ethanol concentration[2] (%), X_3	Campesterol (mg/100 g)	Stigmasterol (mg/100 g)	β-Sitosterol (mg/100 g)
1	15(1)[3]	55(1)	35(0)	1.04	1.80	15.57
2	15(1)	15(-1)	35(0)	0.71	1.43	14.01
3	5(-1)	55(1)	35(0)	1.58	2.56	16.33
4	5(-1)	15(-1)	35(0)	1.68	2.63	17.12
5	15(1)	35(0)	45(1)	0.85	1.64	15.88
6	15(1)	35(0)	25(-1)	1.14	1.84	15.69
7	5(-1)	35(0)	45(1)	1.44	2.35	17.50
8	5(-1)	35(0)	25(-1)	1.46	2.69	17.08
9	10(0)	55(1)	45(1)	0.83	1.67	15.13
10	10(0)	55(1)	25(-1)	1.13	1.89	16.10
11	10(0)	15(-1)	45(1)	0.85	1.70	15.48
12	10(0)	15(-1)	25(-1)	1.04	1.72	14.71
13	10(0)	35(0)	35(0)	1.01	1.95	17.06
14	10(0)	35(0)	35(0)	0.80	1.65	15.75
15	10(0)	35(0)	35(0)	0.94	1.92	16.63

[1]The saponification temperature is fixed at 75°C.
[2]Final ethanol concentration was adjusted by NaCl solution.
[3](-1), (0), and (1) are coded levels.

Table 2. Analysis of extraction variables as linear, quadratic term and interactions on response variables

Source	df	Sum of square		
		Campesterol	Stigmasterol	β-Sitosterol
Model	9	1.220*	2.140**	12.309*
Linear	3	0.823**	1.649**	6.477*
Quadratic	3	0.330	0.428*	3.797*
Cross product	3	0.067	0.063	2.036
Lack of fit	3	0.052	0.034	0.214
Pure error	2	0.023	0.055	0.892
Total error	5	0.075	0.089	1.106
R^2		0.9420	0.9601	0.9175

R^2 = coefficient of determination.
Significant at *P<0.05, **P<0.01.

Effect of extraction conditions on phytosterol content

According to figure 1, the amount of campesterol and stigmasterol decreases slightly as the amount of 60% KOH added increases, but does not have a significant effect on the final ethanol concentration. Therefore, it was confirmed that the amount of 60% KOH in the independent variable had the greatest effect on the content of phytosterol.

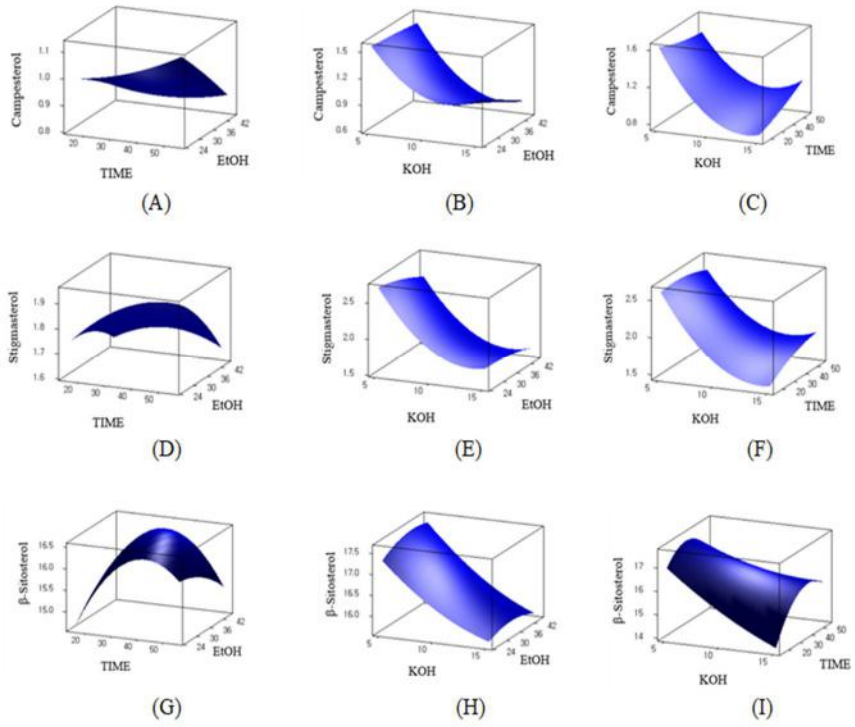

Figure 1. Response surface plots for the levels of campesterol(A-C) and stigmasterol(D-F), β-sitosterol(G-I) depending on three variables, amount of 60% KOH(KOH), saponification time at 75°C(TIME), and final ethanol concentration (EtOH).

Verification of Phytosterol optimal extraction conditions

The optimal extraction conditions for more efficiently extracting campesterol, stigmasterol, and β-sitosterol from quinoa were 5.0 mL of 60% KOH added, 33.4 minutes of saponification time at 75°C, and 34.8% of the final ethanol concentration. Table 3 compares the predicted values obtained under optimal extraction conditions with the actual measurements obtained by actual experiments under those conditions.

Table 3. Predicted value of the responses at optimized conditions

Responses	Optimum conditions[1)]			Predicted value[3)] (mg/100 g)	Experimental value[2)] (mg/100 g)
	Amount of 60% KOH (mL)	Saponification time (min)	Final ethanol concentration (%)		
Campesterol	5.02	33.35	34.66	1.52[a]	1.41 ± 0.04[a]
Stigmasterol	5.04	34.20	33.89	2.61[a]	2.65 ± 0.06[a]
β-Sitosterol	5.06	32.69	35.93	17.59[a]	16.93 ± 0.17[a]

[1)]Optimum conditions were obtained from ridge analysis.

[2)]The values in parenthesis were predicted values 5.0 mL (amount of 60% KOH), 33.4 min (saponification time) and 34.8% (final ethanol concentration) for quinoa.

[3)]Experimental values were obtained using 5.0 mL (amount of 60% KOH), 33.4 min (saponification time) and 34.8% (final ethanol concentration) for quinoa.

Different superscript letters within each column indicate a significant difference between predicted value and experimental value at the $P<0.05$ level.

CONCLUSIONS

The extraction conditions of major phytosterol components from quinoa were optimized using a RSM designed by BBD. For optimization of the extraction and saponification steps, three contents of campesterol, stigmasterol, and β-sitosterol of quinoa was evaluated according to the amount of 60% KOH (5-15 mL), saponification time at 75°C (15-55 min), and final ethanol concentration (25-45%). Based on the results of the regression analysis, a significant coefficient of determination was found, and the significance of the lack of suitability test was rejected, confirming that the response surface model was suitable. The optimal conditions obtained through ridge analysis were 5.02 to 5.06 mL of 60% KOH, a saponification time of 32.69 to 34.20 minutes at 75°C, and a final ethanol concentration of 33.89 to 35.93%. The actual measurements of the content of phytosterol extracted under optimal conditions were 1.41 ± 0.04 mg/100g of campesterol, 2.65 ± 0.06 mg/100g of stigmasterol, and 16.93 ± 0.17 mg/100g of β-sitosterol, which were similar to the predicted value. Therefore, the extraction conditions for analyzing the phytosterol content of quinoa were optimized using the RSM and ridge analysis. In this study, the optimal extraction conditions are considered to be applicable not only quinoa but also various nuts with similar matrix, and are expected to help establish a functional database of phytosterol contents in food.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
Borneo Convention Centre, Kuching, Sarawak

EFFECT OF FERMENTATION TIME USING *RHIZOPUS ORYZAE* ON TEXTURAL PROPERTIES OF CHICKPEA TEMPEH AS DYSPHAGIA FOOD

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Abstract: Tempeh, a fermented food, is highly nutritious and a good source of protein for dysphagia patients. However, tempeh uses large and non-uniform bean seed sizes and requires texture modification for dysphagia diets. This study evaluated the effect of texture modification of tempeh for dysphagia diet according to fermentation time using *Rhizopus oryzae* and ground chickpeas. Tempeh was prepared by grinding boiled chickpeas (particle sizes<4 mm) and varying fermentation time to 24h, 36h, 48h, 60h, and 72h at 33±2°C. The physical properties of tempeh before and after cooking by fermentation time were measured, and modified texture was analyzed using the International Dysphagia Diet Standardisation Initiative (IDDSI) Framework, which is a global guideline. The increase of mould mycelium (cake) by fermentation time was confirmed by appearance and SEM, and cake binding significantly increased hardness, cohesiveness, and brittleness. After cooking, hardness, cohesiveness, and brittleness decreased and shear force increased by fermentation time ($P<0.05$). IDDSI results classified 24h, 36h, and 48h fermentation as level 7 and 60h and 72h fermentation as level 6. Unlike texture modification by adding thickeners, the texture of tempeh was modified by grinding chickpeas and controlling fermentation time. Texture-modified tempeh may contribute to improving the health of dysphagia patients.
Keywords: Tempeh, fermentation time, dysphagia, IDDSI framework, texture

INTRODUCTION

Tempeh is a traditional Indonesian food produce by solid state fermentation (SSF) of soybeans with *Rhizopus* species. During fermentation, soybeans are bound together by white mycelium and macromolecules are hydrolyzed by fungal enzymes (Huang et al, 2019). SSF is a bioprocessing technology that contributes to the development of desirable nutrition, texture, and flavor of various legumes. Chickpea, a legume widely consumed worldwide, are a good source of carbohydrates, proteins, and several bioactive components (Kaur and Prasad, 2021). Various studies have been conducted on tempeh prepared in SSF by *Rhizopus* spp. and chickpea (Erkan et al, 2020; Angulo-Bejarano et al, 2008). Dysphagia refers to the swallowing difficulty, which manifests as an abnormal delay in moving food in the form of an alimentary bolus during swallowing (Alagiakrishnan et al, 2013). Dysphagia causes complications such as malnutrition and aspiration pneumonia, and a texture-modified diet is a convenient and effective treatment method for patients. Chickpea tempeh can be a nutritious food for dysphagia patients, but tempeh requires texture modification for dyspahgia diets. In addition, previous studies on preparing tempeh as a dysphagia food were lacking. This study evaluated the effect of texture modification of tempeh for dysphagia diet according to fermentation time using *Rhizopus oryzae* and ground chickpeas. The physical properties and modified texture of tempeh by fermentation time and heat treatment were analyzed. The modified texture used the global guidline, the International Dysphagia Diet Standardisation Initiative (IDDSI) Framework, which provides standardized terms, descriptors, and measurment criteria for drinks (levels 0–4) and texture-modified food (levels 3–7) (International Dysphagia Diet Standardization Initiative, 2019). Cooked chickpea tempeh were evaluated with the goal of level 6 and level 7 by fermentation time.

MATERIALS AND METHODS

Materials

Commercially imported chickpeas (*Cicer arietinum* L.) cultivated in Canada were purchased from a local food store. The chickpeas were fermented using a commercial tempeh starter (Cultures for Health, Morrisville, NC, USA) and brown rice vinegar (Ottogi, Anyang, Korea) was used.

Preparation of tempeh

Whole seeds of chickpeas were soaked in distilled water (DW) at room temperature for 12 h, and then cooked in DW at 98±2°C for 30 min. After the removal of drain and cooling down to room temperature, then cooked chickpeas were ground to particle size of 3.50±0.22 mm using blender at 13,000 rpm for 20 s. Ground chickpeas were mixed with

vinegar, and inoculated by tempeh starter. The mixture was packed in perforated polyethylene bags of regular size and spacing for aerobic conditions, and then molded. Tempeh were fermented at 33±2°C for 24, 36, 48, 60, and 72 h.

Visual appearance and scanning electron microscopy (SEM)
The white mycelium of tempeh were observed by visual evaluation. Microstructure of tempeh was measured using scanning electron microscope (SU8220, Hitachi, Tokyo, Japan). Lyophilized tempeh were sprayed with platinum and then images were taken at 150× magnification.

Physicochemical properties
Tempeh were diluted 1:4 (w/v) with DW and ground using hand blender for 1 min. The pH and total soluble solids (TSS) were measured with pH meter (Orion 3 star, ThermoFisher Scientific, Brooklyn, NY, USA) and refractometer (Master-a, Atago, Tokyo, Japan), respectively. Viscosity was determined after running at 100 rpm for 1 min using rotational viscometer (DVIM, Brookfield Engineering, Middleborough, MA, USA) with spindle #64.

Texture profile analysis (TPA)
TPA was performed used tempeh before/after cooking using rheometer (Compac-II, Sunscientific, Tokyo, Japan) with 50 mm cylindrical probe. Testing conditions were as follows: a pre-test speed of 2 mm/s, test speed and post-test speed of 1 mm/s, compression distance of 2 mm, clearance of 7 mm, and loadcell of 2 Kgf. The parameters obtained from two-bite compression test included hardness, cohesiveness, and brittleness.

International dysphagia diet standardization initiative (IDDSI) tests
Cooked tempeh (15 mm x 15 mm x 10 mm) were expected to be categorized as level 6 (soft & bite-sized) and level 7 (Easy to chew) described in IDDSI framework (International Dysphagia Diet Standardization Initiative, 2019) according to results of preliminary tests. Samples were measured by fork/spoon pressure test, and fork separation test. The pressure (approximately 17 kPa) balanced thumb nail to white, which is utilized to mimic tongue force during swallowing food. The behavior of samples was observed and compared using IDDSI descriptions to evaluate the level.
Statistical analysis
The experiments were replicated three times and all data were presented as mean±standard deviation. The statistical analyzes were processed using SPSS software package (Version 26, SPSS, Chicago, IL, USA) by one-way analysis of variance (ANOVA) with Duncan's multiple range test and Student's *t*-test. The results were considered statistically significant if $p < 0.05$.

RESULTS AND DISCUSSION

Visual appearance and SEM
The mold mycelium (cake) and microstructure of tempeh by fermentation time are shown in Figure 1. In SSF, the mold forms a compact cake that binds the beans together and causes hydrolysis of macromolecules (Romulo et al, 2021). Chickpea tempeh were confirmed by visual appearance that white cakes were densley formed as fermentation time increased. The microstructure of tempeh showed that surface structure of particles became rough by fermentation time. As reported by Wang et al. (2023), microbal fermentation disruped the microstructure of substrate surface, indicating that nutrients have been decomposed and used. The cake formation and microstructural deformation according to fermetation time are considered to affect physicochemical properties of chickpea tempeh.

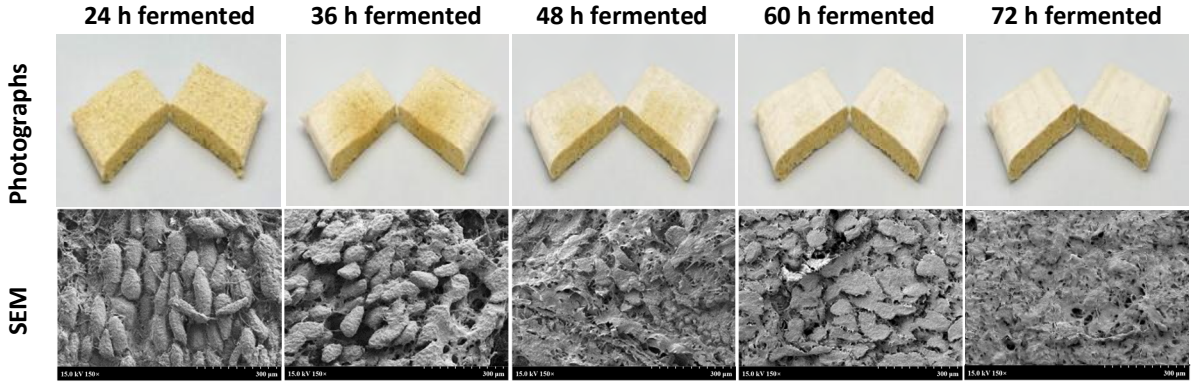


Figure 1. Visual appearance and scanning electron microscopy (SEM, magnification 150×) of tempeh with different fermentation times.

Physicochemical properties

The physicochemical properties of chickpea tempeh are presented in Table 1. The pH, TSS, and viscosity increased significantly with increasing fermentation time. The increase in pH colud be attributed to proteolytic activities and release of ammonia by microorganisms involved in fermentation (Adelekan and Nwadiuto, 2012). SSF increased brix and viscosity by enzymatically breakdown of carbohydrates in chickpeas into simpler forms.

Table 1. Physicochemical properties of tempeh with different fermentation times.

Fermentation time (hour)	pH	Total soluble solids (ºBrix)	Viscosity (cP)
24	5.38±0.03^{a}	3.00±0.00^{a}	148.00±3.46^{a}
36	5.51±0.01^{b}	3.27±0.06^{b}	168.00±6.00^{b}
48	5.60±0.05^{c}	3.50±0.10^{c}	196.00±3.46^{c}
60	5.99±0.02^{d}	3.63±0.06^{cd}	264.00±15.87^{d}
72	6.15±0.01^{e}	3.67±0.12^{d}	340.00±3.46^{e}

Data are expressed as mean±SD (n=3). Different letters within a column present significant differences ($p<0.05$).

TPA

TPA parameters of tempeh before/after cooking are presented in Figure 2. Tempeh before cooking showed a tendency to increase hardness, cohesiveness, and brittleness by fermentation time, which is considered to be formation of compact cakes. Handoyo and Morita (2006) reported mycelium was overgrown and has link network among bean grains becoming strong after fermentation. TPA results of cooked tempeh were confirmed to be the opposite of results before cooking. These results suggest the weakening of cake bond formed in tempeh by heat treatment, and the softening of chickpeas by hydrolysis by fermentation. The hardness and brittleness of tempeh before/after cooking showed a significant effect after 48 h fermendted.

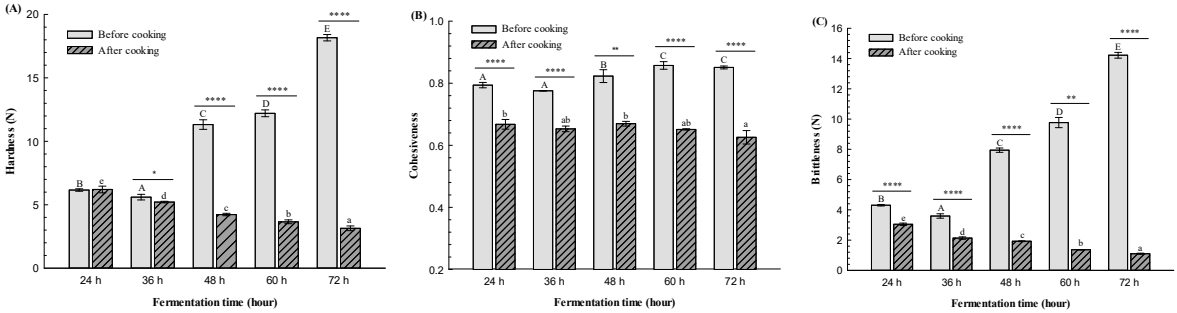
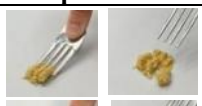
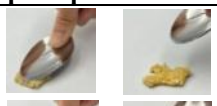
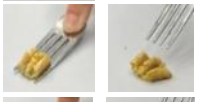
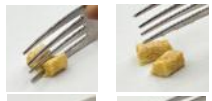
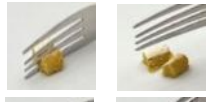
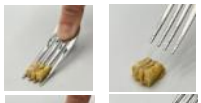


Figure 2. (A) hardness, (B) cohesiveness, and (C) brittleness of tempeh and cooked tempeh with different fermentation times.

IDDSI tests

Table 2. The IDDIS tests of cooked tempeh with different fermentation times.

Fermentation time (hour)	IDDSI tests			
	Fork pressure test	Spoon pressure test	Cut or break apart	Comments
24				Level 7 (Easy to chew)
36				Level 7 (Easy to chew)
48				Level 7 (Easy to chew)
60				Level 6 (Soft & bite-sized)
72				Level 6 (Soft & bite-sized)

Data are expressed as mean±SD (n=3). Different letters within a column present significant differences ($p<0.05$).

The IDDSI test for cooked tempeh is shown in Table 2. For adults, the IDDSI framework recommends that the size of foods for dysphagia are no larger than 15 mm for level 6 and smaller or greater than 15 mm for level 7. Therefore, chickpea tempeh for dysphagia were prepared in a small uniform size and easy to chew through a chickpea crushing process before fermentation. All samples were easily broken and squahsed by fork and spoon with the thumb blanched to white, and unable to recover the shape when the pressure was removed. Samples fermented for 24 h, 36 h, 48 h colud easily broke apart into smaller pieces under pressure, which might be greater than 15 mm as the size of pieces is not restructed. As a consistent result, gel was classified as level 7 because it was easily broken into smaller pieces when the thumbnail turned white (Min et al, 2023). Chickpea tempeh for dysphagia were classified 24 h, 36 h, 48 h fermentation as level 7 and 60 h, 72 h fermentation as level 6.

CONCLUSIONS

Chickpea tempeh were a nutritionally good source for dysphagia patients, but requires a modified texture. In this study, tempeh for dysphagia were developed by preparing small and uniform chickpea particle sizes and varying fermentation times using *Rhizopus oryzae*. The results suggested that tempeh fermented for 24 h, 36 h, 48 h be classified as level 7, and tempeh fermented for 60 h and 72 h as level 6. As the fermentation time increased, tempeh became hard and brittle due to compact cakes. The macromolecules of tempeh by SSF were confirmed through SEM for roughened surface structures under the effect of fungal enzymes, which changed the physicochemical properties of tempeh. The heat treatment made the tempeh softer and less brittle due to the fungal enzymes rather than the effect of cakes. The texture-modified food by IDDSI framework has levels from 3 to 7, and efforts are required to provide tempeh to dysphagia patients with different degrees of disease. Therefore, we plan to conduct study on dysphagia diets using protein-based thickeners and 3D food printing technology to develop tempeh at level 4 and level 5.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
Borneo Convention Centre, Kuching, Sarawak

QUALITY CHARACTERISTICS OF MUFFINS PREPARED WITH THREE TYPES OF SOYBEAN FLOUR

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Abstract: As consumer interest in health, including celiac disease and gluten sensitivity, grows, their preference for gluten-free foods is increasing. This study aimed to investigate the quality properties of muffins prepared using 100% soybean flour as a substitute for regular flour, to assess the feasibility of producing gluten-free food. Different types of soybean flour, such as general soybean flour (GSF), stir-fried soybean flour (SSF), and soaked and dried soybean flour (SDSF), were prepared. Muffins were made using various ingredients, and their proximate composition, quality characteristics, and texture were analyzed. The proximate composition and quality characteristics of muffins made from GSF, SSF, and SDSF showed minimal differences. However, compared to flour muffins, soybean flour muffins had higher protein, fat, and ash content, while their carbohydrate content, height, volume, specific volume, baking loss rate, and pH value were lower. Chromaticity analysis revealed that soybean flour muffins tended to exhibit higher a, and b-values compared to flour muffins. In terms of texture, soybean flour muffins were firmer and chewier than flour muffins, with lower springiness. In conclusion, this study provided essential information for producing gluten-free muffins by highlighting the quality characteristics of muffins made with soybean flour as a substitute for regular flour.
Keywords: muffin, quality, soybean, gluten-free, substitute

INTRODUCTION

Celiac Disease is a permanent autoimmune reaction to gluten present in certain grains such as wheat, rye, barley, and oats. Consumption of these substances leads to damage to the intestinal mucosa and reduces the absorption of important nutrients (1). It affects approximately 1% of the general population, mainly females. It can develop at any age and has a variety of symptoms and manifestations (2). Currently, the only way to manage the condition is to follow a strict gluten-free diet (3). As gluten-containing grains are widely consumed worldwide and the number of people suffering from celiac disease increases, so does the demand for a gluten-free diet. Gluten-free foods are prepared using alternative flours like quinoa, amaranth, buckwheat, and soybean, and include products like bread (4), cookies (5), and cakes (6). Among these, muffins are popular baked goods for consumers due to their convenience, texture, and taste. Various studies are being conducted on gluten-free muffins, including those using rice flour (7), beet fiber (8), and isolated legume protein (9). Soybean is an essential food for human nutrition and is the most popular legume in Asian countries (10). Soybean is rich in protein and contains various physiologically active substances such as isoflavones, saponins, and lecithin, giving it anticancer properties and various physiological benefits (11). Among these, soybean isoflavones have shown positive effects on obesity, cancer, osteoporosis, and cardiovascular diseases (12). Therefore, numerous studies have focused on developing soy-based gluten-free products, although research on 100% soybean muffins is limited.

Thus, this study aimed to investigate the quality characteristics of muffins prepared using 100% soybean flour (general soybean flour (GSF), stir-fried soybean flour (SSF), and soaked and dried soybean flour (SDSF)), as substitutes for wheat flour. The purpose of this study was to assess the feasibility of producing gluten-free food with these soybean flour variations.

MATERIALS AND METHODS

Preparation of soybean flour

The soybeans used in this study were purchased from a local market in Cheongju, Korea. The soybeans were pulverized in step 1 for 5 minutes using a mixer (HMF-3500TG, Hanil Electric Co., Ltd., Seoul, Korea). Subsequently, the pulverized soybeans were sieved using a 30-mesh sieve to obtain flour.

Preparation of muffin

For the production of wheat flour (WF) muffins in this study, the production method outlined by Choi (13) was followed. The procedure was conducted according to the mixing ratios of the raw materials as presented in Table 1. The batters for general soybean flour muffin (GSFM), stir-fried soybean flour muffin (SSFM), and soaked and dried soybean flour muffin (SDSFM) were formulated with soybean flour as substitutes for WF. The mixing ratio of the

samples are detailed in Table 1. Sixty g of batter filled in each paper mold (45 mm in diameter × 37.5 mm in height). The molds were then subjected to baking at 180℃ using upper heat and 150℃ using lower heat for 25 minutes. After baking, the muffins were promptly removed from the oven and allowed to cool at room temperature for 1 hour before being utilized as samples.

Table 1. Mixing ratio of batter for soybean flour muffins.

	Control[1)]	GSFM[2)]	SSFM[3)]	SDSFM[4)]
WF	100	0	0	0
GSF	0	100	0	0
SSF	0	0	100	0
SDSF	0	0	0	100
Butter (g)	60	60	60	60
Sugar (g)	60	60	60	60
Baking Powder (g)	3	3	3	3
Salt（g）	1	1	1	1
Egg (g)	60	60	60	60
Milk (mL)	20	20	20	20

[1]Control: Wheat flour muffin
GSFM: General soybean flour muffin
SSFM: Stir-fried soybean flour muffin
SDSFM: Soaked and dried soybean flour muffin

Proximate analysis of muffins

The proximate composition was determined according to proximate composition methods (14). The moisture content was determined using the atmospheric-pressure drying method at 105℃. Crude protein content was determined semi-micro Kjeldhal method. Crude lipid content was determined soxhlet extraction. Crude ash content was analyzed using an electric furnace (FX-14, Daihan Scientific, Wonju, Korea) at 550℃. Carbohydrate weight was determined as the fraction of the total weight of the sample after excluding moisture, crude protein, crude fat, and mineral contents.

Measurement of volume, weight, height, loss rate, and specific volume of muffins

The weight, height, and volume of the muffins were measured after allowing them to cool at room temperature for 1 hour subsequent to baking in the oven. The height was measured by cutting them longitudinally into two halves from the highest point of the muffin. Volume measurement was performed using the seed substitution method. To calculate the specific volume, the muffin volume (mL) was divided by its weight (g). The baking loss rate of the muffins was calculated using the following formula, which utilizes the batter's weight before baking and the weight after baking. An electronic balance (IB-410, Innotem, Gyeonggi, Korea) was used to determine the weight of the muffins.

$$\text{Baking loss rate (\%)} = \frac{weight\ of\ dough\ before\ baking\ (g) - weight\ of\ muffins\ after\ baking\ (g)}{Weight\ of\ dough\ before\ baking\ (g)} \times 100$$

Chromaticity and pH of muffins

The chromaticity of the muffin was measured using a colorimeter (CR-300, Minolta Co., Tokyo, Japan) for the Hunter L(lightness), a(redness), and b(yellowness) values. All samples were measured 3 times using a white board as the reference color (L = 92.700, a = 0.313, b = 0.396). The pH of the muffin was measured using a pH meter (Docu-pH meter, Sartorius, New York, NY, USA).

Texture profile of muffins

Muffins were cut into cubes with the 2×2×2 cm^3, and the texture was repeatedly measured three times for each experimental group using a texture analyzer (TA-XT2, Stable Micro System Ltd., England) and the texture was measured for hardness, adhesion, adhesiveness, springing, cohesiveness, gumminess, chewiness, and resilience were expressed to the second decimal place. The conditions of TPA were performed as the pretest speed was 2.0 mm/s, the test speed was 1.0 mm/s, the post-test speed was 2.0 mm/s, and the distance was 5 mm.

Statistical analysis

F-values and p-values were calculated using the Statistical Analysis System (SAS) ver. 9.4 (SAS Institute Inc., Cary, NC, USA). For each experiment, average values and standard deviations were calculated by analyzing three or more repetitions, and differences were considered significant at $p < 0.05$ using Duncan's multiple range test.

RESULTS AND DISCUSSION

Comparison of proximate compositions among various muffins.

The four types of muffins prepared with wheat flour, general soybean flour, stir-fried soybean flour, and soaked and dried soybean flour were shown in Figure 1. The proximate values of four types of muffins were compared (Table 2). There was no significant difference in the approximate composition of the four muffins. However, soybean flour muffins are higher in protein, fat, and ash and lower in carbohydrates than flour muffins. Among them, the protein content of soybean flour muffins was more than twice that of the control group, indicating that the use of 100% soybean flour can increase the nutritional value of muffins.

Figure 1. Cross-sections of various muffins

Control GSFM SSFM SDSFM

Control: Wheat flour muffin, GSFM: General soybean flour muffin, SSFM: Stir-fried soybean flour muffin, SDSFM: Soaked and dried soybean flour muffin

Table 2. Proximate composition of the various muffins

	Control[1)]	GSFM[2)]	SSFM[3)]	SDSFM[4)]
Moisture content (%)	19.74±0.04	19.67±0.38	20.04±0.25	18.33±0.53
Crude fat（%）	20.91±0.14	25.42±0.13	25.79±0.17	25.47±0.14
Protein（%）	6.15±0.18	15.85±0.33	15.80±0.20	16.48±0.03
Ash（%）	1.27±0.02	2.82±0.03	1.94±1.44	2.76±0.02
Carbohydrates（%）	51.93±0.28	36.25±0.42	36.43±1.57	36.97±0.43

[1)]Control: Wheat flour muffin
[2)]GSFM: General soybean flour muffin
[3)]SSFM: Stir-fried soybean flour muffin
[4)]SDSFM: Soaked and dried soybean flour muffin

Comparison of quality characteristics among various muffins.

The height, volume, weight, bake loss, and specific volume for the four muffins were compared (Table 3). The volume, height, specific volume, and baking loss rate of all soybean flour muffins were lower than those of the control muffin. Among them, SSFM has the lowest volume, height, and specific volume.

Table 3. Quality characteristics of the various muffins.

	Control[1)]	GSFM[2)]	SSFM[3)]	SDSFM[4)]
Height (cm)	5.47±0.12	4.67±0.06	4.40±0.10	4.53±0.06
Volume (mL)	136.67±5.03	112.33±2.52	102.33±7.57	105.33±3.79
Weight（g）	54.50±0.00	55.50±0.00	55.50±0.00	55.83±0.29
Specific volume (mL/g)	2.51±0.09	2.02±0.05	1.79±0.06	1.89±0.07
Baking loss rate (%)	9.17±0.00	7.50±0.00	7.50±0.00	6.94±0.48

[1)]Control: Wheat flour muffin
[2)]GSFM: General soybean flour muffin
[3)]SSFM: Stir-fried soybean flour muffin
[4)]SDSFM: Soaked and dried soybean flour muffin

Comparison of chromaticity and pH among various muffins

The chromaticity (the crust and crumb) of the four types of muffins were compared (Table 4). There was no significant difference in L-values of the crust and crumb for the three soybean flour muffins, they were all lower than the control muffin. The a-values were higher than those in the control muffin, with the highest a-values for the crust in GSFM and the lowest for the crust in SSFM but the crumb in SSFM is the highest. For the b-value, there was no significant difference in the b-value of the crust of the four types of muffins, and the b-value of the crumb of the three soybean flour muffins was higher than that of WF, and the b-value of the crumb in SSFM was the highest. The pH of the three soybean flour muffins was not significantly different but was lower than that of the control muffin.

Table 4. Chromaticity and pH of the various muffins.

		Control[1)]	GSFM[2)]	SSFM[3)]	SDSFM[4)]
crust	L	73.53±1.18	57.64±2.63	61.58±2.60	60.84±0.69
	a	-2.82±0.34	6.18±2.05	2.05±0.86	5.19±1.14
	b	27.44±0.85	26.36±1.62	26.50±1.15	27.81±0.27
crumb	L	72.82±1.45	60.31±1.20	60.59±0.50	60.72±0.70

	a	-4.50±0.10	-2.09±0.26	-0.89±0.18	-1.55±0.21
	b	20.28±0.71	23.28±0.27	25.40±0.24	24.56±0.13
pH		7.05±0.05	6.64±0.07	6.71±0.14	6.64±0.11

[1)]Control: Wheat flour muffin
[2)]GSFM: General soybean flour muffin
[3)]SSFM: Stir-fried soybean flour muffin
[4)]SDSFM: Soaked and dried soybean flour muffin

Comparison of texture profiles of the various muffins.

The texture of the four types of muffins were compared (Table 5). The results show that the hardness of the three types of soybean flour muffins was higher than that of the control muffin, and the lowest hardness of SSFM was 383.77±41.77. The springiness and chewiness were lower than those of the control muffin, and the chewiness of SSFM was the lowest at 309.46±40.99. There was no significant difference in the springiness of the three types of soybean flour muffins. The cohesiveness of the three types of soybean flour muffins was lower than that of the control muffin, and the highest cohesiveness value of SSFM was 0.77±0.01. There was no significant difference in the resilience values of the four types of muffins.

Table 5. Texture profile of the various muffins

	Control[1)]	GSFM[2)]	SSFM[3)]	SDSFM[4)]
Hardness	118.17±7.22	525.37±32.83	383.77±41.77	536.00±90.50
Springiness	9.22±0.03	1.01±0.01	1.05±0.09	1.02±0.03
Chewiness	878.75±67.91	379.99±19.98	309.46±40.99	403.25±74.19
Cohesiveness	0.80±0.02	0.72±0.00	0.77±0.01	0.74±0.01
Resilience	0.57±0.02	0.51±0.01	0.58±0.01	0.56±0.04

[1)]Control: Wheat flour muffin
[2)]GSFM: General soybean flour muffin
[3)]SSFM: Stir-fried soybean flour muffin
[4)]SDSFM: Soaked and dried soybean flour muffin

CONCLUSION

The study results indicate only slight differences in the proximate composition and quality characteristics of muffins made from GSF, SSF, and SDSF. However, muffins made from soybean flour (three types of soybean flour muffins) exhibited higher levels of protein, fat, and ash and lower carbohydrate content, height, volume, specific volume, bake loss, and pH compared to muffins made from WF. Chromaticity analysis revealed that soybean flour muffins tended to display higher a- and b-values compared to WF. In terms of taste, soybean flour muffins appeared firmer, chewier, and less springiness than wheat flour muffins. In conclusion, this study provides essential information for producing gluten-free muffins by highlighting the quality characteristics of muffins made with soybean flour as a substitute for wheat flour.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
Borneo Convention Centre, Kuching, Sarawak

MINERAL CONTENT CHANGES IN AERIAL PARTS OF *ADENOPHORA TRIPHYLLA* WITH DIFFERENT DRYING AND BLANCHING METHODS

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Abstract: Aerial parts of *Adenophora triphylla* (APAT) are a type of wild vegetable that is commonly used. However, little research has been conducted on how different processing methods, such as drying and blanching, affect nutrient content of APAT. Therefore, this study aimed to compare and analyze the changes in mineral content of APAT resulting from various drying and blanching methods. First, APAT samples were blanched in 100℃ water for 0, 30, 60, and 180 seconds, and dried until constant weight using a hot air dryer (60℃) for 24 hours, a cool air dryer (30℃) for 48 hours, and a freeze dryer (-70℃) for 72 hours. Blanched and dried samples were analyzed for 25 types of mineral content using ICP-OES. The results showed that K, Ca, and P had the highest mineral content in all samples, with dried APAT having the highest total mineral content in the order of hot air, cool air, and freeze drying. Blanched APAT samples showed a decrease in total mineral content as the blanching time increased. In conclusion, this study sheds light on how processing methods affect the mineral content of APAT, which can be useful for enhancing the nutritional value of products made from APAT.

INTRODUCTION

Adenophora triphylla var. japonica is a perennial plant belonging to the dicotyledonous Bellflower family. It is known to be native to East Asia, including regions such as Korea, China, and Japan (1, 2). The Aerial parts of *Adenophora triphylla(*APAT) in spring, as well as the tender leaves and stems in early summer, have been utilized as herbs. Furthermore, its roots have found applications in both culinary and medicinal contexts (2, 3). *Adenophora triphylla* is notably rich in vitamins A and C, along with potassium. Its primary constituents include saponin and inulin (3). Various studies have been conducted on *Adenophora triphylla*, encompassing areas such as the antioxidant effects of ethyl acetate extracts derived from its roots (4), as well as the anti-inflammatory and anti-asthmatic properties of *Adenophora triphylla* extracts (2). However, research on the processing of the APAT is currently insufficient.

Blanching treatment in vegetables, fruits, and mushrooms is witnessing an increase in distribution rates of both raw and blanched products, driven by advancements in production and distribution technologies, as well as consumer preferences for high-quality products. Particularly, with the growth of the food service industry, blanching treatment is increasingly being utilized as various food ingredients (5). The blanching process is one of the processing methods that helps maintain the quality of vegetables by deactivating key enzymes that contribute to quality changes. After blanching, various value-added processing techniques can be applied, allowing the products to be sold as processed foods with higher value (6). However, it's important to note that blanching conditions can lead to changes in color, as well as reductions in nutrients such as vitamins and flavonoids in vegetables (7,8).

In South Korea, the method of drying vegetables such as radish greens, gourd and bracken ferns has been widely employed for preservation (9). The drying process involves the removal of sufficient moisture from the food items to prevent spoilage, making it one of the processing methods used to preserve food (10). The drying process is aimed at preventing the deterioration of food by effectively eliminating moisture, thus aiding in food preservation.

In general, vegetables are consumed after various stages of cultivation and cooking. Vegetables undergo changes in color, taste, aroma, and nutritional content during the cooking process, necessitating research on the nutritional aspects of vegetable processing. However, there is a lack of processing research specifically focused on *Adenophora triphylla*. Therefore, this study aimed to investigate the changes in mineral content of APAT resulting from different drying and blanching methods.

MATERIALS AND METHODS

Materials

The APAT was provided from Chungbuk Agricultural Research and Extension Services.

Preparation of dried APAT

The APAT was dried to a constant weight by different methods: hot air drying at 60°C for 24 hours, freeze drying at -70°C for 72 hours, and cold air drying at 30°C for 48 hours. These processes were employed to prepare dried samples of the APAT.

Preparation of blanched APAT

One hundred g of APAT were blanched in 1 liter of boiling water for 0, 30, 60, and 180 seconds. After blanching, the samples were immediately cooled in cold water. Then, excess moisture on the surface was removed using a paper towel, resulting in blanched APAT samples.

Mineral content of APAT according to processing methods

Drying the APAT and boiling samples are both dried at 105°C. Next, 0.5g of each sample was transferred to a 50 mL conical flask, and 5 mL of nitric acid was added. The mixture was then heated on a hot plate at 140°C for over 5 hours, until 1 mL of solution remained. After that, the solution obtained through nitric acid decomposition was transferred to a 50 mL volumetric flask. Using distilled water, the solution was diluted by a factor of 50. The diluted solution was filtered through a 0.45 μm syringe filter, then, the mineral content was analyzed using ICP. (Inductively coupled plasma-optical-emission spectrometer, Optima 5300 DV, Perkin Elmer, Markham, Canada).

Statistical analysis

Results are reported as mean±standard deviations (n=3). The significance of differences among treatment means was determined using the one-way analysis of variance (ANOVA) and correlation calculated by SAS version 9.3(Statistical Analysiss System, SAS Institute Inc., Cary, NC, USA) with a significance level of $p < 0.05$ by Duncan multiple range's test.

RESULT AND DISCUSSION

Comparison of mineral content by different drying methods in the APAT

The mineral content of 25 different types was analyzed and compared for both the fresh APAT and the dried APAT, based on the drying methods. As a result, both the APAT and dried APAT exhibited a similar distribution pattern of mineral content, with K > Ca > P > Mg... being the predominant order. Particularly, the APAT showed a K content of 5.898 mg/g and a Ca content of 2.449 mg/g. Whereas, in the dried APAT, the K content increased to around 30.781 mg/g, while the Ca content increased to approximately 12.181 mg/g, an increase of about 6-fold. Among the nutritional indicators, K and Ca content are considered appropriate indicators for nutritional assessment of APAT (Table 1).

Table 1. Comparison of mineral contents for APAT according to different drying methods

Minerals (mg/g)	Sample				F-value
	Raw	Hot-air drying (60°C)	Cold-air drying (30°C)	Freezing drying	
Al	0.046±0.014^{b}	0.092±0.003^{a}	0.096±0.008^{a}	0.084±0.003^{a}	21.88***
As	0.001±0.001	0.004±0.003	0.004±0.002	0.006±0.003	-
Ba	0.030±0.004^{b}	0.097±0.005^{a}	0.097±0.003^{a}	0.095±0.002^{a}	251.18***
Be	-	-	-	-	-
Ca	2.449±0.026^{c}	12.966±0.140^{a}	12.181±0.243^{b}	12.669±0.196^{a}	2638.62***
Cd	-	-	-	-	-
Co	-	-	-	-	-
Cr	0.008±0.001	0.008±0.002	0.007±0.000	0.007±0.000	-
Cu	0.010±0.000^{b}	0.010±0.001^{a}	0.011±0.002^{a}	0.004±0.001^{a}	-
Fe	0.087±0.056^{b}	0.130±0.022^{a}	0.131±0.06^{a}	0.126±0.004^{a}	29.73***
Mg	0.753±0.009^{c}	3.702±0.069^{a}	3.519±0.082^{b}	3.643±0.030^{a}	1982.59***
Mn	0.186±0.008^{b}	0.944±0.036^{a}	0.922±0.007^{a}	0.927±0.013^{a}	1079.66***
Mo	-	-	-	-	-
Na	0.487±0.137^{b}	1.448±0.056^{a}	1.557±0.167^{a}	1.452±0.029^{a}	60.09***
Ni	0.007±0.007	0.004±0.001	0.008±0.008	0.004±0.000	-
Pb	0.002±0.001	0.001±0.000	0.001±0.000	0.001±0.000	-
Sb	0.001±0.000	0.001±0.000	0.001±0.001	0.002±0.001	-
Se	-	-	-	-	-

Sn	-	-	-	-	-
Zn	0.032±0.006^{b}	0.104±0.037^{a}	0.102±0.024^{a}	0.086±0.001^{a}	6.75*
P	0.786±0.026^{c}	4.791±0.125^{a}	4.574±0.043^{b}	4.626±0.079^{b}	1840.38***
K	5.898±0.006^{b}	32.587±1.668^{a}	31.602±0.688^{a}	30.781±0.569^{a}	498.88***
B	0.067±0.016	0.079±0.022	0.090±0.009	0.060±0.017	-
Si	0.002±0.001	0.004±0.001	0.003±0.000	0.003±0.001	-
S	0.398±0.015^{b}	2.372±0.046^{a}	2.713±0.746^{a}	2.321±0.069^{a}	23.45***
Total	11.245±0.150^{c}	59.332±1.578^{a}	57.651±0.280^{ab}	56.891±0.813^{b}	2017.76***

$^{a-c}$ Means represented by different superscripts in the same row are significantly different at $p<0.05$.
*,*** Significant at $p<0.05$, $p<0.001$.

Comparison of mineral content by blanched time methods in the APAT

The mineral content of 25 different mineral species was compared and analyzed in the APAT and the blanched APAT using various blanching times. As a result, in the APAT, the distribution of mineral content was K > Ca > P > S... in descending order. Similarly, in the APAT blanched for 30 and 60 seconds, the distribution followed a similar pattern: K > Ca > P > S... However, for the APAT blanched for 180 seconds, the distribution shifted to Ca > K > S > P... in descending order. Analyzing the changes in mineral content as blanching time increased, it was observed that the levels of Ca and S decreased gradually. On the other hand, K and P content exhibited a sharp decrease. Considering the nutritional indicators of the APAT during the blanching process, K and P content appear to be suitable indicators for nutritional assessment (Table 2).

Table 2. Comparison of mineral contents for APAT according to different blanching times

Mineral (mg/g)	Blanching time (s)				F-value
	0	30	60	180	
Al	0.045±0.013	0.035±0.004	0.038±0.002	0.038±0.014	0.55
As	0.008±0.001	0.006±0.001	0.007±0.001	0.008±0.002	1.63
Ba	0.031±0.005^{a}	0.021±0.001^{b}	0.018±0.004^{b}	0.020±0.006^{b}	4.6*
Be	-	-	-	-	-
Ca	3.072±0.140^{a}	1.732±0.030^{b}	1.630±0.080^{b}	1.412±0.042^{c}	236.97***
Cd	-	-	-	-	-
Co	-	-	-	-	-
Cr	0.020±0.001	0.018±0.001	0.018±0.001	0.020±0.002	1.49
Cu	-	-	-	-	-
Fe	0.126±0.014^{a}	0.099±0.006^{b}	0.092±0.007^{b}	0.096±0.012^{b}	7.07*
Mg	0.606±0.027^{a}	0.301±0.007^{b}	0.280±0.008^{b}	0.237±0.006^{c}	381.54***
Mn	0.094±0.008^{a}	0.060±0.004^{b}	0.048±0.004^{c}	0.046±0.002^{c}	58.69***
Mo	-	-	-	-	-
Na	0.430±0.037	0.359±0.033	0.332±0.026	0.319±0.074	3.47
Ni	0.008±0.001	0.008±0.001	0.007±0.001	0.009±0.002	0.86
Pb	-	-	-	-	-
Sb	0.005±0.002	0.006±0.001	0.004±0.001	0.005±0.001	1.01
Se	-	-	-	-	-
Sn	-	-	-	-	-
Zn	0.081±0.009^{a}	0.052±0.004^{b}	0.043±0.004^{b}	0.047±0.005^{b}	27.39***
P	1.251±0.046^{a}	0.547±0.007^{d}	0.485±0.003^{c}	0.348±0.015^{b}	810.57***
K	6.423±0.291^{a}	2.307±0.072^{d}	1.914±0.073^{c}	0.937±0.051^{b}	720.78***
B	0.061±0.016	0.046±0.006	0.033±0.007	0.048±0.022	1.93
Si	0.007±0.001	0.005±0.001	0.007±0.003	0.003±0.001	3.4
S	0.576±0.046^{a}	0.421±0.020^{b}	0.401±0.013^{b}	0.475±0.079^{b}	14.76**
Total	12.844±0.613^{a}	6.021±0.127^{b}	5.355±0.157^{c}	4.067±0.062^{d}	441.65***

$^{a-d}$ Means represented by different superscripts in the same row are significantly different at $p<0.05$.
*,**,*** Significant at $p<0.05$, $p<0.01$, $p<0.001$, respectively.

CONCLUSION

The research findings revealed variations in the mineral content of the APAT based on blanching time and drying methods. This suggests that the mineral content of the APAT can change depending on the chosen blanching time and drying method settings. Depending on the drying method settings of the APAT, the content of K and Ca increased by about 6-fold. Additionally, with increasing blanching time, the content of Ca and S in the APAT decreased gradually, while the content of K and P decreased rapidly. Therefore, these results suggest that the changes in mineral content of the APAT due to drying method settings and blanching time can be used as indicators for nutritional analysis.

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The 17th ASEAN Food Conference 2023 (AFC2023)
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EFFECT OF EXTRACTION METHODS AND TIMES ON QUALITY CHARACTERISTICS OF AERIAL PARTS OF *ADENOPHORA TRIPHYLLA*

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Abstract: The roots of *Adenophora triphylla* are widely used as both food and medicine, but the availability of the aerial parts (leaves and stems) is limited. Therefore, the aim of this study was to investigate the quality characteristics of the aerial parts of *Adenophora triphylla* (APAT) using various extraction methods to increase the utilization of APAT. To prepare the APAT extract (APATE), 10 g of APAT was added to 200 mL of an extraction solvent. The extraction solvents used were water at 60°C, 80°C, and 100°C, as well as 70% ethanol at 80°C, and extraction was conducted for 3, 6, and 9 hours. As the extraction temperature and time increased, the moisture content of APATE decreased and carbohydrate content increased. No significant changes were observed in protein, minerals, and fat. Hot water extraction resulted in increased L-value and total acidity, while a-value, b-value, and pH decreased. Ethanol extraction led to increased L-value, maintained a-value, pH, and total acidity, and decreased b-value. Longer extraction times increased total polyphenol and total flavonoid content but decreased DPPH and ABTS radical scavenging activity. In conclusion, this study provides valuable insights for developing processed products using APAT, considering the extraction processing characteristics and quality changes.
Keywords: *Adenophora triphylla*, aerial parts, extraction, quality, characteristic

INTRODUCTION

Adenophora triphylla var. japonica is a perennial plant belonging to the dicotyledonous Bellflower family. It is known to be native to East Asia, including regions such as Korea, China, and Japan. In the case of the aerial parts of *Adenophora triphylla* (APAT), it is consumed as a vegetable in the spring and is also widely used as an ingredient in bibimbap. Additionally, the roots contain saponin and inulin as main components. Researches on the roots of *Adenophora triphylla* have analyzed effects such as antioxidant and liver protective effects. Furthermore, researches on the APAT have been conducted on the skin-whitening effect of acetic acid ethyl fraction, anti-inflammatory, and anti-asthmatic effects. However, research on APAT is currently insufficient.

Extraction is method to obtain active substances from plants or natural materials, using various methods such as hot water extraction, distillation extraction, and solvent extraction are available. The number of active substances obtained from these methods can vary based on the extraction solvent and methods used. The most common extraction method is hot water extraction, which efficiently converts high molecular weight substances into low molecular weight ones, but it has drawbacks like potential degradation and damage to extracted components due to heat. Researches on extraction have analyzed the antioxidant activity and stability of licorice ethanol extracts, and optimizing the heat extraction conditions of dandelion leaves for enhanced antioxidant activity.

The aim of this study was to investigate the quality characteristics of APAT using various extraction methods to increase the utilization of APAT.

MATERIALS AND METHODS

Materials

The *Adenophora triphylla* was provided by the Chungbuk Agricultural Research and Extension Services.

Preparation of Aerial parts of *adenophora triphylla* extracts

For the preparation of Aerial parts of *adenophora triphylla* extract (APATE), First, APATs were washed using a microbubble washer to remove soil and impurities, and then surface moisture was eliminated. For hot water extracts, 10 g of APATE were placed in a beaker with 200 mL of distilled water, and extraction was conducted at 60, 80, and 100°C for 3, 6, and 9 hours, respectively. The hot water extracts were obtained after filtration. Then, for ethanol extracts, 10 g of APAT were placed in a beaker with 200 mL of 70% ethanol, and extraction was performed at 80°C for 3, 6, and 9 hours each. The ethanol extracts were obtained after filtration.

Proximate composition of different APATEs

The proximate composition was determined according to proximate composition methods (MFDS, 2019), including moisture content by air drying, protein content by semimicro-Kjeldahl method, fat content by ether extraction, and ash content by incineration. Carbohydrate content was calculated by subtracting the sum of moisture, protein, fat, and ash content from the total weight.

Quality characteristics of different APATEs

Chromaticity was measured for APATE at 25±2℃ and using a colorimeter to determine the L (lightness), a (redness), and b (yellowness) values. The standard white plate used for calibration had L=92.7, a=0.3137, and b=0.3960 values. For pH measurement, 10 mL of APATE was taken and pH was measured using a pH meter. Total acidity was determined by taking 10 mL of APATE and neutralizing it with 0.1N NaOH until reaching a pH of 8.3. The acidity was then converted to acetic acid content (%).

Antioxidant characteristics of different APATEs

The hot water extract was freeze-dried and then pulverized using a grinder (80335, Elec-Tech Zhuhai Co., Xiangzhou, China). The ethanol extract was concentrated under nitrogen, freeze-dried, and pulverized. A powdered sample of 1 g was placed in a 50 mL conical tube, followed by the addition of 10 mL of 70% ethanol. The mixture was stirred at 60°C using a thermostatic water bath for 2 hours, then centrifuged at 3,461 × g for 10 minutes. The supernatant was filtered to obtain filtrate for antioxidant and activity analysis. Total polyphenol content was quantified using gallic acid as a standard, expressed as mg of gallic acid equivalent (GAE) per g of sample. Total flavonoid content was quantified using (+)-catechin hydrate as a standard, expressed as mg of catechin equivalent (CE) per g of sample. DPPH radical scavenging activity was measured using L-ascorbic acid as a standard, expressed as mg of L-ascorbic acid equivalent antioxidant capacity (AE) per g of sample. ABTS radical scavenging activity was measured using L-ascorbic acid as a standard, expressed as mg of L-ascorbic acid equivalent antioxidant capacity (AE) per g of sample.

Statistical analysis

F-values and p-values were calculated using Statistical Analysis System (SAS) ver. 9.4 (SAS Institute Inc., Cary, NC, USA). For each experiment, average values and standard deviations were calculated by analyzing three or more repetitions, and differences were considered significant at $p < 0.05$ using Duncan's multiple range test.

RESULTS AND DISCUSSION

Comparison of proximate composition based on extraction methods and times of APATE

Hot water extracts showed lower moisture content as extraction temperatures and times increased. While, ethanol extracts maintained a moisture content of 85.14% to 85.74% even with longer extraction times (Table 1). However, there were no discernible variations based on the extraction conditions for crude ash, crude fat, and crude protein. The results of examining the changes in APATE's composition showed that the extraction time increased, the moisture content decreased. However, there were no changes observed in the content of crude protein, crude ash, and crude fat. Carbohydrate content showed a relative increase as moisture content decreased.

Table 1. Comparison of proximate composition for APATE extracted by hot water and 70% ethyl alcohol according to different extraction temperatures and times

	Temperature (℃)	Time (hr)	Moisture content (%)	Mineral content (%)	Fat content (%)	Protein content (%)	Carbohydrate content (%)
Hot water	60	3	96.78±1.04^{a}	0.04±0.02	0.04±0.01	0.03±0.00	3.10±1.04^{f}
		6	96.61±1.30^{a}	0.07±0.01	0.04±0.01	0.03±0.00	3.25±1.32^{f}
		9	97.02±0.12^{a}	0.06±0.01	0.05±0.02	0.03±0.00	2.84±0.09^{f}
	80	3	94.29±0.60^{b}	0.04±0.01	0.04±0.01	0.03±0.00	5.61±0.58^{e}
		6	91.25±0.76^{c}	0.05±0.01	0.04±0.02	0.03±0.01	8.63±0.77^{d}
		9	91.16±1.63^{c}	0.06±0.02	0.04±0.02	0.03±0.01	8.71±1.61^{d}
	100	3	87.80±1.90^{d}	0.05±0.02	0.03±0.01	0.03±0.00	12.10±1.89^{c}
		6	88.00±0.17^{d}	0.06±0.01	0.04±0.02	0.03±0.00	11.87±0.16^{c}
		9	83.34±1.01^{f}	0.07±0.03	0.05±0.01	0.03±0.00	16.51±1.04^{a}
70% ethanol	80	3	85.14±1.12^{e}	0.04±0.01	0.05±0.01	0.03±0.00	15.29±1.45^{b}
		6	85.32±0.57^{e}	0.07±0.02	0.04±0.02	0.03±0.01	14.55±0.57^{b}
		9	85.74±0.11^{e}	0.07±0.02	0.05±0.01	0.03±0.01	14.12±0.10^{b}
F-value			71.49***	2.08	0.67	0.87	71.49***

$^{a-f}$ Means represented by different superscripts in the same column are significantly different at $p<0.05$.
*** Significant at $p<0.001$.

Comparison of quality characteristics based on extraction methods and times of APATE

APATE from hot water extraction exhibited minimal differences in L-value (ranging from 47.21 to 48.85), with little impact from extraction temperature, particularly (Table 2). Both a-value and b-value decreased with higher extraction temperature. However, a-value showed small changes with extraction time, while b-value increased proportionally. Ethanol extracts showed an increasing trend in L-value (37.72 to 43.03) with longer extraction times, remaining lower compared to hot water extracts. Ethanol extracts also exhibited a constant a-value within a certain range, but b-value sharply increased with longer extraction times. pH and total acidity of hot water extracts slightly decreased and increased, respectively, with rising extraction temperature and time. In contrast, ethanol extracts showed no significant changes in pH and total acidity based on extraction time, indicating higher pH and lower total acidity compared to hot water extracts, pointing to lower extraction efficiency.

Table 2. Comparison of chromaticity, pH and total acidity for APATE by hot water and 70% ethyl alcohol according to different extraction temperatures and times

	Temperature (℃)	Time(hr)	Chromaticity			pH	Total acidity (%)
			L	a	b		
Hot water	60	3	47.21±0.04^{d}	0.03±0.01^{ab}	4.93±0.00^{d}	6.10±0.36^{abc}	0.009±0.002^{bc}
		6	48.33±0.03^{bc}	0.05±0.02^{ab}	3.84±0.04^{e}	5.93±0.10^{abcd}	0.010±0.001^{b}
		9	48.04±0.09^{c}	0.09±0.02^{a}	3.92±0.06^{e}	5.28±0.36^{e}	0.015±0.000^{a}
	80	3	48.33±0.07^{bc}	-0.21±0.00^{ab}	2.91±0.01^{h}	5.92±0.02^{abcd}	0.006±0.004^{e}
		6	48.54±0.03^{ab}	-0.22±0.02^{b}	3.28±0.00^{g}	5.85±0.01^{cd}	0.008±0.001^{bcde}
		9	48.28±0.50^{bc}	-0.21±0.02^{ab}	3.46±0.00^{f}	5.88±0.22^{abcd}	0.007±0.000^{cde}
	100	3	48.74±0.50^{a}	-0.25±0.02^{b}	2.65±0.01^{j}	5.86±0.35^{bcd}	0.007±0.000^{bcde}
		6	48.82±0.02^{a}	-0.24±0.02^{b}	2.77±0.01^{i}	5.56±0.09^{de}	0.009±0.001^{bcd}
		9	48.85±0.09^{a}	-0.25±0.01^{b}	2.93±0.03^{h}	5.41±0.03^{a}	0.010±0.001^{b}
70% ethanol	80	3	37.72±0.04^{f}	-7.32±0.01^{c}	9.68±0.02^{c}	6.24±0.12^{ab}	0.005±0.000^{e}
		6	43.03±0.10^{e}	-8.08±0.54^{d}	14.58±0.05^{a}	6.21±0.13^{abc}	0.006±0.001^{de}
		9	42.97±0.17^{e}	-7.98±0.11^{d}	14.33±0.17^{b}	6.26±0.05^{e}	0.006±0.001^{de}
F-value			786.88***	1424.99***	18453.1***	7.55***	10.48***

$^{a-j}$ Means represented by different superscripts in the same column are significantly different at $p<0.05$.
*** Significant at $p<0.001$.

Comparison of antioxidant characteristics based on extraction methods and times of APATE

For polyphenol content, within the range of hot water extraction conditions, the highest efficiency was observed at a temperature of 80°C. Nevertheless, the most effective extraction method was found to be ethanol extraction. Total flavonoid content increased with higher temperatures and longer extraction times in hot water extraction. Both hot water extracts (80°C) and ethanol extracts exhibited the highest DPPH radical scavenging activity. ABTS radical scavenging activity was highest in the 80°C hot water extraction for 3 hours.

By enhancing the extraction of internally bound compounds within the APAT, both hot water and ethanol extraction methods achieved notably high extraction efficiency for antioxidant compounds. However, prolonged extraction times resulted in comparatively lower antioxidant activity. Considering overall antioxidant properties in APATE based on method, and time, the most effective method for extracting antioxidant compounds with retained activity seems to be the 80°C hot water extraction for 3 hours.

Table 3. Comparison of antioxidant content and activities for APATE by hot water and 70% ethyl alcohol according to different extraction temperatures and times

	Temperature (℃)	Time (hr)	DPPH radical scavenging activity (AE mg/g)	ABTS radical scavenging activity (AE mg/g)	Total polyphenol content (GAE mg/g)	Total flavonoid content (CE mg/g)
Hot water	60	3	0.03 ± 0.01^{g}	0.15 ± 0.00^{bc}	0.28 ± 0.01^{cd}	0.12 ± 0.01^{g}
		6	0.05 ± 0.01^{e}	0.14 ± 0.01^{cd}	0.28 ± 0.00^{cd}	0.14 ± 0.02^{ef}
		9	0.06 ± 0.00^{d}	0.12 ± 0.01^{de}	0.31 ± 0.01^{bcd}	0.17 ± 0.02^{d}
	80	3	0.08 ± 0.00^{bc}	0.16 ± 0.01^{a}	0.26 ± 0.03^{cd}	0.15 ± 0.01^{e}
		6	0.09 ± 0.00^{ab}	0.12 ± 0.02^{e}	0.32 ± 0.00^{bcd}	0.18 ± 0.01^{d}
		9	0.09 ± 0.00^{ab}	0.09 ± 0.00^{f}	0.32 ± 0.01^{ab}	0.19 ± 0.00^{c}
	100	3	0.02 ± 0.01^{g}	0.15 ± 0.01^{ab}	0.28 ± 0.01^{d}	0.10 ± 0.01^{h}
		6	0.04 ± 0.02^{f}	0.12 ± 0.01^{e}	0.29 ± 0.01^{bcd}	0.15 ± 0.01^{e}
		9	0.06 ± 0.01^{d}	0.07 ± 0.02^{g}	0.29 ± 0.02^{cd}	0.23 ± 0.01^{a}
70% ethanol	80	3	0.08 ± 0.01^{c}	0.10 ± 0.00^{f}	0.31 ± 0.01^{bcd}	0.10 ± 0.00^{h}
		6	0.09 ± 0.00^{a}	0.10 ± 0.01^{f}	0.31 ± 0.04^{bc}	0.13 ± 0.01^{fg}
		9	0.10 ± 0.00^{a}	0.07 ± 0.01^{g}	0.35 ± 0.00^{a}	0.20 ± 0.01^{b}
F-value			71.085^{***}	46.55^{***}	4.86^{***}	74.7^{***}

[a-h] Means represented by different superscripts in the same column are significantly different at $p<0.05$.
*** Significant at $p<0.001$.

CONCLUSION

In this study, we analyzed the proximate composition, quality characteristics, and antioxidant characteristics of APATE based on extraction methods and times. Moisture content decreased and carbohydrate content increased in the extracts by increasing extraction temperature and time. Quality was more influenced by extraction time than temperature. We determined extraction efficiency using pH and total acidity, favoring extraction time as a key factor. Maximum extraction efficiency was achieved with 3 hours of hot water extraction at 80°C. This research offers insights for developing processed products utilizing APAT, considering extraction methods and quality variations.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
Borneo Convention Centre, Kuching, Sarawak

PORTABLE ELECTROCHEMICAL BIOSENSOR DEVICE AS POINT-OF-CARE TOOL FOR HAZARDOUS CHEMICAL DETECTION IN AQUACULTURE

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Abstract: Detection of hazardous contaminants by means of portable devices is a strategic approach for practical on-site detection. We report here the detection of malachite green (MG), a carcinogen and teratogen substance, on a handheld electrochemical biosensor device. MG is a toxic triphenylmethane chemical used in aquaculture industry as antifungal agent for fish and shrimps. In aquatic organisms, MG is metabolized into liposoluble leucomalachite green (LMG). Although the usage of MG has been banned in most countries, aquaculture farmers in Asia region still prefers its application as this chemical is very effective in treating fungal, cheap and easily available. Hence its detection is crucial in ensuring the safety of aquaculture products. An enzyme biosensor based on screen-printed carbon electrode (SPCE) is described here for MG detection. The SPCEs were first modified with electrodeposited polypyrrole (Ppy) for butyryl-cholinesterase enzyme (BuChE) immobilization. Inhibition of total MG towards the immobilized BuChE in the presence of butyrylthiocholine substrate was measured via chronoamperometry method. The enzyme-modified SPCEs are then attached to a portable reader, aimed for on-site application. Linear standard curves for total MG had been successfully established in catfish, talapia and prawn matrix; with R^2 values obtained are 0.9453, 0.9422 and 0.9272 respectively.
***Keywords*:** aquaculture, enzyme-based biosensor, enzyme inhibition, portable electrochemical reader, total malachite green

INTRODUCTION

Malachite green (MG), a triphenylmethane dye, is extensively used in aquaculture industry due to its great properties as anti-fungal, anti-protozoan, anti-parasite and anti-bacterial. Although this substance is known as carcinogen and teratogen, MG is still being widely used and favoured by aquaculture farmers due to its low cost, ease of availability and effectiveness in treating fungicides/parasites in fish and shrimps. MG is easily reduced to leucomalachite green (LMG) (Figure 1), highly persistent and remain for a long time in edible fish tissues. Despite being restricted and banned in Malaysia, recent report has shown that fish sold in local market still contain as high as 4 ppb MG (Kwan et al., 2018). Besides, its usage among aquaculture farmers in ASEAN countries such as Vietnam is still reported (Chi et al. 2017).

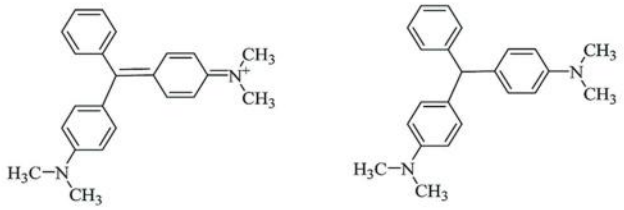

Figure 1. Chemical structures of malachite green (left) and its derivative, leucomalachite green (LMG) (right)

Hitherto, methods such as high-performance liquid chromatography (HPLC), enzyme-linked immunosorbent assays (ELISA), surface-enhanced Raman spectroscopy and spectrophotometry are mainly used for total MG detection

(Nanjappa & Jayaprakash, 2023). However, these methods have several drawbacks such as time consuming, labour intensive, involve hazardous reagents and require highly trained personnel. With this regard, a rapid, simple, sensitive and cost-effective detection of total MG is being sought-after. Of recent, miniaturized biosensor system with screen-printed carbon electrodes (SPCEs) and portable device for on-site detection of contaminants is trending and has garnered attention (Reddicherla et al., 2022). Detection of hazardous contaminants employing portable electrochemical devices is a strategic approach for an efficient and rapid detection.

In this study, an enzyme-based biosensor for the detection of total MG on a portable device is being described. The electrochemical measurement is based on the redox reaction of the enzyme, butyrylcholinesterase enzyme (BuChE), with its substrate where the electron is produced and generated current signal on the modified SPCEs. Inhibition of total MG towards the immobilized BuChE in the presence of butyrylthiocholine substrate was measured via chronoamperometry method. Interference study and matrix effects in various samples were evaluated. Linear standard curves for total MG between 0-10 ppb had been successfully established in catfish, tilapia and prawn matrix; with R^2 values obtained are 0.9453, 0.9422 and 0.9272 respectively.

MATERIALS AND METHODS

Chemicals and reagents

Malachite green oxalate (free zinc) (MG), leucomalachite green (LMG), butyrylthiocholine substrate (BTC), butyrylcholinesterase enzyme (BuChE –221U/mg), pyrrole and sodium hydroxide (NaOH) were obtained from Sigma-Aldrich (Malaysia). Ascorbic acid was from Fluka; while disodium hydrogen phosphate dihydrate, natrium dihydrogen phosphate monohydrate and potassium chloride (KCl) were purchased from Merck.

Electrochemical study

Screen-printed carbon electrodes (SPCEs) used in the study were purchased from Biogenes Technologies, Malaysia. A single strip of the carbon-based SPCE has three (3) channels with a 4-mm combined working, counter and reference electrodes on a circular well with an 8-pin contact type. Electrodeposition of BuChE on SPCEs via polypyrrole (Ppy) network was achieved by chronoamperometry (CA) technique (900 seconds at constant potential of +1.0 V) on a multi-potentiostat (mStat 8000P, Metrohm DropSens). The procedure for enzyme immobilization on SPCE surface modification is adapted from Faridah et al. (2015).

Development of MG standard curves in sample matrix

Non-treated tilapia, catfish and prawn were obtained from organic farm in Hulu Langat. 5 g of fish muscle or prawn was added with 10 mL of 0.1 M phosphate buffer, pH 8.0, and 1 mL of 0.1 M ascorbic acid. Samples were chopped with commercial chopper; and filtered through muslin cloth. Interference study was conducted by comparing the background current generated from the chopped matrix (undiluted and diluted samples of 10x to 50x dilution factors) with buffer system. Standard curves of standard MG was developed by measuring the current changes at different concentration of MGs ranging from 0 to 10 ppb in the sample matrix at a constant potential of -480 mV for 100-200 seconds on portable electrochemical device. The portable reader (Biogenes Technologies, Malaysia) has dimension of 11cm (*L*) x 6 cm (*W*) x 2.5 cm (*H*) and connected to a mobile phone via bluetooth integration (Figure 2).

Figure 2. A portable electrochemical reader with inserted SPCE (right) and a mobile phone unit (right).

RESULTS AND DISCUSSION

Previous study has indicated the success development of enzyme-based biosensor for total MG detection with its high selectivity and sensitivity (Hidayah et al., 2016). However earlier activities were conducted on commercial lab-based

potentiostat and analysis were limited only to tilapia fish. Utilization of portable electrochemical device has becoming a more convenient and strategic approach for researchers to conduct analysis of contaminants on-site. Prior the development of standard curves and real sample application on the portable electrochemical biosensor reader, matrix study of the samples need to be first established. In this study, the choice of sample matrix is widened to catfish, tilapia and prawn. The selection of samples were chosen on the significant amount of MG detected in the aquaculture produces as reported by Bergwerff & Scherpenisse (2003); Kwan et al. (2018) and Pu et al. (2022). From the matrix interfetence study, it was found that samples dilution of 10x is required for chopped fish matrix (i.e. catfish and tilapia). Meanwhile for prawn, no dilution is needed. The current generated from the matrix which were closed to the readings acquired from the buffer systems (i.e. control, 0 ppb) are selected (Figure 3).

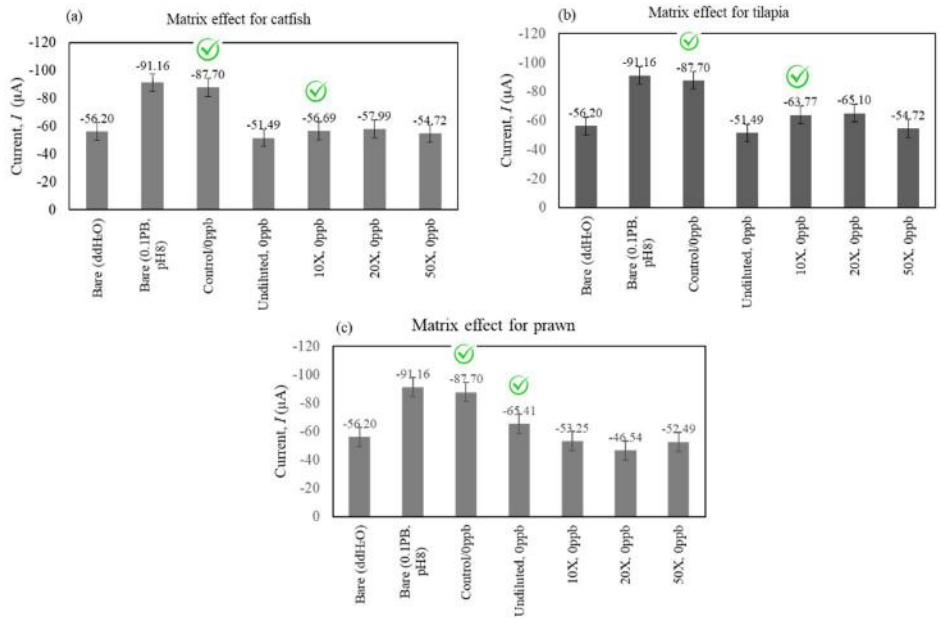

Figure 3. Matrix effect for chopped (a) catfish; (b) tilapia; and (c) prawn towards lectrochemical current. Samples preparation with background current closer to control study are selected.

The enzymatic reaction of immobilized BuChE within the Ppy matrix with butyryl thiocholine iodide (BTC) substrate was studied in the presence of standard total MG solution in various matrix at different concentration from 0 ppb to 10 ppb. A 0.1 M phosphate buffer at pH 8.0 was used as a supporting electrolyte. The calibration curve of total MG inhibitor was plotted as current output (microampere, µA) versus different concentration of standard total MG solution. Standard curves for MG established on the portable device in the three different matrix are presented in Figure 4. Calibration curves of total MG in buffer system showed regression value (R^2) value of 0.9754 in buffer system with current's range difference of 6.46 µA (data not shown). When performed in real sample matrix, the currents were found to be higher as opposed to the currents obtained in buffer system. In catfish and tilapia matrix, R^2 values recorded are 0.9453 and 0.9422 respectively with curent changes range between -79 µA and -53 µA. As for prawn matrix, a wider current range was observed (-80 µA and -42 µA) for MG concentration of 0-10 ppb with R^2 value of 0.9272. The modified enzyme biosensor is sensitive with limit of detection (LOD) as low as 0.25 ppb.

Although homogenized samples exhibited a more stable uniform background current (data not shown), nevertheless when taking into account the feasibility of the analysis to be performed on-site in future, the chopped matrix preparation is preferred. Nevertheless, the standard curves generated from the chopped matrix still give excellent linear standard curves indicating the successful development of the enzyme modified SPCEs. More importantly, its integration with the portable reader is viable and promising.

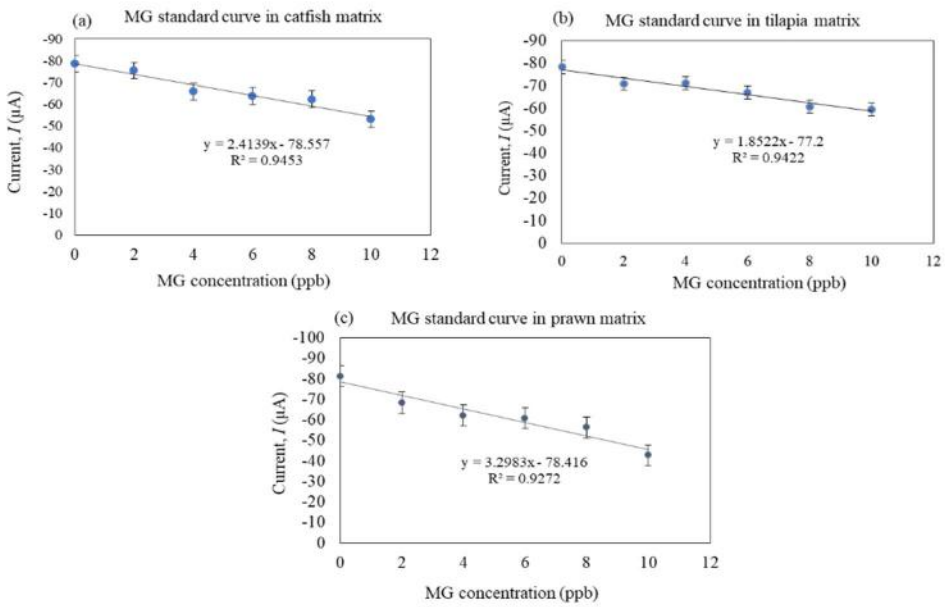

Figure 4. MG standard curves established from the portable electrochemical reader in chopped matrix of (a) catfish; (b) tilapia; and (c) prawn (set potential -480 mV, duration 100-200 seconds).

CONCLUSIONS

A sensitive and reliable modified enzyme-based biosensor for electrochemical detection of MG in fish and prawn matrix has been successfully developed. Furthermore, analysis using portable electrochemical devices offers a great potential as point-of-care testing tool particularly for on-site application. For future work, this enzyme-based biosensor system will be tested on real sample application and results obtained will be validated with conventional methods such as commercial ELISA kits and chromatography technique.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
Borneo Convention Centre, Kuching, Sarawak

CHARACTERIZATION AND LIPASE INHIBITORY ACTIVITY OF RESIN GLYCOSIDES IN THE LEAVES OF DIFFERENT VARIETIES OF SWEET POTATO

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Abstract: Sweet potato (*Ipomoea batatas*) is a staple food crop with numerous varieties and nutritional value of the whole plant. Resin glycosides in sweet potato plants have been shown to have lipase inhibitory activity, but whether there are differences between varieties is unknown. Therefore, 22 varieties of sweet potato leaves from China were selected to extract resin glycosides and determine their lipase inhibitory activity. The highest extraction of resin glycosides was obtained from the sweet potato leaves of variety 1402-12 at 0.8743%. Variety YAN25 showed the highest lipase inhibition activity with an Orlistat equivalent of $1.5985 \pm 0.0678 \times 10^{-4}$. In addition, the structures of the resin glycosides were characterized using LC-QTOF-MS to investigate the effect of the different resin glycosides contents on the lipase inhibitory activity. The results showed that the content of resin glycosides and lipase inhibition activity in different varieties of sweet potato leaves were significantly different and could be used as a basis for the selection of functional food ingredients.
Keywords: *Ipomoea batatas*, Resin glycosides, Pancreatic lipase inhibition, LC-QTOF-MS

INTRODUCTION

Ipomoea batatas, a significant root starch crop, is planted extensively in more than 100 nations. Production is more than 90 million tons annually (Shi et al., 2021). The sweet potato's leaves, which are found above ground, can be picked multiple times annually, though most of them are wasted and the remaining portion is fed to people or animals. The action of several compounds found in sweet potato leaves, such as polyphenols and resin glycosides, has been researched to help prevent resource waste. Recently, it was shown that the resin glycosides in sweet potato leaves have lipase-inhibitory properties (Toy, Song, et al., 2022).

There are now 397 sweet potato varieties registered with the Ministry of Agriculture and Rural Affairs of China's Seed Industry Management Department (Department of Seed Industry Management). These variations vary in a variety of ways in terms of the colour of the flesh, the texture of the root, and the nutrients they contain. Although the resin glycosides in sweet potato leaves have been proven to have lipase inhibitory action, it is still unclear if various types of sweet potatoes differ in the amount of resin glycosides and in their ability to inhibit lipase. Therefore, we explored the structural details of the resin glycosides found in the leaves of 22 different types of sweet potatoes and their lipase inhibitory activity.

MATERIALS AND METHODS

Materials

Ipomoea batatas freeze-dried leaves and stems were grown harvested in the Sweetpotato Research Institute in Xuzhou, China. Vivantis Technologies (Subang Jaya, Selangor, Malaysia) provided the tris-borate-EDTA buffer. Other chemicals from Sigma-Aldrich Co. (St. Louis, MO, USA) included orlistat (PHR1445), p-nitrophenyl palmitate (pNPP) (N2752), 1,3,5-trimethoxybenzene (74599), sodium deoxycholate (D6750), pancreatin from swine pancreas (Cat. No. P754526G), and silica gel.

Extraction and purification of resin glycosides (RG) from sweet potato leaves

The freeze-dried sweet potato leaf samples were shaken continuously in a fixed track shaker for 12 hours while being mixed with dichloromethane. The extracts were dissolved in methanol and agitated in a fixed track shaker for one hour after the dichloromethane layer had been separated and the volatiles eliminated. After being spun at 19,354 g for 10 min, the mixture was dried from the supernatant.

Column chromatography on silica gel was used to separate RG. A gradient of hexane and ethyl acetate at 5:1, 1:1, and 0:1 (v/v) was used to elute 400 mg from a 140g silica gel-packed column. Each gradient had an elution volume of 600 mL. In the end, all remaining samples were eluted with methanol, the methanol phase was kept, the solvent was removed through rotary evaporation, and the samples were then stored at –20 °C.

Using deuteromethane as the solvent and 1,3,5-trimethoxybenzene as the internal standard, the RG content of the extracts was calculated by 1H NMR using an AV500neo spectrometer at 500 MHz.

Determination of pancreatic lipase (PL) inhibitory activity of RG from different varieties of sweet potato leaves

Lipase inhibitory activity was determined by the pNPP method (Toy, Song, et al., 2022). Where buffer was used as a blank control and orlistat was used as a positive control. Determine the sample concentration required to achieve 50% inhibition of enzyme activity (IC_{50}). The final results are presented as orlistat equivalents, units expressed in (ng/μg).

$$\mathrm{OE} = \frac{IC_{50}(Orlistat)}{IC_{50}(Sample)}$$

Identification of RG in sweet potato leaves by LC-QTOF-MS

For preparation as 1 mg/mL solutions with MS-grade methanol and analysis using LC-QTOF-MS, samples of the three species with the highest pancreatic lipase inhibitory activity and the three species with the lowest pancreatic lipase activity were chosen.

On a UPLC system using an ACQUITYT PREMIER BEH C18 1.7 m, VanGuardT FIT Column at 30 °C, chromatographic separations were carried out. Eluent A (water with 0.1% FA) and Eluent B (methanol) were used in the mobile phase at a flow rate of 0.4 mL/min. The following gradient steps were used: 8% A and 92% B from 0 to 7 min, 92%–100% B from 7–8 min, 100% B from 8–15 min, 100%–92% B from 15–15.1 min, and 8% A and 92% B from 15.1–20.1 min.

The Waters Xevo G2-XS QTOF MS was connected to the UPLC system, and the instrument was run in negative ion mode for complete scan monitoring of the m/z 20–4000 range. The following operational settings were used: source temperature of 120 °C, desolvation temperature of 500 °C, and desolvation gas flow rate of 1000 l/h. Capillary voltage was set at 2.5 kV; sample cone voltage was 40 V.

The data were analyzed and processed using Progenesis QI software and EZinfo software.

Statistical analysis

Pancreatic lipase inhibitory activity was performed in triplicate and results are given as mean ± standard deviation. Using SPSS software, a one-way analysis of variance (ANOVA) using Waller-Duncan test ($p < 0.05$) was used to determine the differences in OE values.

RESULTS AND DISCUSSION

Pancreatic lipase inhibitory activity of resin glycosides in sweet potato leaves

The lipase inhibitory activity of RG in sweet potato leaves was expressed in terms of orlistat equivalents to eliminate the effect of some possible factors. The highest activities were found in Yanshu25, Xushu41 and Xushu44. The highest activities were found in Yanshu25, Xushu41 and Xushu44. Higher lipase inhibitory activity than has been reported for different sources of resin glycosides (Toy, Huang, et al., 2022; Toy, Song, et al., 2022). The extraction rates of all varieties were above 0.4% and the highest extraction rates were 1402-12. Xushu44 had both a higher extraction rate and a higher lipase inhibitory activity.

In order to further analyze the effect of RG structure on lipase inhibitory activity, three kinds with high and three kinds with low activity were selected and analysed by LC-QTOF-MS.

Table 1. Extraction rate and lipase inhibition activity of resin glycosides from different varieties of sweet potato leaves

Sample name	Extraction rate	OE (ng/ug)	Sample name	Extraction rate	OE (ng/ug)
Yanshu25	0.62%	1.60±0.07a	Xuzishu8	0.57%	0.96±0.03fg
Xushu41	0.67%	1.48±0.00b	Longshu9	0.64%	0.96±0.02fg
Xushu44	0.74%	1.46±0.01bc	Zishu5	0.80%	0.89±0.05gh
Jishu26	0.63%	1.39±0.05c	Jining18	0.60%	0.87±0.02hi
Xushu27	0.42%	1.27±0.03d	Shulv1	0.53%	0.78±0.02ij
Xushu32	0.55%	1.13±0.01e	Suyu303	0.48%	0.74±0.01jk
QUAN830	0.42%	1.12±0.06e	Jishu25	0.44%	0.70±0.02jk

Xushu18	0.45%	1.11±0.04e	Shangshu19	0.51%	0.68±0.01k
Fushu7-6	0.68%	0.99±0.04f	1402-12	0.87%	0.55±0.01l
Xushu37	0.61%	0.99±0.08f	Sushu8	0.77%	0.45±0.00m
Xushu24	0.49%	0.99±0.04f	Xushu22	0.64%	0.40±0.00m

* Different letters represent significant differences in pancreatic lipase inhibitory activity between varieties by one-way analysis of variance (ANOVA) using Waller-Duncan test ($p<0.05$)

Identification of resin glycosides in sweet potato leaves

Six different RG sample types were chosen for LC-QTOF-MS analysis based on lipase inhibitory activity, including Yanshu25, Xushu41, and Xushu44 with high levels, and 1402-12, Sushu8, and Xushu 22 with low levels, in order to identify the kinds of RGs they contained. Based on the results of LC-QTOF-MS and RG's database, a total of 150 possible substances were identified. As the samples were divided into two groups, the OPLS-DA model was chosen to carry out the analysis of differences between groups. The RGs were filtered using VIP > 1 since a huge amount of data in OPLS-DA is not beneficial for classification and interferes considerably with the analysis process, diminishing the discriminating ability of the model. Variables with VIP > 1 were thought to have the largest impact on the model (Zhou et al., 2022). VIP values are typically used to describe the contribution of variables to the model. The structure of RG with VIP > 1 is likely to have had a substantial impact on its lipase inhibitory action. 24 RGs were screened from the 150 possible RGs that were strongly associated with lipase inhibitory activity.

Structural characterization of screened resin glycosides

To investigate the connection between the RGs' structure and their lipase inhibitory activity, structural analysis was used to the screened RGs. The two structures can be used to summarize twenty of them. The unique distinctions are in the R_1-R_6 groups, which are all four or five glycosyl groups and fatty chains in their primary structures. Oxhydryl and cinnamoyl are the major groups at the R_4 and R_5 groups, respectively, whereas methyl or hydroxymethyl predominates at the R_1 group. Except for Muricatin V and VI, the remaining 18 RGs have a rhamnopyranosyl or glucopyranosyl at the R_3 group.

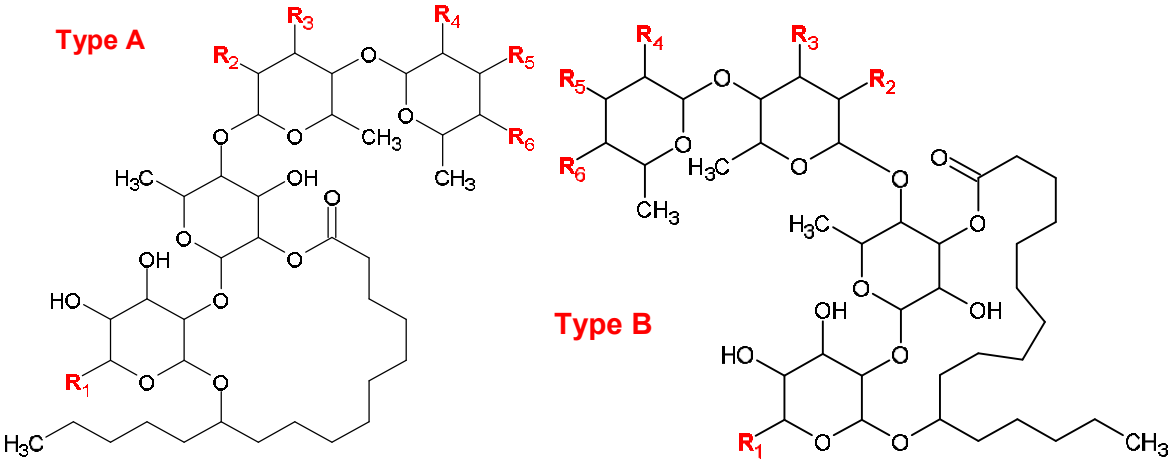

Figure 1. Structures of resin glycosides (RG)

CONCLUSIONS

The highest lipase inhibitory action was found in Yanshu 25 when RG was isolated from the leaves of 22 different kinds of sweet potatoes. 150 potential RG structures were found in the samples of Yanshu 25, Xushu 41, Xushu 44, 1402-12, Sushu 8, and Xushu 22 by LC-QTOF-MS. Leptophyllin A had the biggest impact out of a total of 24 potential structures that were tested for their lipase inhibitory action utilizing OPLS-DA. This research could be useful in helping choose the raw ingredients for the extraction of RG, which has the potential to be evolved into a functional food.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
Borneo Convention Centre, Kuching, Sarawak

EFFECT OF ISOLATED PEA PROTEIN ON HONEYED GINSENG MANUFACTIURED BY 3D PRINTING FOR PATIENTS WITH DYSPHAGIA

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Abstract: Honeyed red ginseng (HRG), dried by boiling down steamed ginseng in honey, is a promising functional food for older adults because of its high saponin content; however, HRG has a gummy texture. Foods for patients with dysphagia need to be modified to a paste-like consistency; however, the foods' sensory properties are consequently reduced. A new method of preparing HRGs for patients with dysphagia was proposed. Isolated pea protein (IPP) was used as a thickener to optimize the texture and a key nutrient supplement for older adults. This study aimed to manufacture dysphagia HRGs via reverse engineering and 3D food printing and investigated the potential of IPP at a ratio of 0%, 3%, 6%, and 9% (w/w) for dysphagia foods. Polysaccharide–protein interaction was observed using scanning electron microscopy and Fourier transform infrared spectroscopy. IPP and 3D printing demonstrated a promising feasibility of use. IPP improved the storage modulus and shear thinning, and printing parameters were optimized using response surface methodology. The addition of 6% IPP could be classified as a level 5 dysphagia diet and had the highest sensory similarity to commercial HRGs. This study presented a nutrition supply system and future food manufacturing technology for dysphagia using 3D printing technology.
Keywords: Pea protein, red ginseng, dysphagia, IDDSI, 3D printing, reverse engineering

INTRODUCTION

Dysphagia, causing swallowing difficulties, leads to malnutrition, weight loss, and dehydration due to reduced food intake (Merino et al., 2020). Dysphagia foods alter texture, often via pureeing and mincing, yielding visually distinct and potentially unappetizing products (Schwartz et al., 2018). IDDSI standardizes texture-modified foods (TMFs) at various levels, aiding assessment (IDDSI, 2019). 3D food printing (3DFP) creates personalized foods with edible inks, offering potential dysphagia patient benefits. It enables nutrient augmentation through tailored food inks and enhances meal aesthetics. Yet, printing dysphagia foods mandates rheological food ink property consideration and varied printing parameter setting (e.g., printing speed, flow rate). Recent studies explored dysphagia foods via 3DFP (Dick et al., 2021). More research is needed to enhance printable dysphagia foods with protein hydrocolloids for optimal older adult nutrient supplementation. Protein is vital for older adults, recommended at 1.2–1.5 g protein/kg body weight/day (García et al., 2019). Pea protein, rich in essential amino acids, boosts dysphagia food protein content. Pea protein is preferable over soybean protein for 3DFP thickening due to its high protein content and ink property control (Masiá et al., 2023). Ginseng (Panax ginseng Meyer) holds bioactive saponin and polysaccharides, displaying immunity enhancement, energy revitalization, fatigue alleviation (Lee et al., 2015). Honey red ginseng (HRG) forms by steaming, slicing, boiling with sugar, honey, and drying, yielding a hard, tough, occasionally sticky texture (Lee et al., 2017). The study aimed to develop printable texture-modified foods using HRG, pea protein, and ginseng polysaccharides. Rheological properties were optimized through response surface methodology, and the resulting 3D-printed HRGs were evaluated using the IDDSI framework.

MATERIALS AND METHODS

Materials Six-year-old fibrous root ginseng obtained from Keumsan market (Dec. 2022) was used. IPP (protein 80.00%, fat 8.30%, carbohydrate 3.00%) was from Almi GmbH (Austria). Powder-form IPP was made through alkaline isoelectric precipitation and spray drying. Commercial HRG slices (Korea Ginseng Corporation, Daejeon, Korea) were the control.

Food-ink Preparation Ginseng was steamed (7 hrs), chopped (5 min, 1,750 rpm). HRG paste mixed with sepia and beet powder for 5 mins. HRG paste combined with different IPP-to-HRG ratios (0%, 3%, 6%, 9% w/w). HRG ink stored at 4°C. Samples: IPP0, IPP3, IPP6, IPP9.

Rheology analysis Rotational rheometer (HR-10, TA Instruments, New Castle, DE, USA) with 20-mm-diameter plate-plate system and 1 mm gap.

Optimization of printing parameter by response surface methodology (RSM) Minitab Software (Version 16, LLC, PA, USA) used for RSM design. Cube points (1-4), axial points (5-8), center points (9-13). Data analyzed with regression equation: $Y = \beta0 + \beta1A + \beta2B + \beta11A^2 + \beta22B^2 + \beta12AB$. Y=response (printed linewidth), A=flow rate, B=printing speed. Model fit: R^2, β coefficients.

3D printing process 3DFP was performed by extrusion-based 3D printer (Makerbox 2.0, Makerbox, Seoul, Korea). The 3D models used were designed using Fusion 360 (Autodesk, San Rafael, CA, USA). G-codes of all 3D models for 3DFP were generated by slicing using Cura (Ultimaker B.V., Geldermalsen, The Netherlands).

Visual appearance, scanning electron microscopy (SEM), and Fourier transform infrared (FT–IR) analysis Visual appearance captured with smartphone camera (iPhone 13; Apple, Inc., CA, USA). SEM (SU8220, Hitachi, Tokyo, Japan) at 50×. FT-IR (PerkinElmer) analyzed intermolecular interactions (4000–400 cm^{-1}).

International dysphagia diet standardization initiative (IDDSI) tests 3D Printed HRG, and commercial HRG slices were expected to be categorized as Level 5 (minced and moist) described in IDDSI framework (IDDSI, 2019) according to results of preliminary tests. Samples were measured by spoon tilt, and fork drip/pressure test.

Similarity sensory analysis Printed HRGs and IPP0 not printing (NP) were prepared for comparison on the effect of 3DFP. Participants rated samples (1-7 scale) for smell, color, appearance, overall similarity (Exemption No. 2023-0022 from KNU Institutional Review Board).

Statistical analysis The statistical analyzes were processed using SPSS software package (Version 26, SPSS, Chicago, IL, USA) by one-way analysis of variance (ANOVA) with Duncan's multiple range test.

RESULTS AND DISCUSSION

Rheology analysis, and Printing parameter optimization using RSM

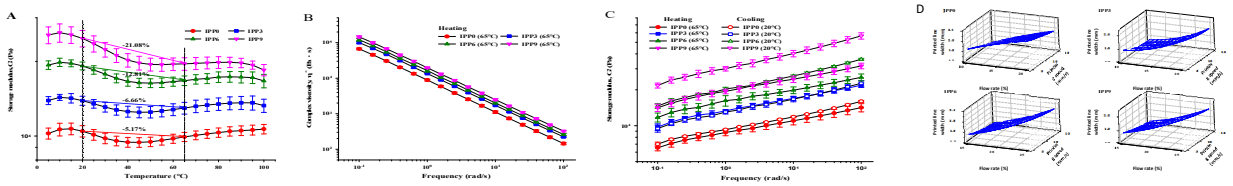

Figure 1. Dynamic rheological properties of temperature sweep test (A), complex viscosity at 65°C (B), and storage modulus at 65°C and 20°C (C), and 3D response surface plot for printed line width (mm) as a function of flow rate (%) and printing speed (mm/s) (D) of honeyed red ginseng inks.

The temperature-dependent viscoelastic properties and printability of HRG inks for dysphagia foods were assessed via rheology (Fig. 1). Adjustments in food ink properties were needed for extrusion from a 3D printer's nozzle. G' changes with temperature showed higher values with added IPP, indicating a similar trend (Fig. 1A). All HRG inks exhibited shear-thinning behavior at 65°C (Fig. 1B), suitable for printing. In Fig. 1C, G' for HRG inks exceeded G'' at 65°C, showing solid-like behavior, both with frequency-dependent trends. IPP boosted mechanical strength, indicating stronger polysaccharide-protein interactions. Thus, HRG ink formulations can print a rigid self-supporting structure. Cooling to 20°C increased G', with rapid growth above 6% IPP based on steamed ginseng. RSM is commonly used to optimize food processes (Ba & Boyaci, 2007). Comparing mechanical properties like TPA and IDDSI becomes challenging when different rheological properties are used with the same printing parameters. To print HRGs of similar size and shape to CONT for dysphagia, we optimized flow rate (%) and printing speed (mm/s) using RSM to achieve consistent printed linewidth (mm) for HRGs similar to CONT.

Visual appearance, SEM, and FT-IR

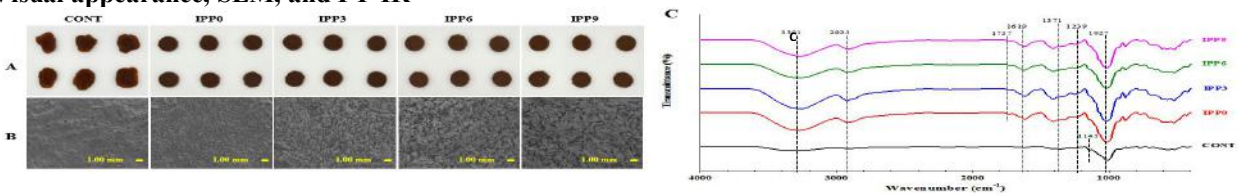

Figure 2. Visual appearance (A) scanning electron microscopy (SEM, magnification 50×) (B) , and FT–IR spectroscopy (C) of honeyed red ginseng.

Optimal HRG printing (Fig. 2A), CONT compact with gel layer, firm microtexture. Dysphagia HRGs showed uniform reticular, porous microstructures, slight variations. Pea protein, mostly globulin, forms insoluble oligomer structure. Higher IPP% led to less porosity, forming large dense protein aggregates resembling globulin structures.

Similar findings elsewhere (Moreno et al., 2020), denser structure from protein-polysaccharide aggregation, IPP's influence (Fig. 2B). HRGs' FT–IR closely matched ginseng's reported peaks (Jiang et al., 2020). Peak ~3301 cm^{-1} signifies O–H stretch vibration, showing phenolic structures. This band links to hydrogen-bonded hydroxyl groups, including water (Jivan, Yarmand, & Madadlou, 2014). Peak ~1027 cm^{-1} suggests C-C-O or C-C-OH bending vibrations in ginsenosides, starch, indicating starch crystallinity (Lu et al., 2008).

IDDSI tests

Table 6. Categorization of honeyed red ginseng using IDDSI evaluation methods.

Samples	IDDSI test			
	Spoon tilt test	Fork drip test	Fork pressure test	Level
CONT				Solid foods (not classified)
IPP0				Not classified
IPP3				Not classified
IPP6				Level 5 (minced and moist)
IPP9				Level 5 (minced and moist)

IDDSI (2019); (n = 5).

HRGs for dysphagia in this study resemble soft, smooth pastes, with a slight lump visible. They do not separate liquids from solids, meeting the IDDSI level 5 requirement (Yu et al., 2023). CONT used here lacks adhesiveness, and its hardness and gumminess. During IDDSI testing, CONT didn't adhere to the tool, making biting and swallowing challenging. Spoon tilt tests assessed HRGs' viscosity and cohesiveness. All dysphagia HRGs maintain shape on the spoon. IPP6 and IPP9 tend to slip on tilting or light shaking, meeting level 5. In contrast, IPP0 and IPP3 left some food on the spoon due to high cohesiveness. In fork drip tests, all HRGs stacked on the fork without flowing through tines/prongs. In fork press tests, dysphagia HRGs smoothly passed through fork tines/prongs, leaving clear marks on the surface. Samples easily mashed with little pressure, retaining shape after fork removal. Thus, all dysphagia HRGs fit IDDSI level 5 for fork drip and pressure tests. IPP6 and IPP9 aced all three, aligning with IDDSI level 5 – highly suitable for dysphagia diets.

Similarity sensory analysis

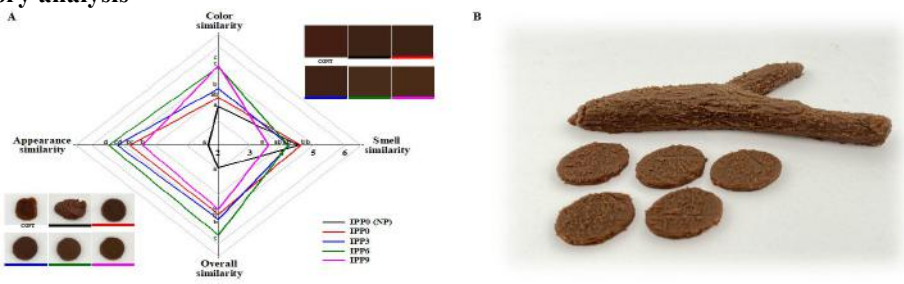

Figure 3. Sensory similarity evaluation, and Photograph of honeyed red ginseng with 6% IPP (whole and slice products) manufactured by 3D printing for patients with dysphagia. NP, not printing. Rating scale: 1 (samples are completely different) ↔ 7 (samples are the same); (n=30).

TMF often does not stimulate appetite and may lack sensory appeal. This study aimed to determine the shape and sensory properties of TMFs compared to commercial HRGs, as shown in Fig. 6. A hand-scooped IPP0 (NP) of red ginseng paste was prepared to assess the sensory effects of 3D printing. Notably, significant differences ($p<0.05$) in appearance similarity were observed between printed and non-printed TMFs. Non-printed TMFs, such as IPP0 (NP), received low scores for smell, color, and overall similarity, suggesting their unappealing nature for dysphagia patients. Among printed HRGs, IPP6 scored the highest appearance similarity at 5.47. Increasing IPP percentage resulted in higher color similarity, enhancing brightness and yellowness while reducing redness, achieving a light reddish-brown color. Similar color properties can reduce consumer rejection and promote appetite and acceptance (Dantec et al., 2022). Smell similarity gradually decreased with increasing IPP percentage, with IPP9 scoring the lowest. Although red ginseng typically has a bunt, molasses, and woody aroma, the added IPP masked this aroma with a bean-like smell, deviating from the typical profile (Chung et al., 2011). Overall similarity scores were highest for HRGs with IPP6,

followed by IPP3, IPP0, and IPP9. Applying 3D printing with appropriate IPP content to HRGs can enhance acceptance depending on the health status and preferences of dysphagia patients.

CONCLUSIONS

Commercial HRGs are unsuitable for older adults with dysphagia due to their hard and gummy texture, but they hold promise as functional food. This study aimed to develop visually appealing dysphagia food with enhanced nutrition using IPP as a thickener and 3DFP. Optimal conditions with 6% IPP resulted in the most suitable HRG for dysphagia diet. And provided the possibility of manufacturing similar to the actual form of red ginseng through reverse engineering (Fig. 3B). FT–IR and SEM analysis revealed increased interaction between IPP and ginseng polysaccharide, impacting HRG's printability and texture. According to the IDDSI framework, the developed TMFs were classified as level 5 and showed sensory similarity to commercial HRGs. Overcoming cognitive and technical barriers in 3DFP is crucial for widespread consumer adoption.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
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QUALITY CHARATERISTICS OF HYBRID CHICKEN NUGGETS: EFFECT OF ISOLATED RICE, PEA, SOY PROTEINS

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Abstract: The development of hybrid-meat is required as an alternative to reducing the amount of animal meat, and the current demand for the product encourages the replacement of plant-based ingredients. This study compared the quality and processing characteristics of hybrid chicken nuggets using isolated rice, pea, and soy proteins. Isolated rice, peas, and soy protein were replaced by 20% of the weight of the chicken breast to prepare the chicken protein emulsion gel, which is the batter of hybrid nuggets. Transglutaminase, NaCl, and dibasic sodium phosphate were added to improve cross-linking, and the batter was heated at 180°C for 15 minutes (central temperature of 72°C). Gel strength, and rheological properties of nugget batter before cooking, mechanical properties were high in the order of soy > peas > rice protein among plant-based proteins. As for the post-cooking characteristics, soy protein had the lowest cooking loss and water-holding capacity, while the diameter shrinkage was the largest. In addition, hybrid nuggets with high cohesiveness, springiness, and share force of plant protein-added samples were manufactured. Therefore, the possibility of manufacturing hybrid-meat according to the application of various plant-based proteins can be identified, and it can be used as data for sustainable food research.
Keywords: Hybrid meat, Chicken Nuggets, Plant-based protein, Cross-linking

INTRODUCTION

With global population growth and evolving lifestyles, the inherent connection between sustainable food system challenges and a healthy planet is being acknowledged (FAO et al., 2020). The production of meat substitutes using plant proteins poses various challenges due to significant differences between the characteristics of plant and animal proteins (Broucke et al., 2022). One solution is to partially replace animal proteins in the conventional meat diet with plant proteins, thereby reducing resistance to plant proteins. Hybrid meats are food products composed of plant-based ingredients designed to replicate the functionality, nutritional composition, and sensory attributes of traditional meat items (Ismail et al., 2020). These products are better received by meat consumers than meat analogues, as they offer a traditional meat flavor while curbing meat consumption (Neville et al., 2017). Soybean, pea, and rice proteins are commonly utilized as raw materials for producing meat substitutes (Sá, Moreno et al., 2020). Plant-based resources contribute to an environmentally sustainable protein supply chain and offer added health benefits, including lower saturated fat content compared to animal-based proteins and fiber content (Lemken et al., 2019). These proteins undergo denaturation and aggregation upon heating, forming a protein network that primarily retains water through capillary forces. This protein gelation significantly influences the final structure of meat products (Tornberg, 2005). Researchers have extensively investigated the technical aspects of hybrid meat products (Scholliers et al., 2020, Aviles et al., 2023). However, the application of diverse plant-based proteins to chicken has been relatively limited. Considering these factors, our research objective was to analyze the physicochemical properties of meat emulsion gel by incorporating various isolated plant proteins into hybrid chicken products. Additionally, we aimed to assess the impact of different types of plant proteins on the quality characteristics of plant-based meat replacement in hybrid chicken nuggets.

MATERIALS AND METHODS

Materials Chicken breast from local market (Harim Co., Ltd., Iksan, Republic of Korea) was used. Isolated rice (IRP), Isolated pea protein (IPP), and Isolated soy protein (ISP) were from Almi GmbH (Austria). Microbial transglutaminase (TG) with an enzymatic activity of 100U/g were purchased form Ajinomoto Co. Inc. (Tokyo, Japan).

Preparation of hybrid chicken First, remove connective tissue and fat to preprocess the chicken. Isolated rice, peas, and soy protein were replaced by 20% of the weight of the chicken breast to prepare the chicken protein emulsion gel, which is the batter of hybrid nuggets. Transglutaminase, NaCl, and dibasic sodium phosphate were added to improve cross-linking, and the batter was heated at 180°C for 15 minutes (central temperature of 72°C).

Gel strength, emulsion stability, and rheology analysis Gel strength were performed using a rheometer (Compac-II, Sunscientific, Tokyo, Japan) with a cylinder probe (20 mm diameter). Emulsion stability was measured amount of solvent released after centrifuge (10,000 g/4 ℃/30 min). The rheological properties of hybrid chicken before cooking were measured using a rotational rheometer (HR-10, TA Instruments, New Castle, DE, USA) with 20-mm-diameter plate-plate system and 1 mm gap. Viscosity was determined within shear rate range from 0.1 to 100 s^{-1}. The frequency sweep test assessed within angular frequency range from 0.1 to 100 rad/s.

Visual appearance, Water holding capacity, cooking lose, and shirinkage rate Visual appearance captured with smartphone camera (iPhone 13; Apple, Inc., CA, USA). Water holding capacity was analyzed based on the filter-paper press method (Joo, 2018). Cooking loss was determined by measuring the weight of each sample before and after cooking, and was calculated as percentage of lost moisture from initial sample weight. Shrinkage rate of each sample by cooking was measured with a digital caliper. The length, width, and hight shrinkage of hybrid chicken after cooking were calculated as the percentage reduction in the cooked sample length (diameter).

Texture profile analysis (TPA) and Warner-Bratzler Shear force TPA and Warner-Bratzler shear force of hybrid chicken after cooking were performed using a rheometer with a cylinder probe (50 mm diameter) and Warner-Bratzler shear force probe according to the modified method by Wu et al. (2022).

Statistical analysis The statistical analyzes were processed using SPSS software package (Version 26, SPSS, Chicago, IL, USA) by one-way analysis of variance (ANOVA) with Duncan's multiple range test.

RESULTS AND DISCUSSION

Gel strength, and emulsion stability, and rheology analysis of the samples before cooking

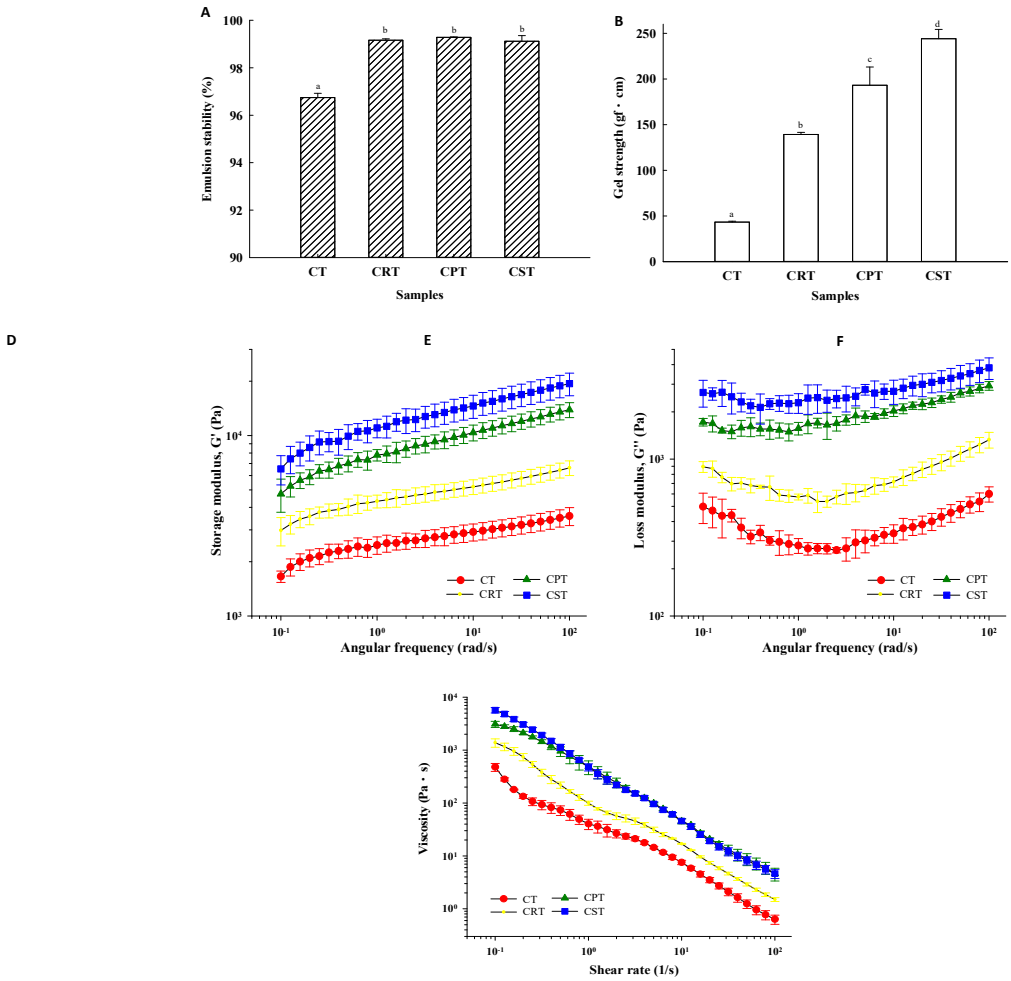

Figure 1. Visual appearance, gel strength (A), and emulsion stability (B) Storage modulus (C), lose modulus (D), and viscosity versus shear rate (E) of hybrid chicken meat with different isolated plant proteins.

As a result of evaluating the characteristics of the samples before cooking, the emulsion stability and gel strength were significantly higher in samples containing plant protein compared to CT, and the most stable and elastic gel was produced in CST (Fig. 1B and Fig. 1C). As shown in Fig. 1D and 1E, G' > G" properties were found in all samples, indicating that they mainly had elastic properties (Tabilo-Munizaga & Barbosa-Cannovas, 2005). Furthermore, since all G' and G" results showed frequency-dependent correlation in the order of CT<CRT<CPT<CST in the range studied (0.1–100 Hz), the structures formed in the model system can be characterized by strong gels (Rao, 2007). All emulsion gel showed shear-thinning properties in which viscosity decreases depending on shear rate, and these results are considered suitable for processing (Fig. 1F). The viscosity of each sample affected the final viscosity depending on the plant protein, with higher viscosity requiring stronger extrusion pressure during processing (Rao, 2007). The rheological properties of meat emersion gel reflected in the final textural properties.

Water holding capacity, cooking lose, and shirinkage ratio of the samples after cooking

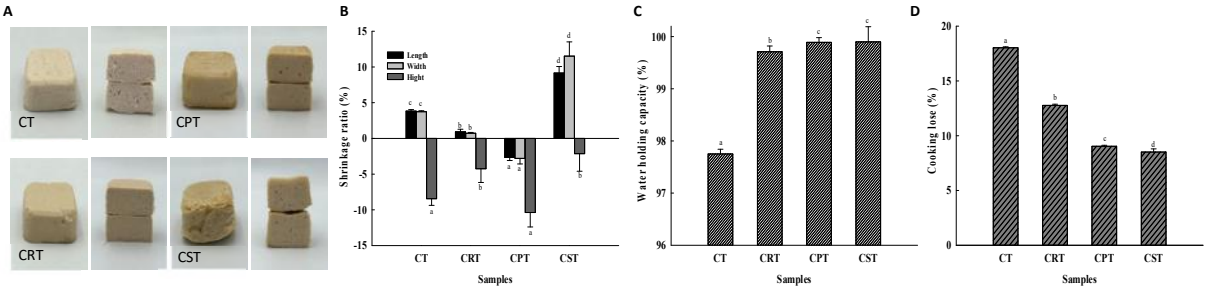

Figure 2. Visual appearance (A), shirinkage ratio (B) Water holding capacity (C), and cooking lose (D) of hybrid chicken meat with different isolated plant proteins.

The visual appearance of the samples was compared to CT, CRT was able to identify relatively dense structures, while CPT and CST were unbalanced and slightly inflated (Fig. 2A). The same could be confirmed in the shrinkage data from cooking. Additionally, it was confirmed that the overall height had expanded (Fig. 2B). According to Fig. 2C, water holding capacity showed overall stronger binding in samples using plant protein compared to CT. As shown in Fig. 2D, CPT and CST showed significantly lower cooking losses. From a technical point of view, low cooking losses can lead to economically advantageous high yields. Hence, when an external force was applied, these model systems easily released the held water, suggesting that a substantial amount of the remaining water was rather weakly bound during heating (Scholliers et al., 2020).

TPA and Warner-Bratzler Shear force of the samples after cooking

Table 1. Texture profile analysis and Warner-Bratzler Shear force of hybrid chicken meat with different isolated plant proteins.

	Hardness (N)	Cohesiveness (%)	Springness (%)	Chewiness (N)	Warner-Bratzler Shear force (N)
CT	132.25±3.87^{a}	80.57±0.64^{a}	86.40±6.20^{a}	1819.23±115.35^{a}	1327.82±113.10^{a}
CRT	357.12±22.18^{b}	85.87±0.78^{b}	91.50±0.17^{ab}	5750.18±500.35^{c}	2573.26±136.93^{b}
CPT	221.53±29.62^{a}	89.33±0.42^{c}	93.87±0.06^{b}	3574.58±590.52^{b}	2559.54±198.40^{b}
CST	354.90±113.79^{b}	90.70±1.65^{c}	96.17±0.38^{c}	2507.66±861.87^{ab}	3297.00±391.85^{c}

Data are expressed as mean±SD (n=3). Different letters within a column present significant differences ($p<0.05$).

The textural properties, including hardness, cohesiveness, springenss, chewiness, and shear force, of hybrid chickens after cooking are shown in Table 1. Meat alternatives are required to not only have a meat-like taste, but also a fibrous, muscular meat-like structure that closely resembles real meat, with similar moisture level, bite resistance and mouthfeel (Akdogan, 1999). TPA and shear force of samples after cooking showed significant differences depending on the kind plant proteins, hardness, chewiness, and shear force were higher in the order of CT<CPT<CRT<CST.

Cohesiveness and elasticity were correlated with the viscoelasticity of meat emulsion gel, which is thought to be because intermolecular binding after cooking varies from plant protein to plant protein and soybean protein has the highest intermolecular interaction power compared to other proteins.

CONCLUSION

In this study, we analysed the structural and quality characteristics of hybrid chickens prepared with different plant proteins. Plant proteins have been shown to improve physicochemical cross-linking, which can have a positive impact on the final product. Therefore, this study suggests that plant protein can be used as an effective non-meat ingredient in emulsified meat products by replacing meat within 20% level.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
Borneo Convention Centre, Kuching, Sarawak

DEVELOPMENT OF PLANT-BASED SCALLOP ADDUCTOR MUSCLE USING 3D FOOD PRINTING: EFFECT OF AMYLOSE CONTENT AND PASTING PROPERTIES OF RICE FLOUR

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Abstract: Seafood diets need to be replaced with plant-based diets for health and sustainability. Appropriate plant-based materials and manufacturing technology should be applied to mimic texture and internal structure of seafood. This study investigated printability and texture properties of plant-based scallop adductor muscle (SAM) by 3D printing and rice flour of various amylose contents and pasting properties. Plant-based SAMs were prepared as food-ink by mixing rice flour from four cultivars (Miho, Saeilmi, Saemimyeon, and Dodamssal) with composite sols (κ-carrageenan/konjac glucomannan). The pasting properties of rice flour were measured by rapid visco analyser (RVA). 3D printing was performed by rheological properties of food-inks, and texture properties after cooking were compared with actual SAMs. The high viscosity of rice flour in RVA affected rheological properties of food-ink and required higher extrusion pressure in printing. Each food-ink was improved printing precision by optimizing printing parameters with response surface methodology and was printed as striated-muscle structure. After cooking, plant-based SAMs showed significant increase in hardness and tendency to increase springiness and cohesiveness by amylose content of rice flour. This study provides a method for analog processing of SAM-like morphology and improved texture using 3D printing and physicochemical properties of rice flour.
Keywords: Scallop adductor muscle, 3D food printing, rice flour, amylose content, texture properties

INTRODUCTION

Scallops are commercially important marine fishery resource whose consumption is increasing owing to its distinctive flavor and delicious taste, as well as its health-beneficial substances (Wu et al, 2022). The main edible part, the scallop adductor muscle (SAM), is muscle food, and muscle texture is one of the most important quality indicators in seafood products (Sun et al, 2022).The rising seafood consumption prompts marine farming and its production, resulting in environmental destruction, and has negative effects on human health such as allergies and heavy metals by intakes. These concerns encourage the development of seafood analogs that mimic the texture charateristics (Ran et al, 2022). Therefore, appropriate plant-based materials and maufacturing technology should be applied to mimic texture and internal structure of SAM. Starch of rice (*Oryza sativa* L.) is composed of polysaccharides amylose and amylopectin. Understanding the structure and functional properties of starch is industrially useflu, and is very importnat for selecting suitable rice cultivars (Park et al. 2020). The functional properties of rice starch, such as amylose content, gelatinization temperature, and pasting viscosity, are important for processability of rice. 3D food printing is a technology that can imitate internal structure of food by free design and additive manufacutring. Food-ink used in 3D printing affects printability depending on characteristics of materials (Ma and Zhang, 2022). This study investigated printability and textural properties of SAM by 3D printing and rice flour of various amylose contnets and pasting properties.

MATERIALS AND METHODS

Materials

The four rice varieties used were Miho, Saeilmi, Saemimyeon, and Dodamssal, which were provided by the Rural Development Administration (Miryang, Korea). Konjac glucomannan (KGM, Haena, Seongnam, Korea), κ-carrageenan (KC), $CaCO_3$, and yeast extract (ESfood, Gunpo, Korea) were purchased. Frozen scallop adductor muscles (SAMs) produced in China were prepared to compare texture properties with plant-based SAMs and stored frozen.

Physicochemical properties of rice flour

Amylose content was determined colorimetrically modifed from the method of Govindaraju et al. (2022). A rapid viscosity analyzer (RVA 4500, Perten Instruments, Haqersten, Sweden) was used for determining the pasting properties from the method of Seong et al. (2023).

Food-ink preparation

Plant-based SAMs for 3D printing, were prepared as follows. KC-KGM dispersion was prepared by stirring equal amount of 6% KC solution and 4% KGM solution and $CaCO_3$ (1% w/w of total weight), and then heated at 80℃ for 30 min. Each rice flour and hot KC-KGM dispersion were prepared in a ratio of 1:7 (w/w), yeast extract (0.8% w/w of total weight) was added, and constant mixing for 1 min. The mixtures were cooled down to room temperature after filling 50 mL syringes, and kept at 4℃ overnight to prepare food-inks (labeled as MH, SIM, SMM, and DDS).

Rheology properties

Rheological properties of food-inks were determined using a rotational rheometer (HR-10, TA instruments, New Castle, DE, USA) using a 20 mm plate–plate system with a gap of 1000 μm. Viscosity was determined within shear rate range from 0.01 s^{-1} to 100 s^{-1} at test temperature of 85℃ after heating food-ink in syringe at 85℃ for 30 min. The frequency sweep test assessed within angular frequency range from 0.1 rad/s to 100 rad/s at test temperature of 25℃ after heating the food-ink in syringe at 85℃ for 30 min.

3D printing process

An extrusion-based 3D printer (Makerbox 2.0, Makerbox, Seoul, Korea) with a modified feeder system was applied for printing test. Among various printing parameters, nozzle diameter (1.9 mm), layer height (1.5 mm), and printing temperature (85℃) were fixed. Flow rate and printing speed of food-inks were each set to obtain the same printed line width of 2 mm (±1%). 3D model was developed with an octagonal-shape (width/height, 30 mm; length, 20mm) and striated muscle as internal structure. The printed SAMs before cooking were observed by visual evaluation. The printed and cooled down SAMs were frozen, and quality evaluation after cooking was performed by completely thawing frozen SAMs at 4℃ for 12 h.

Quality characteristics after cooking

Cooking method was performed at 75℃ for 20 min using water bath cooking. SAMs were placed in polyethylene bags and cooked to an internal temperature of 70℃. After heating, samples were cooled in water bath at 20±3℃ for 10 min to stop the temperature increase. The SAMs after cooking were observed by visual evaluation.

Textural properties

Texture profile analysis (TPA) and shear force were performed using a rheometer (Compac-II, Sunscientific, Tokyo, Japan) according to the modified method by Wu et al. (2022).

Statistical analysis

All data are presented as mean±standard deviation for at least three repeated-measures. The statistical analyzes were processed one-way analysis of variance (ANOVA) and/or Duncan's multiple range test using the Minitab statistical software and SPSS software package (Version 26, SPSS, Chicago, IL, USA). Differences of $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Physicochemical properties of rice flours

Table 1. Amylose content and pasting properties of rice flours.

Rice flours	Amylose content (%)	Pasting properties					
		Peak viscosity (cP)	Trough viscosity (cP)	Breakdown (cP)	Final viscosity (cP)	Setback (cP)	Pasting temperature (℃)
Miho	11.70 ±0.28^{a}	247.25 ±2.89^{b}	104.89 ±1.78^{b}	147.75 ±4.67^{b}	168.39 ±2.65^{b}	-78.86 ±5.53^{a}	70.97 ±0.47^{a}
Saeilmi	19.50 ±0.21^{b}	305.03 ±8.31^{c}	145.39 ±5.72^{c}	159.64 ±2.60^{c}	245.44 ±5.85^{c}	-59.58 ±3.38^{b}	75.07 ±0.46^{b}
Saemimyeon	32.01 ±0.28^{c}	324.06 ±0.84^{d}	180.19 ±1.42^{d}	143.86 ±0.62^{b}	355.47 ±4.67^{d}	31.42 ±3.85^{c}	81.80 ±0.52^{c}
Dodamssal	44.55 ±0.69^{d}	88.50 ±0.60^{a}	72.61 ±0.80^{a}	15.89 ±0.34^{a}	119.45 ±0.54^{a}	30.95 ±1.13^{c}	87.37 ±0.49^{d}

Data are expressed as mean±SD (n=3). Different letters within a column present significant differences ($p<0.05$).

The amylose content and pasting properties of rice flours are presented in Table 1. Amylose content is a key indicator of cooking quality of rice grains (Govindaraju et al, 2022). Amylose content of Miho, Saeilmi, Saemimyeon, and Dodamssal was 11.70%, 19.50%, 32.01%, and 44.55%, respectively. Miho was classified as low amylose rice, Seailmi as medium amylose rice, Saemimyeon and Dodamssal as high amylose rice. Peak viscosity, trough viscosity, breakdown, final viscosity, setback tended to increase with amylose content in Miho, Saeilmi, and Saemimyeon, while Dodamssal showed the lowest values ($p<0.05$). Pasting temperature increased with amylose content. 3D network that occurs by re-association of amylose upon cooling can increase the setback value in rice flour (Jamal et al, 2016). The printability and quality characteristics after cooking of plant-based SAMs are expected to affect differences in amylose content and pasting properties depending on rice varieties.

Rheology analysis

The printability of food-inks for SAM manufacturing was characterized by rheological properites and are shown in Figure 1. All food-inks shoewd shear-thinning properties in which viscosity decreases depending on shear rate, and these results are considered suitable for printing. The viscosity of each food-ink affected the final viscosity of rice flour, with higher viscosity requiring stronger extrusion pressure during printing. Food-inks with enough mechanical strength could withstand the printed shape over time and possess good resolution (Liu et al, 2018). The G′ of all food-inks was higher than the G″, indicating a solid-like gel behavior. When loss tangent is less than 1, this indicates that sample exhibits gel-like properties with a dominant elastic behavior (Yang et al, 2018). SMM had the strongest elasticity, and Miho had the lowest elasticity. The rheological properties of food-ink reflected the pasting properties of rice flour used.

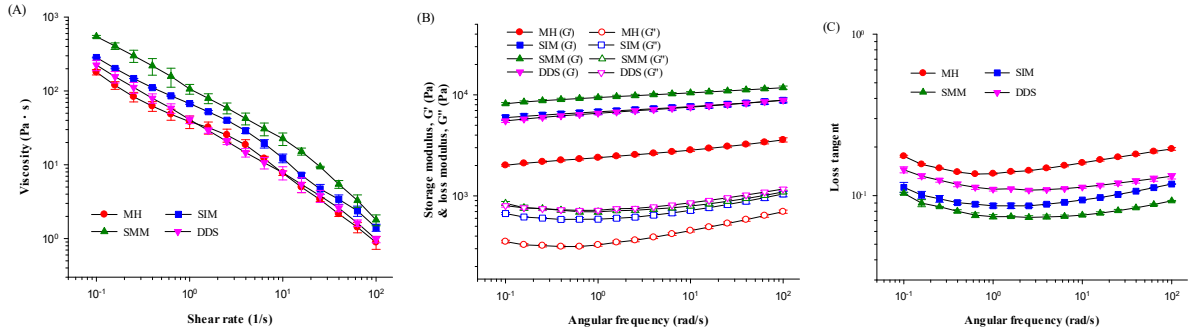
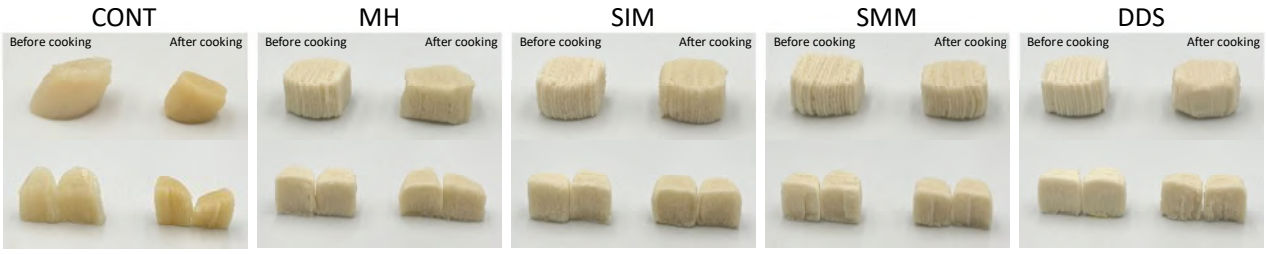


Figure 1. Rheology properties of food-ink with different rice flours. The viscosity (A), storage modulus and loss modulus (B), and loss tangent (C).

Visual appearance

The structures of commercial SAM (CONT) and plant-based SAM before and after cooking are illustrated in Figure 2. The internal strucutre of SAM exhibited a striated-muscle pattern (Sun et al, 2022). Plant-based SAMs were prepared in the same size under each optimal printing parameters, and printed by imitating the internal strucutre of CONT. The interanl sturcture of printed SAMs were maintained even after cooking, because it was printed at a high temperature of 85℃, which is above the gelatinization temperature of rice flours.

Figure 2. Visual appearance of scallop adductor muscle before and after cooking.



Textural properties

The textural properties, including hardness, gumminess, springiness, cohesiveness, brittleness, and shear force, of SAMs after cooking are shown in Table 2. The muscles of small aquatic animals, such as shellfish, generally have lower amounts of collagen compared to larger terrestrial animals giving them a softer texture (Wu et al, 2022). The hardness, gumminess, and brittleness of plant-based SAMs increased with amylose content of rice flour, and CONT,

MH showed low values ($p<0.05$). Springiness was higher in CONT, SMM, DDS, and cohesiveness was significantly higher in order of CONT and DDS. The shear force was highest in CONT, which is considered to be bacasue the molecular bonds of CONT were stronger than those of plant-based SAMs after cooking. The amylose content of rice starch contributed to improving the physical properties of the product.

Table 2. Textural properties of scallop adductor muscle.

Samples	Hardness (gf)	Gumminess (gf)	Springiness	Cohesiveness	Brittleness (gf)	Shear force (gf)
CONT	293.00±16.09^{a}	268.49±15.33^{a}	93.77±0.81^{bc}	91.63±0.25^{d}	251.86±16.46^{b}	3910.00±70.00^{e}
MH	314.00±1.00^{a}	255.62±2.29^{a}	87.07±0.64^{a}	81.40±0.96^{a}	222.53±1.95^{a}	416.67±5.77^{a}
SIM	508.00±15.72^{b}	407.41±14.97^{b}	86.67±1.35^{a}	80.20±1.15^{a}	353.22±18.41^{c}	533.33±11.55^{b}
SMM	728.33±11.68^{c}	637.34±3.81^{c}	93.33±0.06^{b}	87.50±1.11^{b}	595.04±3.85^{d}	953.33±30.55^{d}
DDS	1068.67±10.79^{d}	960.74±6.55^{d}	95.10±0.78^{c}	89.90±0.89^{c}	913.55±11.60^{e}	730.00±26.46^{c}

Data are expressed as mean±SD (n=3). Different letters within a column present significant differences ($p<0.05$).

CONCLUSIONS

As the consumption of SAMs increases, environmental and health concerns are increasing the need for sustainable alternatives. In this study, plant-based SAMs were developed using rice flour of different varieties and 3D food printing technology, and texture after cooking was compared with commercial SAM. Different viscosity of rice flours affected rheological properties of food-inks and caused differences in extrusion amount during printing. Therefore, optimal printing parameters were necessary to obtain SAMs of the same size. Plant-based SAM was able to produce an internal structure similar to CONT through 3D printing and maintain its structure even after cooking. The amylose content and pasting properties of rice flour influenced texture properties of plant-based SAM. CONT had a soft and tough texture after cooking, and SMM was harder but had the most similar texture. Plant-based SAM with a various texture can improve consumer acceptance. Further research is needed to mimic the smell and flavor of scallops.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
Borneo Convention Centre, Kuching, Sarawak

QUALITY CHARACTERISTICS OF CHICKPEA AQUAFABA MAYONNAISE WITH OPTIMAL PROCESSING TECHNOLOGY

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Abstract: Recently, there is a trend to replace eggs with plant-based ingredients to make mayonnaise. In this study, different techniques (pH-shifting, ultrasonication, and combination of pH-shifting and ultrasonication) were applied to canned chickpea aquafaba to improve the functionality as an emulsifier, and the effect of pH-shifting treatment (pH4, pH12) in the production of vegan mayonnaise using prepared chickpea aquafaba was investigated. Protein solubility in freeze-dried canned aquafaba powder (AP) treated with different techniques showed significant differences. Water absorption capacity showed that the pH-shifting treatment was the highest, while the ultrasonic treatment was the lowest. Oil absorption capacity was highest in the pH 4 treatment. Cooking chickpeas in a chickpea:water ratio of 1:6 (w/w) at 115°C and 80 kPa for 30 min produced aquafaba with the highest yield. The pH-shifting treatment resulted in a significant difference in pH and color. Mayonnaise made from pH-shifted AP had smaller droplet size than mayonnaise made from untreated AP. pH 4 treatment recorded the highest gumminess and emulsion stability of the mayonnaise. These results suggest that pH-shifting treated aquafaba can be used to produce mayonnaise with improved emulsion capacity, and pH-shifting treatment is likely to be effective in extreme acidic conditions as a processing technique.
Keywords: Aquafaba, chickpea, mayonnaise, pH-shifting, emulsion capacity

INTRODUCTION

Mayonnaise is an oil-in-water emulsion-based sauce produced worldwide. The globular proteins and phospholipids of egg yolk used in mayonnaise play an important role as emulsion stabilizer (Taslikh et al, 2022). Recently, as plant-based diets have become popular across the population, various studies are being conducted on plant-based mayonnaise to replace the egg yolk (Eveleigh et al, 2023). Aquafaba is a compound word of aqua meaning water and faba meaning family Fabaceae (Erem et al, 2023). Chickpea aquafaba can be produced by separating the liquid from canned or pressure cooked chickpeas, and functional properties include foam generation, emulsification, and thickening (Mustafa et al, 2018). However, plant-based emulsions are generally unstable (Patill et al, 2022). Therefore, the application of technology capable of improving the functionality of chickpea aquafaba is required. Ultrasound and pH-shifting are techniques that can improve the functional properties of proteins, affecting stability such as emulsifiability and foam generation (Figueroa-González et al, 2022). In this study, various techniques (pH-shifting, ultrasonication, and combination of pH-shifting and ultrasonication) were applied to canned chickpea aquafaba. The effect of pH-shifting (pH4 and pH12) in the manufacturing of plant-based mayonnaise using prepared chickpea aquafaba was investigated. We found the optimum conditions among several techniques and tried to confirm the possibility of improving the quality of mayonnaise using aquafaba.

MATERIALS AND METHODS

Materials

Canned chickpeas (Fratelli Longobardi S.R.L, SA, Italy) were purchased and used aquafaba obtained after separating chickpeas. Commercially imported chickpeas cultivated in Canada were purchased from a local food store. For mayonnaise, canola oil (Ottogi, Seoul, Korea), lemon juice (Polenghi Las, Lo, Italy), and salt (Chungjungone, Seoul, Korea) were used. Citric acid and calcium hydroxide (Esfood, Gunpo, Korea) were used for pH-shifting treatment.

Analysis of canned aquafaba treated with different techniques

Aqufaba from canned chickpeas was treated with pH-shifitng, ultrasonication, combination of pH-shifitng and ultrasonication, and then lyophilized AP was analyzed. pH-shifting treatment was performed using pH meter (Orion 3 star, ThermoFisher Scientific, Brooklyn, NY, USA), 50% (w/v) citric acid, calcium hydroxide saturated solution,

and aquafaba solutions were adjusted to pH4, pH8, pH12, and then titrated back to pH7 (labeled as pH4, pH8, pH12). Ultrasonic treatment was performed for 3 min, 5 min, 8 min with sonicator (BKUP-900K, Bio Koncision, Gwacheon, Korea) at 24 kHz and 360 W (labeled as U3, U5, U8). Combination treatement by ultrasound by pre, post, online meant that ultrasound was conducted before, after, during pH-shifting at pH12. Protein solubility, water holding capacity (WHC), and oil holding capacity (OHC) were analyzed by modifying method of Alsalman et al. (2020).

Preparation of chickpea aquafaba

Chickpeas were soacked for 24 h at 4°C. Aquafaba solutions were obtained by cooking in an autoclave (115°C, 30 min) at different ratios of raw chickpeas and water. The AP yield was calculated as a percentage of the mount of aquafaba solids obtained after cooking in the amount of raw chickpeas.

Preparation of mayonnaise

APs used for plant-based mayonnaise were treated with pH-shifting (untreated, pH4, and pH12) in the same method as in Section 2.2 to aquafaba prepared in Section 2.3. Plant-based mayonnaises were prepared by the method of Kim et al. (2022).

Optical microscopy

A drop of mayonnaise was placed on a microscope slide, covered with a coverslip. Then, microscope (IN480T-FL, Amscope, microsystem, Irvine, CA, USA) was used to obseve the microstructure at a magnification of 400×.

Physicochemical properties

The pH was measured with pH meter, and color values (CIE LAB) were measured using a colorimeter (CR-400, Minolta, Osaka, Japan) calibrated with standard white plate (L^*=83.83, a^*=0.29, b^*=28.97). L^*, a^*, and b^* values indicate lightness, redness, and yellowness, respectively. Protein solubility was analyzed by modifying method of Alsalman et al. (2020). Emulsion stability index was determined according to the mothod of Drozłowska et al. (2020).

Statistical analysis

The experiments were replicated three times and all data were presented as mean±standard deviation. The statistical analyzes were processed using SPSS software package (Version 26, SPSS, Chicago, IL, USA) by one-way analysis of variance (ANOVA) with Duncan's multiple range test. The results were considered statistically significant if $p < 0.05$.

RESULTS AND DISCUSSION

Analysis of canned aquafaba treated with different tchniques

Figure 1. shows the protein solubility, WHC, and OHC of canned AP treated with pH-shifting, ultrasonication, and combination of pH-shifting and ultrasonication. The protein solubility was significantly highest at pH8. The reason for the lowest value at pH4 was the effect of isoelectric point, and similar results were confirmed in other study (Pelegrine and Gasparetto, 2005). In the WHC, the pH-shifting treatment was able to obtain overall high values, while the ultrasonic treatment showed the lowest values. The higher the WHC, the better the protein structuring behavior (Bühler et al, 2020), which is expected to have a positive impact on pH-shifting aquafaba. The OHC were highest at pH4 ($p<0.05$). Hydrophobic proteins of aquafaba play an important role in oil absorption (Zielińska et al, 2018). These results confirmed the possibility of pH-shifting treatment alone to improve the functionality of AP.

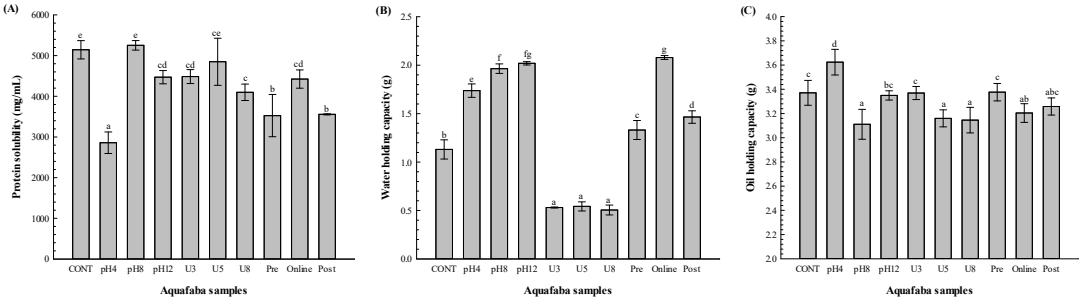

Figure 1. Protein solubility (A), water holding capacity (B), and oil holding capacity (C) of aquafaba powder from canned chickpea with differernt treatments (pH-shifting, ultrasonication, and combination of pH-shifting and ultrasonication).

Yield of chickpea aquafaba

Table 1. shows the yield of aquafaba produced with different ratios of chickpea and water. As the water ratio increased, the aquafaba yield increased, showing the greatest difference when increasing from a 1:5 ratio to a 1:6 ratio. Yields

tend to increase over time, but freeze-drying is less efficient with more water, so a 1:6 ratio of chickpea to water is considered the most efficient.

Table 1. Yield of chickpea aquafaba prepared with different ratios of chickpea and water.

Chickpea	Water	Aquafaba yield (%)
1	3	10.82
1	4	12.25
1	5	13.61
1	6	17.36
1	7	18.24

Optical microscopy of mayonnaise

A optical micrographs of aquafaba powder prepared by pH-shifting treatment are illustrated in Figure 2. Oil droplets with smaller diameter than CONT were observed in mayonnaises using AP treated with pH-shifting, and finer structure was observed in pH4 treatment than in pH12 treatment. Densely packed droplets contribute to the stability of mayonnaise strucutre (Depree and Savage, 2001). The emulsion stability of mayonnaise using AP treated with pH4 is expected to be higher.

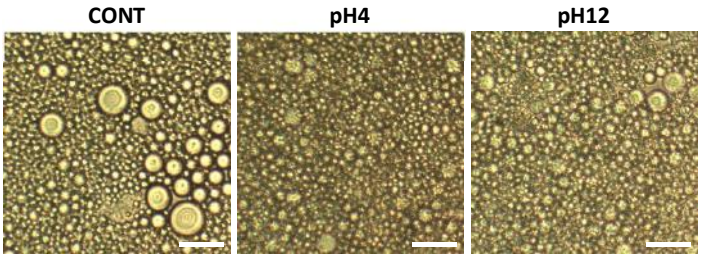

Figure 2. Optical micrographs (magnification 400×) of mayonnaise using aquafaba powder with pH-shifting treatements. The bars denote 20 μm in scale.

Physicochemcial properties of mayonnaise

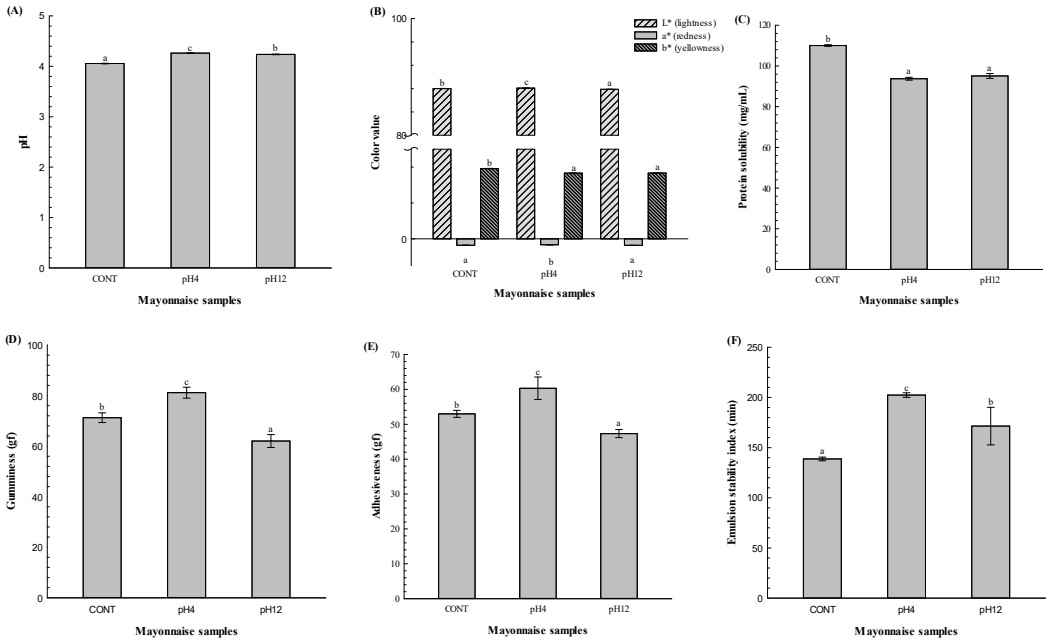

Figure 3. The pH (A), color value (B), protein solubility (C), gumminess (D), adhesiveness (E), and emulsion stability index (F) of mayonnaise using aquafaba powder with pH-shifting treatments.

The physicochemical properties of mayonnaise prepared by pH-shifting treatment on AP are shown in Figure 3. The pH results of mayonnaise were not significantly different when treated with pH-shifting. Commercially available

mayonnaise has a pH value of about 4.5 (Song et al, 2013). By treating AP with pH-shifting, the L^* of mayonnaise increased at pH4 and decreased at pH12. The a^* was the highest at pH4, and b^* decreased by pH-shifting treatment. The protein solubility of mayonnaise using AP treated with pH-shifting was significantly decreased. The gumminess and adhesiveness of mayonnaise presented the highest values at pH4 ($p<0.05$), and pH-shifting treatment affected the physical properties of mayonnaise. The viscosity of mayonnaise is one of main factors in the emulsion property, and Kim et al. (2022) reported that high viscosity of plant-based mayonnaise has high emulsion stability. The emulsion stability index of mayonnaise using AP treated with pH-shifting was higher than that of CONT, and the pH4 sample showed the highest emulsion stability. The pH-shifting treatment affected the protein structure of AP, which contributed to the quality characteristics of mayonnaise.

CONCLUSIONS

Among the AP treated with various techniques (pH-shifting, ultrasonication, and combination of pH-shifting and ultrasonication), the pH-shifting treatment showed high WHC and OHC, and these results were considered a suitable technique for improving the functionality of AP. The production of chickpea aquafaba was effectively obtained high yield of AP at a 1:6 ratio (w/v) of chickpea to water. Plant-based mayonnaises were developed by applying pH-shifting treatment to the prepared AP. As results of analyzing the physicochemical properties, AP treated with pH4 was determined to be the most reasonable emulsifier, as it had a higher emulsion stability index and formed smaller oil droplets. Mayonnaises using AP treated with pH-shifting presented higher emulsion stability than CONT, suggesting that pH-shifting treatment can improve the functionality of AP. For the commercialization of plant-based mayonnaise using pH-shifting, additianl research on quality comparison with mayonnaise using egg yolk is needed.

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24-27th October 2023
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PROXIMATE NUTRIENT COMPOSITION OF WASTE WATERMELON

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Abstract: In the Philippines, the bulk of watermelon production comes from Western Visayas at around 59,366 MT or 44.0% of the total watermelon production in the country (PSA, 2022). Due to the abundance in its production, the generated wastes that comes from the watermelon peels, seeds, and rinds also increases. This study aims to assess the proximate nutrient composition of 3 variety of waste watermelon namely Sweet 16, Sweet Gold, and Green. The collected waste watermelon fruits in the farm were washed, chopped, peels and rinds were separated, and oven-dried at varying temperatures. All 3 varieties undergone various pre-treament conditions including soaking in 8% NaCl to optimize the amount of antioxidant present, steam blanching, soaking in calamansi juice, and untreated-(UR) samples were analyzed for comparison. The proximate nutrient composition of watermelon wastes were determined based on the modified AOAC standards methods (2019). Results show that the watermelon has a very high combined amount of fiber and nitrogen free extract content (NFE or carbohydrate) ranging from 54.1% to 79.3%, moisture content(6.2%-16.0%), ash content(9.8%-38.5%), crude protein(4.6%-15.8%), and crude fat content(0.65%-1.5%). Due to notable protein content of the waste watermelon peels and rinds, the researchers recommend that it can be an alternative protein source for various application such as animal feed and plant-based protein studies.
***Keywords*:** composition, nutrient, proximate, watermelon, waste

INTRODUCTION

Western Visayas is the highest producer of watermelon in the Philippines amounting to 59,366 MT in 2021 (PSA, 2022), accounting for 44% of the total watermelon production in the country. With every production, comes waste due to watermelons being rejected at the field based on the quality, weight, and grading classification. Due to the generated food wastes and losses (FLW), its conversion to a value-additive strengthens the positive socioeconomic and environmental impact of the extracts, including its beneficial effect when added to a variety of food products.

Various research aimed at utilizing watermelon wastes have been generated, focusing on food fortification and substitution of ingredients to maximize the nutrient profile of food products. One food research conducted by Naknaen et al. (2018), analyzed the use of watermelon rind wastes as a potential source of dietary fiber to improve the health properties of cookies. Results of their research showed that an increase in the watermelon rind powder substantially increased the dietary fiber, total phenolic content, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activities and the ferric reducing antioxidant power in the cookies. Another research, utilized the impact of watermelon seed flour on the physicochemical and sensory qualities of ice cream evaluated by Qayyum et al. (2016). Analysis of the ice cream showed a marked increase in the fat, protein, total solids, solid-not-fat, and ash content while the moisture, viscosity, meltdown and over run decreased. Further research was also conducted in assessing the watermelon peel, its antioxidant activity and mineral content is considered a good source of natural polyphenols, antioxidants and minerals as evidenced by the research of Feizy et al. (2020), wherein the watermelon peel macro- and micronutrients has 6.77g/100g Protein, 0.92g/100g Fat, 13.2g/100g Ash, 24g/100g Fiber.

The main objective of this study is to utilize FLW coming from three cultivars of watermelon peels and rinds by characterizing the proximate nutrient composition of the pre-treated, and untreated watermelon wastes. This project focuses in the conversion of the waste watermelon to a powdered food ingredient for value-adding, and food preservative for high-fat foods, thereby mitigating the impact of food losses. This project is aligned with the priority agenda and funded by the Department of Science and Technology - Philippine Council for Industry, Energy, and Emerging Technology Research and Development (DOST-PCIEERD), specifically for food and nutrition security, under the research area of functional food program, and in the development of market competitiveness in Western

Visayas. The results of this study shows the best pre-treatments, and drying temperature per cultivar to either increase, decrease, and/ or improve a specific parameter in terms of nutrient composition such as moisture, ash, crude protein, crude fat, crude fiber, and carbohydrates of the sweet gold, sweet 16, and buffalo watermelon cultivars.

MATERIALS AND METHODS

Collection and Preparation of Samples

Around 100 pcs. were collected for the red-fleshed watermelon (*Citrullus sp.*) samples, "*Sweet 16*" and *"Buffalo",* and yellow-fleshed, "*Sweet Gold*" watermelons in Cubay-Napultan, Sibalom, Antique last February 2023. Only the watermelons that were considered as rejects, left on the field, and without visible fungal growth were collected as samples. All samples were transferred to the Integrated Research and Development Laboratory (IRDL) of the University of Antique – Main Campus for its processing.

Preparation of Watermelon Samples

The watermelons are washed, soaked in 100ppm chlorinated water solution for 10-15 minutes, and rinsed with potable tap water. Once rinsed, the watermelons are stored in a well-ventilated area at room temperature until processing.

Watermelon Rinds and Peels

Watermelons are sliced into 8 sections, and the pulp part, which is the red or yellow flesh, is removed using a kitchen knife. The flesh of the watermelons is separated from the rinds and peels portion which is cut lengthwise, and then diced to produce a 1 cm^3 size. The following are the conditions (1, 2, 3, 4) with the pre-treatments that the watermelon peels and rinds were subjected to:

- Condition 1.1: Drying at 60°C
- Condition 1.2: Soaking in 8% NaCl and drying at 60°C
- Condition 2.1: Drying at 40°C
- Condition 2.2: Soaking in 8% NaCl and drying at 40°C
- Condition 3.1: Steam blanching (BLA) for 1 minute and drying at 40°C
- Condition 3.2: Steam blanching for 1 minute, soaking in 8% NaCl, and drying at 40°C
- Condition 4.1: Soaking in calamansi juice (CAL) for 10 minutes, and drying at 40°C
- Condition 4.2: Soaking in calamansi juice for 10 minutes, soaking in 8% NaCl, and drying at 40°C.

All of the watermelon samples were loaded onto a drying rack and dried in a forced air dryer. The dried watermelon extracts were powdered using a blender, packed in aluminum polyethylene sealable pouches, and stored at room temperature away from direct sunlight.

Determination of Proximate Nutrient Composition

For the complete proximate nutrient composition of the powdered samples, the standard method from Official Methods of Analysis of AOAC International or AOAC, 21st ed. (2019) were adapted using the specified equipment as shown in Table 1 below.

Table 1. List of Method Reference and Equipment

Test Parameter	Reference Method	Method Title	Equipment Used	Remarks
Moisture	AOAC Method 925.10	Total Solids and Loss on Drying (Moisture) in Flour	Infrared Moisture Analyzer	Modified
Ash	AOAC Method 942.05	Ash of Animal Feed	Muffle Furnace	-
Crude Protein	AOAC Method 2001.11	CrudeProtein in Animal Feeds, Forage (Plant Tissue), Grain, and Oilseeds	Kjedahl Analyzer K9860	Modified Block Digestion Method Using Copper Catalyst and Steam Distillation into Boric Acid; Factor used: 6.25
Crude Fat	AOAC Method 2003.06	Crude Fat in Feeds, Cereal grains, and Forages	Soxhlet Analyzer SOX406	Modified Submersion method;
Crude Fiber	AOAC Method 978.10	Crude Fiber in Animal Feed and Pet Food	Fiber Analyzer F800	Fritted glass crucible method
Nitrogen Free Extract	Not Applicable	By Arithmetic Difference	Not Applicable	-

Statistical Analysis

The two-way analysis of variance (ANOVA) was performed, with Fisher's least significant difference (LSD) as the post-hoc using the StatPlus software.

RESULTS AND DISCUSSION

The moisture content (%MC) of the watermelon powder from peels and rinds is shown in Table 1 (Column 4). The %MC of the sweet gold, and sweet 16 cultivar were reduced when steam blanched, and for sweet 16, the addition of a pre-treatment method of soaking in 8% NaCl lowered its MC%. The lowest MC% was found in the buffalo cultivar, pre-treated with 8% NaCl and dried at 60°C. The pre-treatments of steam blanching, and soaking in 8% NaCl to reduce the water content present in the peels and rinds prior to dehydration, resulted in a final low moisture content, leading to an increased storage stability of the samples.

Lowest ash values were recorded for sweet gold with samples soaked in calamansi and dried at 40C, while for sweet 16, and buffalo, samples dried at 40C, and 60C respectively have the lowest ash content. The ash content of the peels and rinds samples as presented in Table 1 (Column 5) were highest in sweet gold dried at 60°C, in sweet 16 that was steam blanched then soaked in 8% NaCl and dried at 40°C, and in buffalo soaked in calamansi then 8% NaCl, and dried at 40°C; the ash content for each were higher compared to the normal ash of watermelon peels and rinds between 2.90 to 14.46% (Cansu & Fatma, 2023; Zubairu et al. 2018). This indicates that the initial minerals present in the specific batch of samples, depending on its maturity, may have contributed to the overall ash. The pre-treatment with 8% NaCl, and its combination with calamansi further increased its mineral content that would contribute to the overall flavor profile, and micronutrients of the watermelon extracts.

As seen in Table 1, Column 6, the % Crude Protein (%CP) of the peels and rinds watermelon extract is highest in the sweet gold cultivar at 15.79% for samples soaked in calamansi dried at 40°C, which have been stored for 2 months, higher than the reported values of Ho et al. (2016) for yellow watermelon rinds that were hot air dried at 40°C with %CP reported at 13.37 + 0.14%. In terms of the red-fleshed watermelon cultivars, the sweet 16 also had its highest %CP with the calamansi treatment dried at 40°C, and for the buffalo variety, it resulted in a 11.7 %CP dried at 40°C. The pre-treated hot air dried samples had higher %CP from the study, compared with studies using freeze drying technique, with only 11.82% (Ho et al., 2016). All samples were stored 1-2 months before the analysis, and still had higher %CP than the air dried samples stored from the study of El-Behairy et al. (2022), with %CP ranging from 12.4 to 11.8% during the first to second month of storage. This indicates that pre-treating the watermelon peels and rinds with calamansi is an inventive step as the initial addition of calamansi was only included to lower the pH of the samples, and increase its shelf-life stability; however, in the study, its use as a pre-treatment also increased the %CP and may be due to the phenolic amines present in citrus fruits such as calamansi, significantly increasing the value of the extracts due to its higher %CP.

As shown in Table 1 (Column 7), drying the watermelon peels and rinds at 60°C reduced its %Crude Fat (%CFt). For the buffalo and sweet 16 variety, pre-treatment with 8% NaCl further reduced its %CFt to 0.87 and 0.64 respectively while for sweet gold, its %CFt lowered without the need for a 8% NaCl pre-treatment. The salt absorption for each samples is affected by the type of cultivar, as sweet 16 and buffalo better retained the salt, causing it to break down fat resulting in a lower fat content, while the addition of salt in sweet gold increased its fat content, explaining how salt is indirectly affecting its overall %CFt, and can only be due to the maturity of the sweet gold used during processing. The lower fat content in the samples reduces lipid oxidation during storage, therefore increasing its shelf-life stability, and when added to high fat foods, the samples will not affect its fat content and flavor profile as much. A high % CP, and low %CFt can be achieved when pre-treating the watermelon peels and rinds with calamansi and 8% NaCl. In addition, the %CFt of seeds were quite high, and even increased when dried at 60°C for the sweet gold, and sweet 16 variety, while for the buffalo variety, the higher fat content is found in samples dried at 40°C. The difference between the effect of the temperature on the CF% of the seeds from the different cultivars may be due to the type of fatty acid present in the samples, and how they are affected by certain drying temperatures.

Based on Table 1 (Column 8), the highest Crude Fiber + CHO% was found in the steam blanched peels and rinds samples of the sweet gold, and buffalo varieties, and samples dried at 40°C for both sweet gold, and sweet 16. With seeds, lower temperature gave higher Crude Fiber + CHO% to the sweet gold and sweet 16 varieties, while for the buffalo, higher values were obtained from samples dried at 60°C. The high values of Crude Fiber + CHO% may be due to the maturity of the samples, and how steam blanching stopped the enzymatic reactions that could contribute to its degradation. The difference in the Crude Fiber + CHO% values in varying temperatures are not significant for most samples.

Table 2. Nutrient Composition of Watermelon Peels and Rinds

Sample	Type	Condition	Moisture (%)	Ash (%)	Crude Protein (%)	Crude Fat (%)	Crude Fiber + Nitrogen Free Extract (%)
Percent Dry Basis (%)							
Yellow-fleshed "*Sweet Gold*"	Peels and Rinds	40°C UR	$12.39\pm1.06^{a,A}$	$12.53\pm0.27^{a,b,D,E}$	$12.10\pm0.05^{a,D}$	$1.46\pm0.10^{a,A}$	$73.90\pm0.37^{b,A}$
		60°C UR	$8.86\pm0.67^{a,D}$	$30.25\pm0.36^{a,A}$	$8.44\pm0.29^{a,F}$	$0.65\pm0.09^{b,D}$	$60.67\pm0.14^{b,B}$
		40°C 8%	$12.48+0.44^{a,A}$	$28.95\pm0.12^{c,A,B}$	$7.70+0.33^{a,G}$	$1.22+0.07^{a,A,B}$	$62.13+0.44^{a,B}$
		60°C 8%	$9.48\pm0.37^{a,D}$	$28.38\pm0.25^{b,B}$	$7.81\pm0.29^{a,G}$	$1.10\pm0.20^{a,B}$	$62.71\pm0.58^{a,B}$
		BLA UR	$8.17+1.50^{a,D}$	$13.66+0.03^{a,D}$	$9.88+0.12^{c,E}$	$0.79+0.05^{a,C,D}$	$78.97+5.73^{a,A}$
		BLA 8%	$9.32\pm0.08^{a,D}$	$11.95+3.47^{c,E}$	$12.91+0.07^{a,C}$	$1.21\pm0.13^{a,A,B}$	$73.92+3.60^{a,A}$
		CAL UR	$16.02\pm0.70^{a,B}$	$9.83\pm0.05^{b,F}$	$15.79+0.21^{a,A}$	$1.00\pm0.12^{a,b,B,C}$	$73.38\pm0.26^{a,A}$
		CAL 8%	$14.42+2.38^{a,C}$	$26.72+0.29^{b,C}$	$14.60+0.19^{a,B}$	$1.03+0.06^{a,B,C}$	$57.65+0.16^{b,B}$
Red-fleshed "*Sweet 16*"	Peels and Rinds	40°C UR	$8.63\pm0.30^{b,B,C}$	$11.13\pm0.20^{b,F}$	$7.27\pm0.22^{b,C}$	$1.46\pm0.10^{a,A}$	$80.38\pm0.60^{a,A}$
		60°C UR	$7.77\pm0.41^{b,C,D}$	$14.38+0.04^{b,D}$	$6.99\pm0.18^{b,C}$	$0.65\pm0.09^{b,D}$	$77.75\pm0.72^{a,A}$
		40°C 8%	$8.83+0.40^{c,B,C}$	$30.76+0.53^{b,B}$	$6.25+0.09^{b,D}$	$1.22+0.07^{a,A,B}$	$61.87+0.43^{a,B}$
		60°C 8%	$7.54\pm0.21^{b,C,D}$	$27.16\pm0.10^{b,C}$	$5.72\pm0.48^{b,E}$	$1.10\pm0.20^{a,B}$	$66.47\pm0.45^{a,B}$
		BLA UR	$8.84\pm0.16^{a,B,C}$	$13.63\pm0.21^{a,D,E}$	$11.46\pm0.27^{a,B}$	$0.79\pm0.05^{a,C,D}$	$78.37\pm7.72^{a,A}$

		BLA 8%	$6.58\pm0.41^{b,D}$	$38.53\pm1.01^{a,A}$	$6.14\pm0.16^{b,D,E}$	$1.21\pm0.13^{a,A,B}$	$54.68\pm0.93^{b,C}$
		CAL UR	$9.89\pm0.33^{b,A,B}$	$11.19\pm0.11^{b,F}$	$12.05\pm0.17^{b,A}$	$1.00\pm0.12^{a,b,B,C}$	$76.07\pm0.13^{a,A}$
		CAL 8%	$10.64\pm0.13^{b,A}$	$12.48\pm0.14^{c,E,F}$	$11.03\pm0.24^{b,B}$	$1.03\pm0.06^{a,B,C}$	$75.77\pm0.19^{a,A}$
Green *"Buffalo"*	Pees and Rinds	40°C UR	$8.92\pm0.12^{b,B,C}$	$13.34\pm0.10^{a,C}$	$11.70\pm0.35^{a,A}$	$1.41\pm0.11^{a,A}$	$77.45\pm6.72^{a,b,A}$
		60°C UR	$9.96+0.42^{a,A,B}$	$12.41+0.30^{c,D}$	$7.00+0.19^{b,D}$	$1.26+0.10^{a,A}$	$79.33+0.37^{a,A}$
		40°C 8%	$10.43+0.43^{b,A}$	$33.63+0.44^{a,B}$	$7.41\pm0.31^{a,D,E}$	$1.02\pm0.20^{a,B}$	$57.93+0.67^{a,B,C}$
		60°C 8%	$6.20\pm0.32^{c,E}$	$33.27\pm0.21^{a,B}$	$4.59\pm0.14^{c,G}$	$0.87\pm0.19^{a,b,B}$	$61.27\pm0.20^{a,B}$
		BLA UR	$9.11\pm0.48^{a,A,B,C}$	$13.59\pm0.02^{a,C,D}$	$10.85\pm0.07^{b,B}$	$1.10\pm0.06^{a,A,B}$	$78.07\pm6.30^{a,A}$
		BLA 8%	$8.05+0.46^{a,C,D}$	$36.88+1.05^{b,A}$	$6.32+0.05^{b,F}$	$1.12+0.07^{a,A,B}$	$55.68+1.05^{b,B,C}$
		CAL UR	$7.91\pm0.05^{c,C,D}$	$14.17\pm0.16^{a,C}$	$10.01\pm0.21^{c,C}$	$1.23\pm0.02^{a,A}$	$77.93\pm5.72^{a,A}$
		CAL 8%	$6.73+0.29^{c,D,E}$	$37.58+0.91^{a,A}$	$7.51\pm0.27^{c,E}$	$0.81\pm0.08^{a,B}$	$54.10+0.80^{b,C}$

CONCLUSIONS

This study concludes that the present identified the best pre-treatments, and drying temperature per cultivar to either increase, decrease, and/ or improve a specific parameter in terms of nutrient composition such as moisture, ash, crude protein, crude fat, crude fiber, and carbohydrates of the sweet gold, sweet 16, and buffalo watermelon cultivars. It was found out that steam blanching is the most efficient method to lower the moisture content of the watermelon powder regardless of cultivar type, thus increasing its shelf-life stability. The high ash values in the peels and rinds is due to the initial minerals, and pre-treatments used. Drying temperature at 60^0C reduced the %CFt of all samples which implies that samples with lower fat content reduces lipid oxidation during storage, therefore increasing its shelf-life stability, and when added to high fat foods, the samples will not affect its fat content and flavor profile as much. In summary, the interaction between the pretreatment conditions, and the specific cultivar were found to be unique as it produced technical effects that have not been previously found in watermelon peels and rinds extracts. This study also confirms the increase in the value of the watermelon wastes, and the production of a value-additive that can be added to several food products due to its nutritional benefit.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
Borneo Convention Centre, Kuching, Sarawak

EFFECT OF DIFFERENT PROCESSING CONDITIONS ON THE TOTAL PHENOLIC CONTENT AND FREE RADICAL SCAVENGING ACTIVITY OF THREE CULTIVARS OF WATERMELON RINDS AND PEELS

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Abstract: Western Visayas is the top watermelon producer in the Philippines with 59, 366 MT or 44.0% of the overall production. Current interventions focus on pectin and candy production from watermelon rejects. This study is the first to explore the use of watermelon peels and rinds from Sweet 16, Buffalo, and Sweet Gold, pre-treated with steam blanching (SB), calamansi juice extract (CJE), and untreated (UR) as control, wherein 50% of the samples were further treated with 8% NaCl to reduce its drying time. The SB and CJE underwent 40^{0}C oven drying (OD), while UR, with and without 8% NaCl were subjected to 40^{0}C and 60^{0}C OD. Dried samples were blended to produce watermelon rinds and peels (WMRP) and analyzed for total phenolic content (TPC), and DPPH free radical scavenging activity (RSA%) using UV-vis spectrophotometer. Based on TPC, 8% NaCl-60^{0}C sweet gold WMPR yielded the highest (21.73 GAE mg/g), while SB 8% NaCl-40^{0}C buffalo WMRP produced the lowest (6.01 GAE mg/g). The WMRP had 64.59 to 89.60% RSA, forming most of the TPC. The study found certain processing conditions are best combined with specific cultivars to increase its TPC and RSA, maximizing the use of WMRP as a potential natural food preservative and value additive. Screening of phytochemicals responsible for the specific antioxidant activity is recommended to fully understand how to better utilize the WMRP in food products.
***Keywords*:** watermelon rejects, total phenolic content, antioxidant activity, natural food preservative, food additive

INTRODUCTION

Biodegradable wastes are derived from natural organic compounds that have been degraded by biological mechanisms (Maji et al., 2020). The decomposition process that constitutes biodegradable wastes comparatively impacts the environment due to the production of greenhouse gases such as methane. In the Philippines, considerable watermelon rejects are generated from its production in Western Visayas, amounting to 41.9% or 11,250 pcs of watermelon losses, as surveyed in a harvest site in Cubay-Napultan, Sibalom, Antique, last February 2023. Current interventions in reducing the watermelon losses and wastage were limited to researches on pectin extraction, production of watermelon rind candy, and its use as a rejuvenating agent (Lo et al., 2019; Back et al., 2016), while its utilization in adding value to food products, and as a food preservative, has not yet been studied in the Philippines.

The watermelon varieties used for its conversion as dried and powdered extracts were the red-fleshed with striped outer peels "*Sweet 16*" watermelon, red-fleshed with dark green peels "*Buffalo*" watermelon, and the yellow-fleshed with green peels "*Sweet Gold*" watermelon. The purpose of the combined peels and rinds in the study was to reduce the time needed to prepare the watermelon waste for drying, and ease during technology transfer. A preliminary study was conducted to determine the desired percentage of the iodized sodium chloride (NaCl) treatment. It was found that 8% iodized NaCl did not alter the taste of the watermelon rinds and peels powder (WMRP), allowing osmotic dehydration, lowering the water content of the samples, and decreasing its overall drying time. With steam blanching as one of the conditions used, its purpose was to stop the enzymatic processes in the watermelon rinds and peels, to prevent degradation of its phenolic compounds, responsible for its antioxidant activity (Ndie & Okaka, 2018; Wickramasinghe et al., 2020). In terms of the pH values of most food products, the pH of safe foods is normally pH <4.5, and to achieve this, the watermelon peels and rinds were soaked in pH ≤4 calamansi (*Citrus × microcarpa*) juice.

The objective of this research was to utilize and characterize the effect of the different treatments, processing conditions, and its effect on the watermelon peels and rinds, in order to reduce the generated food losses during watermelon harvest season. Through this research, it was found that the antioxidant activities of the watermelon

cultivars can be enhanced, depending on the type of cultivar, treatment, and condition, which greatly adds value to the rejected parts of the watermelon, further strengthening food sustainability and security in the Philippines.

MATERIALS AND METHODS

Collection and Preparation of Samples

Around 100 pcs. were collected for the red-fleshed watermelon (*Citrullus sp.*) samples, "*Sweet 16*" and *"Buffalo",* and yellow-fleshed, "*Sweet Gold*" watermelons in Cubay-Napultan, Sibalom, Antique last February 2023. Only the watermelons that were considered as rejects and left on the field were collected as samples. All samples were transferred to the Integrated Research and Development Laboratory (IRDL) of the University of Antique – Main Campus for its processing.

Preparation of Watermelon Samples

The watermelons were washed with potable water, dipped in 100ppm of chlorine solution for 5 minutes, rinsed with potable water, air dried, and stored in a well-ventilated area prior to the start of the preparation.

Watermelon Rinds and Peels

The red and yellow flesh of the watermelons were separated, with only the white rinds, and the green peels remaining. The watermelon rinds and peels were cut by $1cm^3$ to undergo four conditions and two treatments as detailed in Table 1.

Table 1. Treatments and Conditions for WMRP

Treatments	Condition 1	Condition 2	Condition 3	Condition 4
Untreated (.1)	Drying at 40°C	Drying at 60°C	Steam blanching for 1 minute and drying at 40°C	Soaking of WMPR in calamansi juice extract for 10 minutes and drying at 40°C
Soaking in 8% NaCl (2-5mins) (.2)				

Determination of total phenolic compounds

The modified methodology for determining the total phenolic compounds was based on Feizy et al. (2020), wherein the homogenized powder at 0.5g of WMRP was extracted with 25mL of aqueous-ethanol solution (70% v/v). The mixture was completely stirred for 1 minute on a vortex mixer and incubated under the dark for 30 minutes. The resultant suspension was centrifuged at 4000 rpm for 15 minutes. A part of the collected supernatant (200uL) was mixed with 1:00 Folin Ciocalteu reagent (1.5mL). The test tubes were then vortexed for 5 minutes, and added with 1.5mL of sodium bicarbonate (Na_2CO_3) solution (6% w/v), and incubated at a dark place for 60 minutes. Control sample(s) were also prepared, and the absorbance of all samples based on Neglo et al. (2021) was read at 760nm with a calibration curve using 20, 40, 60, 80, and 100mg/mL gallic acid in methanol. The results were expressed as g of gallic acid equivalent per g of dry matter (mg gallic acid/ dry weight of the sample).

DPPH Free Radical Scavenging Activity (RSA)

The determination of the DPPH Free Radical Scavenging Activity (RSA) was based on the modified method by Feizy et al. (2020) will be used. Around 5g of the WMRP was extracted with 25mL of ethanol solution (70% v/v), vortexed for 1 minute with 30 minute intervals for the first 8 hours, and stored in a dark place for 24 hours. and gently stirred for 24 hours. The resulting suspension was centrifuged at 4000 rpm for 15 minutes; the collected supernatant (0.1mL) was diluted in methanol (8.9mL), added with 1mL DPPH solution (0.004% v/v in methanol), and incubated in a dark place for 30 minutes. A control was prepared, and the absorbance of all samples were measured at 517nm. The percentage of inhibition was expressed with the following equation:

$$\% \text{ Inhibition} = \frac{[A_{control} - A_{sample}]}{[A_{control}]} \times 100$$

Statistical Analysis

The two-way analysis of variance (ANOVA) was performed, with Fisher's least significant difference (LSD) as the post-hoc using the StatPlus software.

RESULTS AND DISCUSSION

The RSA% of the sweet 16 for the peels and rinds, were highest at 60°C, the buffalo variety at 40°C, while the sweet gold produced an enhanced RSA% in the samples treated with calamansi and dried at 40°C. A significant increase in the RSA% in the sweet gold samples treated with calamansi, is due to the presence of Vitamin C in the citrus fruit (Venkatachalam et al., 2023), which contributed to its RSA%, and preserved even when heated at 40°C. In sweet gold, its RSA% was also high with samples treated with 8% iodized NaCl and dried at 60°C, which indicates soaking the samples in NaCl improved its radical scavenging activity due to the iodine present in the 8% iodized NaCl, which has shown to possess reducing properties due to its ability to act as an electric donor (Karbownik-Lewińska et al., 2022).

The effect of the iodine from the iodized NaCl differs per cultivar as for the sweet 16, the addition of 8% NaCl significantly lowered its DPPH RSA%. For the buffalo samples, the effect of the 8% NaCl is similar with the untreated samples. The variations in the results may be linked to the specific effect of the iodine and its concentration, the calamansi juice on the cultivar, and on its degree of ripeness. Iodized NaCl and calamansi can accumulate better inside the more mature cultivars, due to how the rinds and peels become softer over time, leading to higher membrane permeability.

Table 2. RSA(%) of the WMRP

Type	Condition	Yellow-Fleshed *"Sweet Gold"*	Red-Fleshed *"Sweet 16"*	Red-Fleshed *"Buffalo"*
		RSA (%)		
Peels and Rinds	40°C UR	$44.24\pm1.04^{b,C}$	$45.75\pm1.92^{b,C}$	$64.32\pm1.75^{a,A}$
	60°C UR	$25.09\pm0.73^{b,D}$	$69.85\pm2.48^{a,A}$	$56.38\pm6.89^{a,A}$
	40°C 8%	$70.11\pm1.04^{a,B}$	$54.39\pm5.52^{b,B}$	$64.59\pm1.36^{a,A}$
	60°C 8%	$77.06\pm5.51^{a,A,B}$	$40.96\pm1.35^{b,C}$	$32.38\pm1.36^{b,B}$
	BLA UR	$36.75\pm0.30^{b,C,D}$	$50.81\pm1.49^{a,b,B}$	$56.14\pm2.71^{a,A}$
	BLA 8%	$73.94\pm0.08^{a,A,B}$	$49.44\pm4.99^{b,B}$	$50.90\pm0.7^{b,A}$
	CAL UR	$89.60\pm0.94^{a,A}$	$-1.78\pm0.19^{b,D}$	$7.54\pm9.23^{b,C}$
	CAL 8%	$83.84\pm1.26^{a,A,B}$	$54.92\pm2.81^{b,B}$	$34.63\pm2.50^{c,B}$

Data are presented as mean ± standard deviation (n=3). Mean values in the same row with similar superscript small letters (a-c) are not significantly different at $p < 0.05$. Mean values in the same column with similar superscript capital letters (A-D) are significantly different at $p<0.05$.

The highest RSA% in the study ranged from 64.59 to 89.60% per cultivar, and were found to be higher in comparison to the published studies by Abdulazeez et al. (2020) for sun-dried and powdered peels and rinds, with RSA% at 35.24% to 38.73%, and Neglo et al. (2021), with a 34.48% RSA for fresh watermelon peels, both of which having no prior treatments. This indicates that the watermelon cultivars in Antique, Philippines had considerably higher initial RSA% even without any treatments, and its RSA% can even be increased with the novel conditions that were applied in the study, leading to better product characteristics, and added value.

Table 3. TPC of the WMRP

Type	Condition	Yellow-Fleshed "Sweet Gold"	Red-Fleshed "Sweet 16"	Red-Fleshed "Buffalo"
		TPC (GAE mg/g)		
Peels and Rinds	40°C UR	$16.91\pm0.66^{a,B}$	$13.35\pm0.75^{b,B}$	$11.39\pm0.43^{c,B}$
	60°C UR	$17.52\pm0.52^{a,B}$	$16.00\pm0.91^{b,A}$	$14.01\pm1.01^{c,A}$
	40°C 8%	$11.19\pm0.97^{a,C}$	$10.68\pm0.38^{a,C}$	$9.13\pm0.38^{b,C}$
	60°C 8%	$21.73\pm0.61^{a,A}$	$12.40\pm0.29^{b,B}$	$7.77\pm0.94^{c,D}$
	BLA UR	$10.41\pm0.50^{a,D}$	$-0.71\pm0.03^{b,E}$	$10.70\pm0.27^{a,B}$
	BLA 8%	$9.66\pm0.15^{a,D}$	$9.48\pm0.86^{a,D}$	$6.01\pm0.30^{b,E}$
	CAL UR	$21.16\pm0.46^{a,A}$	$10.57\pm0.32^{b,C}$	$10.11\pm0.31^{b,B,C}$
	CAL 8%	$16.94\pm0.04^{a,B}$	$12.91\pm0.27^{b,B}$	$11.18\pm0.62^{c,B}$

Data are presented as mean ± standard deviation (n=3). Mean values in the same row with similar superscript small letters (a-c) are not significantly different at $p < 0.05$. Mean values in the same column with similar superscript capital letters (A-D) are significantly different at $p<0.05$.

The control samples that were untreated, and dried at 40°C had significantly lower TPC values, indicating the condition(s) applied per cultivar enhanced its TPC value. The WMRP dried at a higher temperature of 60°C had higher TPC for all watermelon cultivars. For the sweet gold variety, WMRP soaked in 8% NaCl, and in the calamansi juice extract had significantly higher TPC values compared to the other samples, while in sweet 16 and buffalo, its TPC values were highest for untreated samples dried at 60°C. The increased TPC values in higher temperatures was attributed to the reduced enzymatic activities that would have otherwise contributed to the oxidation of the phenols present in the WMRP. The TPC values of the WMRP were higher, in comparison to the red and yellow-fleshed watermelon rind powders by Ho et al. (2018), indicating that the origin, conditions used, and inclusion of the peels with the rinds affects its TPC values.

CONCLUSIONS

There are certain treatments, conditions, and its combination, that increase the TPC and RSA% of a specific cultivar, as each watermelon rinds, and peels, interacts differently to the treatments and conditions. Lower initial TPC, and RSA% values can be enhanced through the pre-treatments, and conditions as shown in the study, which are less costly compared to the current studies proposing further extraction methods, and the use of several equipment in terms of antioxidant preservation. Through this study, the utilization of watermelon rinds and peels, have a great potential to

be commercialized and used in a wide variety of food products, further reducing the socioeconomic impact brought about by food losses and wastes, and adding value to a previously discarded food material.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
Borneo Convention Centre, Kuching, Sarawak

INNOVATIVE APPLICATION OF RICE-BASED INGREDIENTS IN DEVELOPING CHEESE ANALOG FLAVOURED CAKES: AN APPROACH TO ENHANCE BAKERY PRODUCT DIVERSITY

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Abstract: Malaysia, particularly the state of Sarawak, has various local rice varieties that can be utilized in value-added food processing. Rice is suitable for bakery products due to its versatility and gluten-free nature. This study focuses on developing a rice-based layered cake and a rice-based cake (non-layered) using a commercial procedure. The best formulation consists of 6.9% rice flour and 9.2% fermented rice of the total ingredients. This formulation produces a strong cheese flavor without the addition of cheese. Sensory evaluations indicate that both developed cakes receive high scores for cheese taste and overall acceptance compared to commercial cheesecake. The analysis reveals that both developed rice cakes contain all nine essential amino acids required by the human body. The total amount of essential amino acids in the rice layered cake and non-layered rice cake is 29.69 mg/100g and 30.91 mg/100g, respectively. Additionally, the rice cakes exhibit γ-aminobutyric acid (GABA) with a value ranging from 1.27 to 1.93 mg/100g, an organic compound that has potential health benefits. These findings demonstrate the potential of using local rice varieties to create diverse bakery products that cater to different dietary needs and preferences, offering gluten-free options with a desirable cheese flavor and nutritional value.
Keywords: Rice-based cakes, cheese analog, essential amino acids, GABA

INTRODUCTION

Malaysia, particularly within the state of Sarawak, boasts a diverse range of indigenous rice varieties **(Figure 1)** that offer significant promise for applications in value-added food processing (Nicholas et al., 2015). A recent study, conducted with the support of the Malaysian Agricultural Research and Development Institute's (MARDI) 2022 Budget Initiative Project, has focused the spotlight on the pronounced potential of rice as a valuable resource in product development.

Significantly, the intrinsic versatility and gluten-free characteristics inherent in these indigenous rice varieties have sparked investigations into their compatibility for incorporation within bakery products. The strategic utilization of rice-based ingredients in the realm of bakery industry not only offers a way to enhance product diversity but also serves as an optimization strategy for harnessing local resources (Jan et al., 2021).

This study is dedicated to the exploration of innovative applications for rice-based ingredients, with a specific focus on rice flour, and the fermented rice known as 'tapai beras'. The core objective revolves around the creation of cheese analog flavoured cakes, encompassing both basic and multi-layered rice-based variations, employing established commercial techniques. This work involves a thorough evaluation of sensory attributes and nutritional composition, with a focus on γ-aminobutyric acid (GABA) content, as well as microbiological analysis and shelf-life study. The primary aim is to expand the variety of bakery choices for consumers.

Figure 1. Various indigenous rice varieties of Sarawak

MATERIALS AND METHODS

Development of Rice-Based Cheese Analog Flavoured Cakes

The formulation and processing method of rice-based cheese analog flavoured cakes is based on the information from previous research conducted by MARDI, with some adjusted depending on the requirements. Rice flour and fermented rice 'tapai beras' are used in the cake formulation to assess their suitability for use in producing layered rice cakes.

The production of rice flour is carried out using the 'dry milling method'. Rice is finely ground using a grinding machine and sifted through a metal sieve sized at number 80 - 100 mesh. The study involves several formulations with varying ratios of rice flour to wheat flour i.e., 80:20, 60:40, and 50:50, to determine the optimal formulation for layered rice cake.

Sensory Evaluation

Sensory evaluation for determining the best formulation of layered rice cake involved 30 participants, utilizing a 7-point hedonic sensory test that encompassed sensory attributes such as aroma, color, texture, sweetness, and overall acceptance (Poste et al. 1991). Sensory testing was also conducted to assess a comparison between the developed rice-based cheese analog flavoured cakes and several commercial cheese cakes.

Proximate, Nutritional Composition and Microbiological Analysis

Proximate analysis conducted included the determination of moisture, protein, fat, carbohydrate, energy, sodium, sugar, fiber, and ash content. Additionally, analysis of the amino acid profile and γ-aminobutyric acid (GABA) was carried out for the produced rice cake by using ultra-performance liquid chromatography. Microbiological testing covered Total Plate Count, Coliform Count, and Yeast and Moulds Count (AOAC, 2023)

Shelf-life Study

The study involves the packaging and determination of the shelf life of developed rice cake. All the samples are stored in a refrigerated environment at temperatures ranging from 4 to 10 °C. The targeted shelf life for the cake is set at 3 months. The assessment of shelf life encompasses various aspects, including microbiological analysis as mentioned above, colour analysis expressed in the L*, a*, b* notation, measurement of moisture content, water activity (Aw), pH levels, and sensory evaluation.

RESUITS AND DISCUSSION

Development of Rice-Based Cheese Analog Flavoured Cakes

After several formulation development studies and a series of sensory evaluations, the study carried out in this project revealed that the optimal ratio of rice flour to wheat flour in the formulation of layered rice cake is 60:40. The basic formulation of the rice-based cheese analog flavoured cake **(Figure 2)** is as shown in **Table 1**. Potassium sorbate of not exceeding 2000mg/kg can be added (optional) to the cake as a permitted food preservative.

Figure 2. Rice-based cheese analog flavoured cake

Table 1. Basic formulation of rice-based cheese analog flavoured cake

Ingredients	%
Rice flour	6.9
Wheat flour	4.6
Butter	23.0
Egg	23.0
Fermented rice 'tapai beras'	9.3
Sugar	8.3
Condensed milk	9.2
Evaporated milk	3.7
Ovallete	1.4
Salt	0.4
Citric acid	0.2
Others (flavouring, colouring)	10.0

Sensory Evaluation

A sensory evaluation was conducted to evaluate rice cakes (basic and layered), compared to commercial cheese cakes (basic and layered). The sensory test results **(Table 2)** indicate that both the rice cakes received the highest scores for aroma, texture (softness), sweetness, cheese flavour, and overall acceptance.

In terms of cheese flavour, both the basic and layered rice cakes received high sensory scores (5.73 ± 1.04 and 5.90 ± 0.88 respectively). Both rice cakes exhibited a strong cheese flavor that significantly differed from the cheese flavour in the commercial cheese cake (5.13 ± 0.97) and commercial layered cheese cake (5.06 ± 0.86). Regarding overall acceptance, both the basic and layered rice cakes received high sensory scores (5.83 ± 0.98 and 6.33 ± 0.66, respectively), and they significantly differed from the overall acceptance scores of the commercial cheese cake (5.43 ± 1.00) and commercial layered cheese cake (5.26 ± 0.78).

This indicates that even without using cheese ingredients in the formulation of the rice cakes, the cheese flavour in both cakes is strong and can be significantly distinguished from the cheese flavour in the commercial cheese cakes.

Table 2. Sensory evaluation results of rice cakes (basic and layered) compared to commercial cheese cakes (basic and layered)

Attributes	Basic Rice Cake	Commercial Basic Cheese Cake	Layered Rice Cake	Commercial Layered Cheese Cake
Colour	5.66 ± 0.92 b	5.60 ± 0.93 b	6.50 ± 0.57 a	5.90 ± 0.95 b
Aroma	5.63 ± 1.03 ab	5.86 ± 1.13 a	5.96 ± 0.85 a	5.10 ± 1.15 b
Texture	5.66 ± 1.12 ab	5.26 ± 1.55 b	6.16 ± 0.83 a	5.46 ± 1.10 ab
Sweetness	5.66 ± 1.18 a	5.20 ± 1.37 a	5.83 ± 1.14 a	5.33 ± 0.88 a
Cheese Flavour	5.73 ± 1.04 ab	5.13 ± 0.97 bc	5.90 ± 0.88 a	5.06 ± 0.86 c
Overall Acceptance	5.83 ± 0.98 ab	5.43 ± 1.00 b	6.33 ± 0.66 a	5.26 ± 0.78 b

** Mean in the same row with the same letter are not significantly different (p >0.05)*

Proximate, Nutritional Composition and Microbiological Analysis

Analysis results shown in **Table 3** indicates that the proximate values and nutritional content of layered rice cake and rice cake are nearly similar to those of commercial layered cheese cake and commercial cheese cake. However, the ash content in layered rice cake and rice cake was found to be slightly higher (3.0 – 3.8%) compared to the ash content in commercial layered cheese cake (0.5%) and commercial cheese cake (1.2%). This higher ash content indicates a relatively elevated mineral content in both the rice cakes (basic and layered) compared to the commercial cheese cakes (basic and layered).

Table 3. Proximate, nutritional composition and microbiological analysis results of rice cakes (basic and layered) compared to commercial cheese cakes (basic and layered)

Attributes	Basic Rice Cake	Commercial Basic Cheese Cake	Layered Rice Cake	Commercial Layered Cheese Cake
Energy (kcal/100g)	418 ± 1.41	488.5 ± 3.53	460.5 ± 2.12	373 ± 38.18
Protein (g/100g)	6.2 ± 0.14	7.3 ± 0.28	4.75 ± 0.21	5.95 ± 0.21
Carbohydrate (g/100g)	45.4 ± 0.70	48.95 ± 0.35	46.95 ± 0.77	61.05 ± 1.62
Fat (g/100g)	18.35 ± 0.35	24.7 ± 0.42	21.35 ± 0.21	8.78 ± 0.59
Moisture (g/100g)	27.85 ± 0.35	17.55 ± 0.63	23.95 ± 0.21	23.65 ± 0.77
Ash (g/100g)	3.08 ± 0.02	1.22 ± 0.02	3.0 ± 0.14	0.595 ± 0.02
Total sugar (g/100g)	10.1 ± 0.00	9.8 ± 0.00	9.4 ± 0.14	9.65 ± 0.07
Sodium (mg/100g)	3.226 ± 0.17	4.223 ± 0.37	4.01 ± 0.39	1.25 ± 0.11
Dietary fibre (g/100g)	4.0	2.7	5.3	8.8
pH value	5.38 ± 0.02	5.36 ± 0.01	5.08 ± 0.02	5.48 ± 0.007
Total Plate Count (cfu/g)	NG (<10)	NG (<10)	NG (<10)	NG (<10)
Coliform Count (MPN/g)	NG (<3.0)	NG (<3.0)	NG (<3.0)	NG (<3.0)
Yeast and Mould (cfu/g)	NG (<10)	NG (<10)	NG (<10)	NG (<10)

* NG – *No Growth*

The human body requires 20 different amino acids, consisting of essential amino acids and non-essential amino acids, to grow and function properly. There are 9 essential amino acids. While the body can synthesize non-essential amino acids, it cannot produce essential amino acids. Therefore, essential amino acids must be obtained from the diet (Kathy & Jilian, 2023).

Analysis shows that basic rice cake contains 30.91 mg/100 g of essential amino acids, while layered rice cake contains a total of 29.69 mg/100 g. Furthermore, the analysis also indicates the presence of γ-aminobutyric acid

(GABA) in rice cakes, with respective values of 1.27 mg/100 g in basic rice cake, and 1.93 mg/100 g in layered rice cake (**Table 4**).

Both rice cakes are found to contain all 9 essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine) necessary for proper growth and functioning of the body. The amino acid content in these rice cakes is potentially contributed by raw materials based on rice, especially fermented rice 'tapai'.

Table 4. Total essential amino acids, non-essential amino acids, soluble amino acids and GABA content in rice cakes (basic and layered)

Samples	(mg/100 g sample)			
	Essential amino acids	Non-essential amino acids	Soluble amino acids	γ-aminobutyric acid (GABA)
Basic rice cake	30.91	43.44	74.34	1.27
Layered rice cake	29.69	51.82	81.50	1.93

Shelf-life Study

For both basic and layered rice cake, there is a slight decrease in moisture content after being stored for 3 months. The values of water activity (Aw) and pH for both cakes remain relatively unchanged. As for the colour attribute, there is a minor change in the L, a, b values for basic rice cake throughout the storage period, while there is no change in the L and b colour attributes for layered rice cake during the storage study. In terms of microbiological testing, there is no growth of *E. coli*, and no increase in yeast and mold in layered rice cake during the 4-month storage period. The Total Plate Count remains at 10^2 cfu/g in the 4-month of storage.

Sensory evaluation of both rice cakes shows that the minimum scores for all sensory attributes exceed the scale of 5.0 (slightly liked) during the 3-month storage. However, significant differences start to occur in the scores for color, aroma, texture, taste, and overall acceptance attributes in the samples that have been stored for 3 months. By the 4th month of storage, low minimum scores of 4.0 (neither liked nor disliked) are obtained for aroma and taste attributes. In the 2-3 month storage range, the sensory values for the cake start to decline but are still acceptable, and the shelf life of rice cake does not exceed 3 months.

CONCLUSION AND RECOMMENDATIONS

The study demonstrates the potential of rice-based ingredients, specifically rice flour and fermented rice 'tapai beras' in producing cheese analog flavored cakes. These cakes offer a unique cheese-like flavor and aroma profile, even in the absence of actual cheese. Sensory evaluations reveal that the cheese analog cakes, formulated with rice flour and tapai beras, achieved higher scores for cheese aroma, texture, sweetness, and overall acceptability compared to commercially available cheese cakes. The rice cakes also contain γ-aminobutyric acid (GABA), an organic compound that has potential health benefits. The successful development of these cakes opens up new avenues for bakery product diversification, promoting the use of local resources, and stimulating innovation in the food processing industry. The findings of this research are expected to contribute to enhancing the landscape of bakery product diversity and creating opportunities for local entrepreneurs to explore novel product formulations.

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The 17th ASEAN Food Conference 2023 (AFC2023)
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POTENTIAL EFFECTS OF SINGLE AND MIXED CULTURED JACKFRUIT BEVERAGES IN TERMS OF NUTRITIONAL VALUE AND ANTIMICROBIAL ACTIVITY AGAINST SELECTED FOODBORNE PATHOGENS

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Abstract: Jackfruit, a nutritious substantial tropical fruit has gained increasing interest among food specialist when more evidence of this fruit health benefits studies was reported. Two types of lactic acid bacteria, namely *L. plantarum* UALp-05 (LP) and *Streptococcus thermophilus* 0046 (ST) were selected to prepare cultured jackfruit beverage to evaluate the potential effects of single and mixed cultured jackfruit beverages as a new functional drinks. During three days of fermentation, there was a significant increase in total reducing sugar and total phenolic content in both single-(LP, ST) and mixed-cultured (LPST) jackfruit beverages. The increment of total reducing sugar was attributed to the microbial action of breaking down sucrose and converts it to glucose and fructose. The concentration of lactic acid in cultured jackfruit beverages also showed a significant increment after subjected to microbial fermentation. The high content of lactic acid and phenolic acid have contributed to the potent antimicrobial effect against selected foodborne pathogens such as *Escherichia coli O157:H7*, *Salmonella typhimurium, Staphylococcus aureus*, and *Listeria monocytogenes*. These findings indicated that the lactic acid bacteria (LAB) fermented jackfruit beverages can result in the increment of nutritional value, enhanced antimicrobial properties, and contains higher concentrations of total phenolic compounds such as gallic acid and protocatechuic acid.
Keywords: jackfruit beverage, lactic acid bacteria, fermentation, foodborne pathogens, antimicrobial effect

INTRODUCTION

Jackfruit has indeed gained increasing interest among food specialists and consumers due to its nutritional value and potential health benefits. It is low in calorie and fat, provides good source of vitamin (vitamin A, C, B), dietary fiber and minerals (magnesium, phosphorus, potassium and etc.) and primarily consumed fresh in tropical regions (Ranasinghe et al., 2019). As more evidences on the health benefits of jackfruit are reported, it is likely that the consumer market for both fresh and processed jackfruit products will continue to grow in the future.

Fermentation is a traditional and simple way to enhance substrate flavor and texture by breaking down complex food matrix in jackfruit and making it easier to digest. The fermentation process also increases the bioavailability of nutrients, making them more accessible for absorption. Some studies have suggested that certain fermented foods may have anti-microbial properties. Mohd danial et al (2020) reported potent antimicrobial effect of fermented papaya leaf against opportunistic skin pathogenic microbes due to the presence of multiple organic acids, produced by the microbial action. Incorporating a variety of fermented foods into your diet, along with a healthy eating pattern, may contribute to promote gut health and potentially reducing inflammation as reported by Sew et al. (2020) and Koh et al. (2021). In this study, two types of lactic acid bacteria namely *L. plantarum* UALp-05 (LP) and *Streptococcus thermophilus* 0046 (ST) were selected to produce fermented jackfruit beverage which potentially may produce bioactive compounds that have anti-microbial and enhance the nutritional value. The aim of this study is to evaluate the potential effect of single and mixed cultured jackfruit fermentation in terms of nutritional value and anti-microbial properties, particularly against selected foodborne pathogens such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella typhimurium* and *Escherichia coli O157:H7*.

MATERIALS AND METHODS

Preparation of cultured jackfruit drink

A 200 mL of 5% (w/v) jackfruit solution was prepared and inoculated with 10% LAB inoculum of either single culture (*L. plantarum* UALp-05 (LP) and *Streptococcus thermophilus* 0046 (ST) or mixed culture (LPST) with initial colony of 10^8 cfu/mL. The jackfruit solution was subjected to 3 days of fermentation with the sampling taken at the interval of 1 day. The fermentation process was end by pasteurize the jackfruit beverage at 90◦C for 30 min and kept in chilled condition for future analysis.

Total reducing sugar content

The dinitrosalicylic acid (DNS) method was employed to determine the reducing sugar content of jackfruit samples. Standard for calibration curve was prepared by adding 1 ml of DNS reagent to a series of diluted glucose as a standard solution (0 – 10 mg/mL). A suitable wavelength of 540 nm was selected to analyze the reducing-sugar concentration using a UV–Vis spectrophotometer.

Total phenolic content

Total phenolic content (TPC) of jackfruit samples was determined according to Mohd Ali et al. (2015) with minor modifications. A total of 1 mL of water extract (2 mg/mL) was mixed with 5 mL Folin–Ciocalteu reagent (1:1 with water) and incubated for 5 min at room temperature. Then, 4 mL of sodium carbonate solution (7.5%, w/v) was added and vortexed. The mixture was incubated in the dark for 2 h and the absorbance was read at 765 nm. The total phenolic content was expressed as gallic acid equivalents (mg GAE g/mL).

Quantification of targeted compounds using liquid chromatography technique

The sugar composition of cultured and non-cultured jackfruit solution was quantified using Rezex monosaccharide (Pb^{2+}) with dimension column of 300 x 7.8 mm and then subjected to constantly flow rate of distilled water at 0.5 mL/min under oven temperature of 85◦C. The analyses of organic acids of jackfruit samples was determined using Synergi 4 µm, Hydro-RP80A (250 × 4.6 mm) with the temperature controlled at 30°C. The mobile phase consists of mobile phase A (20 mM KH_2PO_4 with adjusted pH 2.9) and mobile phase B (water) with a flow rate of 0.6 mL/min. Gradient elution was performed as follows: from 0 to 30 min, 100% A; from 30 to 45 min, linear gradients from 100 to 0% A; from 45 to 55 min, linear gradient from 0 to 100% A. Peak identification was made by comparing retention times and UV spectra at 190 nm with authentic tartaric acid, lactic acid, citric acid, oxalic acid and malic acid.

The gallic acid and protocatechuic acid was analyzed using ultra performance liquid chromatography by running 1 µL sample solution through 1.7 µm x 2.1 x 150 mm Kinetex column. The gradient mobile phase consists of mobile phase A (3% acetic acid, v/v) and mobile phase B (Methanol) with a flow rate of 0.35 mL/min under oven temperature of 40°C. Gradient elution was performed as follows: from 0 to 0.6 min, 100% A; from 0.6 to 12 min, linear gradients from 100 to 40% A; from 12 to 16 min, linear gradient from 40 to 100% A and remain 2 min at 100%A. Peak identification was confirmed by comparing retention times and UV spectra at 270 nm with authentic gallic acid and protocatechuic acid and quantification was determined from the external calibration curve.

Determination of minimum bactericidal concentration (MBC)

Escherichia coli O157: H7 UPMEC32 (local isolate), *Salmonella typhimurium* ATCC®53648™ and *Listeria monocytogenes* ATCC®51772™ were cultured onto tryptone soy agar individually and incubated overnight to prepare stock culture solution according to Koh et al. (2019) procedure. The preparation of *Staphylococcus aureus* (ATCC 49775™) culture solution in Mueller Hinton (MH) broth was done following Mohd Danial et al. (2020). Both cultured and non-cultured jackfruit beverage (LP, ST & LPST) were used as an initial working concentration to determine its MBC. Two wells served as control which consists of culture without treatment and sterilized growth media. The MBC for each microbe was determined using broth microdilution technique as described in Koh et al. (2019).

RESULTS AND DISCUSSION

The introduction of LAB cultures in fermented food and beverage production is a common practice because of the positive impacts they have on flavor, texture, and even the nutritional value of the final product. The use of beneficial lactic acid bacteria (LAB) like *Lactobacillus plantarum* (UALp-05) and *Streptococcus thermophilus* (0046) as inoculum cultures in the production of cultured jackfruit beverage can lead to the transformation of its taste and aroma profile to a sweet sourness level as a consequence of the metabolic activities of these LAB. LAB are known for their ability to ferment sugars and produce lactic acid as a major metabolic product. The lactic acid content increased drastically within 1 day and reached the highest concentration range in between 10,300 and 11,200 ppm after day 3 of fermentation as observed in both single- (LP, ST) and mixed culture (LPST) jackfruit beverages (Table 1). Other organic acids also detected in culture jackfruit beverage including tartaric acid, citric acid, oxalic acid and malic acid.

Jackfruit initially contain abundant of sucrose with minor component of glucose and fructose. However, during lactic acid fermentation, enzymes like invertase was produced to hydrolyze sucrose into its constituent monosaccharides such as glucose, and fructose through microbial action, consequently resulting in the increment of total reducing sugar as summarized in Figure 1a &1b. These monosaccharides are reducing sugars because they have free aldehyde or ketone groups, allowing them to react with the reagents used in reducing sugar tests.

Indeed, the increase in the total phenolic content of cultured jackfruit beverages was contributed by the metabolic activities of the lactic acid bacteria (Figure 1c). Phenolic compounds are a group of secondary metabolites found in plants, and they often possess antimicrobial and antioxidant properties and contribute to various sensory attributes such as color, flavor, and aroma. The rising of total phenolic content in all cultured jackfruits beverage was attributed by the increment of gallic acid and protocatechuic acid after fermentation. The fermentation process, guided by the metabolic activities of LAB, can bring about changes in the composition of the beverage, resulting in the accumulation of beneficial compounds like gallic acid and protocatechuic acid in cultured jackfruit beverage after subjected to LAB fermentation (Figure 1d). These compounds are naturally occurring in plants, offer a range of potential health advantages, including antioxidant effects, anti-inflammatory properties, and possible contributions to cardiovascular and metabolic health.

As shown in Figure 2, it was discovered that single- (LP & ST) and mixed cultured (LPST) jackfruit beverages had varying degrees of antibacterial activity against *Escherichia coli O157:H7*, *Salmonella typhimurium*, *Listeria monocytogenes* and *Staphyococcus aureus*. However, non-cultured jackfruit beverages were completely devoid of anti-microbial properties (data not

shown). The phenolic compounds and organic acids that were created during microbial fermentation contributed to the antibacterial capabilities of cultured jackfruit drinks. In addition to having the potential to damage microbial cell membranes and obstruct microbial development and replication, gallic acid and protocatechuic acid were reported exhibited antioxidant and antibacterial activity against a variety of pathogenic microorganisms (Lobiuc et al., 2023; Kakkar and Bais, 2014). It is crucial to noted that depending on variables like concentration, the precise microorganisms targeted, and the environment in which they are employed, their effectiveness can be changed. The abundance of lactic acid and citric acid in cultured jackfruit drinks also helps to generate an acidic environment that is unfriendly to many germs, extending the shelf life of food and avoiding bacterial contamination. It is important to mention that these compounds' antibacterial abilities might not be as strong as those of particular commercial antimicrobial drugs. This is why diluting cultured jackfruit drinks made it less antibacterial effective. These compounds are frequently used in conjunction with other preservation techniques to increase the overall safety and shelf life of the product. In natural foods and beverages, the antimicrobial effects of phenolic compounds and organic acids can contribute to the preservation of the product and the inhibition of potentially harmful microorganisms.

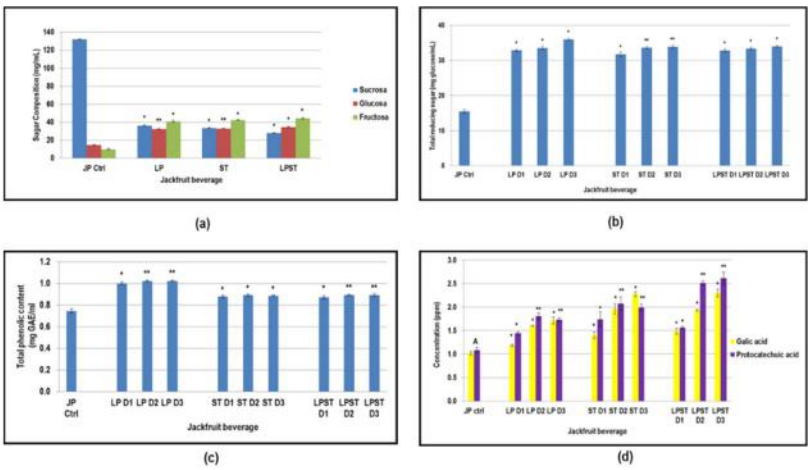

Figure 1. Comparison of non-cultured and cultured jackfruit beverages: a) sugar composition after 3 days fermentation; b) total reducing sugar; c) total phenolic content; d) gallic acid & protocatechuic acid content. Abbreviations: JP CTRL: non-cultured jackfruit beverage; LP: *L. plantarum* UALp-05 (LP) jackfruit beverage; ST: *Streptococcus thermophilus 0046* (ST) jackfruit beverage; LPST: mixed culture LP & ST jackfruit beverage. Significant differences between cultured and non-cultured jackfruit beverages are marked with * (p<0.05). No significant differences between different fermentation day are marked with ** (p>0.05).

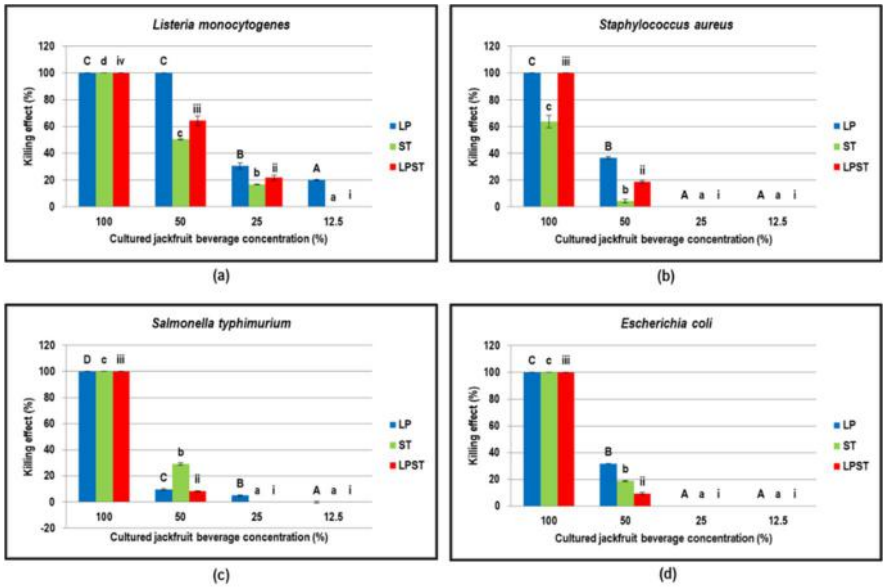

Figure 2. The killing effect of non-cultured and cultured jackfruit beverages against selected pathogenic microorganisms: a) *L. monocytogenes*; b) *S. aureus*; c) *S. typhimurium*; d) *E. coli* 0157:H7. Abbreviations: JP CTRL: non-cultured jackfruit beverage; LP: *L. plantarum UALp-05* (LP) jackfruit beverage; ST: *Streptococcus thermophilus 0046* (ST) jackfruit beverage; LPST: mixed culture LP & ST jackfruit beverage. Bars of the same colour with different notations are significantly different between samples (p<0.05).

Table 1. Comparison of organic acid profile between cultured and non-cultured jackfruit beverages after 3 days fermentation

	Concentration (ppm)				
Samples	**Tartaric acid**	**Lactic acid**	**Citric acid**	**Oxalic acid**	**Malic acid**
JP CTRL	223.98 ± 15.41[a]	336.72 ±16.78[a]	824.58 ± 28.01[d]	24.18 ± 1.83[b]	71.76 ± 3.83[a]
LP	513.60 ± 13.82[c]	10894.41 ± 741.21[b]	460.79 ± 28.32[c]	27.85 ± 1.29[c]	408.98 ± 4.00[b]
ST	464.86 ± 6.52[b]	10329.55 ± 315.97[b]	412.30 ± 17.30[b]	18.85 ± 0.81[a]	470.20 ± 6.31[b]
LPST	455.06 ± 5.51[b]	11159.92 ± 1055.25[b]	362.94 ± 13.28[a]	16.77 ± 0.33[a]	423.73 ± 3.67[c]

[a]Each value in the Table represents the mean ± standard deviation from triplicate analyses. Mean values with different superscripts in the same column are significantly different at $p<0.05$. Abbreviations: JP CTRL: non-cultured jackfruit beverage; LP: *L. plantarum* UALp-05 (LP) jackfruit beverage; ST: *Streptococcus thermophilus* 0*046* (ST) jackfruit beverage; LPST: mixed culture LP & ST jackfruit beverage

CONCLUSION

Utilization of LAB in fermentation not only transform the sensory qualities of the jackfruit beverage but also enhance its nutritional value and inhibit the growth of harmful microorganisms by increasing the presence of health-promoting compounds such as phenolic compounds and organic acids, which can enhance the nutritional value of the beverage and extending the shelf life of jackfruit beverage. By consuming cultured jackfruit beverage, it can contribute to the overall health benefits and support gut health by maintaining a healthy balance of gut flora, which can improve digestion, boost the immune system, and enhance nutrient absorption. A balanced gut microbiome is associated with overall better digestive health and can have positive effects on overall well-being. Indeed, fermentation can influence the sensory qualities of the beverage and transform the product to more appealing to consumers, making it a valuable addition to the diet.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
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OPTIMIZATION OF MATRIX EFFECT ON THE DEVELOPMENT OF IMMUNOASSAY FOR FUMONISIN B1 IN CORN GRAINS

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Abstract: Fumonisin is a mycotoxin that has been produced by Fusarium fungus and is generally contaminated in corn, corn grains and corn-based products. The contamination of fumonisin has impaired food safety and security due to its toxicity effects. Therefore, a simple immunoassay was developed for fumonisin detection based on the direct competitive immunoassay format. The decreased colour signal was interpreted as an increase in fumonisin concentration. However, the matrix effect affected the antibody-toxin interaction. This paper presents an optimization study of the corn matrix effect in developing a calibration curve of the immunoassay. The result showed that the activity signal was increased by 39% due to the corn-matrix effect and a 1:20 dilution ratio was the optimum dilution to discard the matrix effect on the detection assay. The calibration curve in the corn matrix was successfully plotted with the goodness of fit (R^2 = 0.9798) in a ranging concentration of 0 to 2500 ppb. The limit of detection (LOD) and quantitation (LOQ) were calculated to be 690 ppb and 2091 ppb respectively. This assay could be established as a 96-well immunoassay screening for large-quantity samples in a one-time test.
***Keywords*:** Mycotoxin, fumonisin B1, antibodies, diagnostic, corn grains

INTRODUCTION

The contamination of mycotoxins can occur in all stages of the food, feed and agricultural produce chain. This is a global issue of toxic contaminants that can be impacted by international trade. Mycotoxins are by-products of secondary metabolites that are naturally produced by specific fungi such as genera of Fusarium, Aspergillus, Penicillium, Altemaria and Claviceps (Agriopoulou et al. 2020a). Aflatoxin, fumonisin, zearalenone, ochratoxin and others are among the major mycotoxins produced by toxigenic fungi that are frequently observed in food and feed due to the weather, climate and poor production practices during plantation, postharvest or storage. The contamination of mycotoxins should be monitored extensively to ensure the safety and security of foods and feeds. Fumonisin is the highest mycotoxin produced by the fungus *Fusarium verticilloides* which is preferable to high content in corn crops (Garcia-Diaz et al. 2020). Fuminisin B1 also has been classified as group 2B of human carcinogens by the International Agency for Research on Cancer (IARC). Any contamination of fumonisin B1 in corn grains will affect the quality and safety of products. It will affect the food chains in terms of food sustainability and security in future. The presence of FB1 in grain corn not only constitutes a threat to animal and human health, but it may cause serious economic impact losses to farmers as well as to the livestock industry (Hejri et al 2013). Analytical instrumental-based analysis such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), liquid chromatography-tandem mass spectrometry (LC-MS/MS) and gas chromatography-tandem mass spectrometry (GC-MS/MS) is standard procedure following AOAC and CODEX Alimentarius guidelines (Manzanares et al 2014, Park et al. 2018). However, all these analytical approaches require high costs to manage large-scale samples due to the reagent used and maintenance. Therefore, the detection of mycotoxins in food and feed by using rapid tools is highly needed. In this study, an immuno-diagnostic kit for fumonisin detection in a buffer system was successfully developed as the option of analytical approach that can be used for large sample tests, affordable and low cost. However, the detection of fumonisin in the corn grains matrix should be optimized in the complex medium. Therefore, this study was conducted to determine the matrix effect and develop a matrix-matched calibration curve for the enhancement of high-sensitivity detection.

MATERIALS AND METHODS

Sample extraction

500 g corn grains were sterilized by soaking in 30% (v/v) sodium hypochlorite for 4 hours and rinsed with distilled water. The sample was autoclaved at 121ºC for 15 minutes, room-dried for 24 hours and homogenously ground. Then, 3g of ground sample was used for the matrix effect and calibration curve development studies respectively.

Direct competitive enzyme-linked immunosorbent assay (ELISA) for Fumonisin B1 determination

100 µL of Anti-FB1 antibodies (0.1 mg mL^{-1}) was coated onto 96-well microplate and incubated overnight at 4ºC. The microplate was three times washed with 0.01M phosphate buffer saline (Tween-20) (PBST) and blocked with 250 µL of 0.05% (w/v) non-fat dried milk for 1 hour at 37ºC. After the incubation, the microplate was washed again and added with 50 µL sample or standard solution, followed by 50 µL FB1-conjugated horse radish peroxidase (HRP) for 2 hours incubation at 37ºC. After the incubation, the microplate was washed and 100 µL of TMB substrate was added for the 15 mins of incubation. The activity was determined at an absorbance of 370 nm by using a microplate spectrophotometer (Thermo Fisher Multiskan).

Matrix effect analysis

3 g of ground sample was added with acetonitrile 80:20 (v/v) and vortexed for 5 minutes, followed by centrifuging at 3000 rpm for 10 minutes. The pellet was discarded and the supernatant was collected for serial dilution ratios (1:1, 1:10, 1:20) with 0.01 M phosphate buffer saline (PBS). The sample was proceeded for ELISA as described. The matrix effect (ME) was calculated as ME (%) = absorbance sample $_{A370nm}$/ absorbance control $_{A370nm}$ x 100%.

Development of standard calibration curve in corn grain matrix

The corn grains extract was spiked with standard FB1 in the concentration range of 0 ppb to 2500 ppb. These standard solutions were then used in the direct competitive ELISA assay for the calibration curve development. The standard calibration graph was plotted between absorbance activity (%) and FB1 concentration.

RESULTS AND DISCUSSION

The direct competitive ELISA was developed in a buffer system based on the competitive binding between the target FB1 with FB1-conjugated HRP enzymes towards the coated antibodies that specifically recognized FB1. The decreased activity of absorbance assay was inversely proportional to the increase of FB1. However, the determination of FB1 from contaminated corn grains has increased the interference in the interaction between analyte and bioreceptor antibodies, induced the non-specific binding and decreased the sensitivity of detection. Therefore, the matrix effect analysis was primarily determined to estimate the appropriate sample matrix for high-sensitivity detection of the target analyte. The matrix effect analysis was determined at a negative control level through the analysis of sample dilution ratios. The crude extract was diluted in a few ratios (1:1, 1:10 and 1:20). As shown in Figure 1, the absorbance activity of ELISA for negative control (buffer) was measured at absorbance 2.62 au, meanwhile, the activity of the crude sample (undiluted sample) had increased the absorbance up to 3.65 au with the increase of 39% of matrix effect (Table 1). This result could give a false negative interpretation. However, the matrix effect was gradually decreased as the dilution ratio increased up to 1:20 and optimally equal to the control level. However, further dilution of the sample may result in a quantitative loss of molecules of interest (Adil & Shamsi 2023). Therefore, a 1:20 dilution ratio was the optimum dilution to discard the matrix effect on the detection assay. The development of a standard calibration curve in the sample matrix is important because a buffer-based calibration model usually does not relate to a real sample that generally has a complex matrix. In this study, the calibration curve in the corn matrix was successfully plotted with the goodness of fit (R^2 = 0.9798) in a ranging concentration of 0 to 2500 ppb (Figure 2). The calibration model was applied to 1:20 dilution ratios of the sample matrix. The response of the concentrations to the activity signal was accomplished in the reduced complexity of the corn medium. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated to be 690 ppb and 2091 ppb respectively, which accommodate for the assessment of FB1 in corn samples that averagely reported in a range of 1.5 ppm to 2.6 ppm due to 30 – 70% contamination during post-harvest storage (Zentai et al 2019).

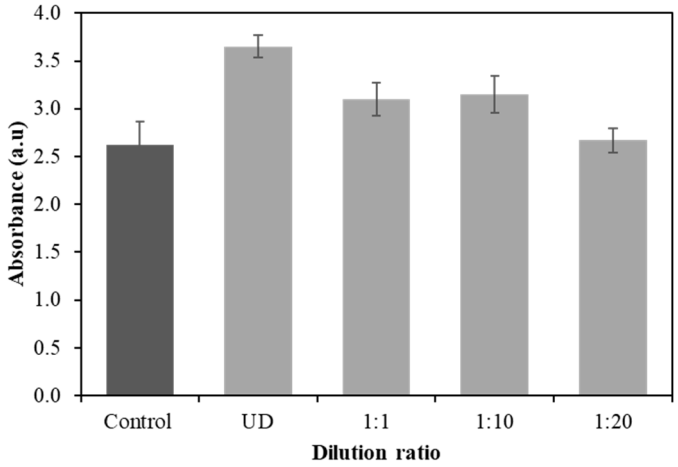

Figure 1. The effect of corn matrix in immunoassay of Fumonisin B1 detection; UD (undiluted)

Table 1. The percentage of matrix effect on immunoassay based on the dilution ratio

Dilution ratio	Absorbance $_{A370\ nm}$ (a.u)	Activity (%)	Matrix effect (%)
Control	2.62	100	nil
UD	3.65	139.16	39
1:1	3.10	118.23	18
1:10	3.15	120.00	20
1:20	2.67	101.72	2

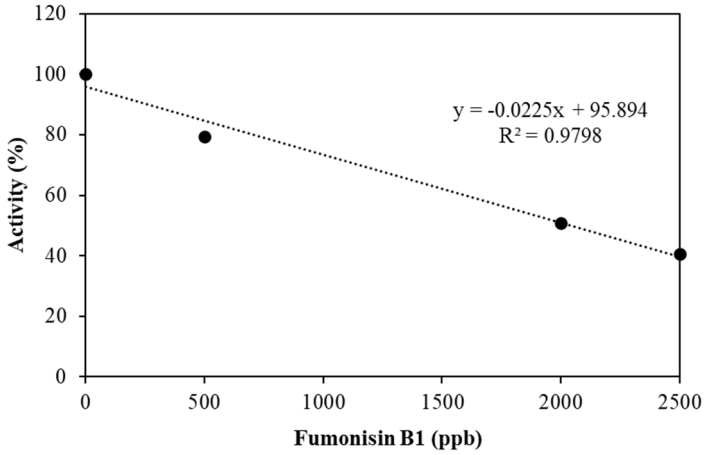

Figure 2. A standard calibration curve of direct competitive immunoassay for the detection of Fumonisin B1

CONCLUSIONS

The sample matrix is one of the parameters that will affect the sensitivity of immunoassay. The matrix effect of corn grains has been evaluated and the dilution ratio of 1:20 is recommended to discard the high matrix interference. This result significantly improved the sensitivity of detection with the established calibration curve in corn grain medium. Therefore, the contamination of fumonisin in corn grains can be rapidly detected by using an immunoassay technology platform with less maintenance and low cost. This also provides a simple tool for accessing food and feed safety due to mycotoxins issues.

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The 17th ASEAN Food Conference 2023 (AFC2023)
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MODIFICATION OF IMMUNOSENSOR-SURFACE ELECTRODE FOR HIGH-REACTIVITY DETECTION OF MARINE BIOTOXIN IN SHELLFISH

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Abstract: Contamination of marine biotoxin in shellfish due to harmful algal blooms has high impacts on seafood biosecurity and human health. Therefore, an electrochemical biosensor based on the specific antibodies against marine biotoxin-saxitoxin has been developed for simple and rapid detection in shellfish. A modification of the sensor-surface electrode was constructed based on the component of high conductivity polypyrrole (PPy) polymer, gold nanoparticles (Au) and immobilized saxitoxin-specific antibodies (STX-Ab) as biorecognition elements. The Ppy/Au/STX-Ab-modified electrode was electrochemically characterized based on the differential pulse voltammetry (DPV) by using the AutoLab system. The peak potential of the sensor strip was obtained at -40 mV with a peak current of 5.36 µA. A standard calibration curve was developed in the concentration range of 0 to 20 ppb (R^2=0.967) and achieved a limit of detection (LOD) and quantitation (LOQ) of 5.55 ppb and 16.81 ppb respectively. The change of DPV current signal was inversely proportional to the saxitoxin level and supported the antibody-toxin binding interaction that hindered the electron transfer to the sensor surface for the determination of biotoxin.
Keywords: Mycotoxin, fumonisin B1, antibodies, diagnostic, corn grains

INTRODUCTION

The high density of microalgal species in the freshwater or marine environment has contributed to the harmful algal blooms (HABs) phenomenon (Lau et al. 2017; Lim et al. 2012). Eutrophication of the coastal water, urbanization and commercial agricultural activities led to the nutrient enrichment in the freshwater and marine coast that subsequently promoted alga blooms. The massive algae can cause fish kills due to hypoxia in the surrounding environment and the excretion of bioactive compounds such as biotoxin in filter-feeding shellfish that give a high risk of human food poisoning. The occurrence of HABs with identified phytoplankton species was reported in Sabah, Malacca, Johor and Pahang (Adam et al. 2011; Din et al. 2022; Hamzah et al. 2019; Razali et al. 2015; Suleiman et al. 2017). Paralytic shellfish poisoning (PSP) describes a type of seafood poisoning due to contamination of marine biotoxin produced by several species of dinoflagellate during HABs (Suleiman et al. 2017). Saxitoxin (STX) is a harmful marine biotoxin and is classified as a neurotoxin (Nordin et al. 2022). As reported in the year 2001, six persons were hospitalized and one casualty due to the severe intoxication caused by *Allexandrium minutum* (Lim et al. 2004). Meanwhile, six episodes involving 58 cases of paralytic shellfish poisoning (PSP) or saxitoxin (STX) poisoning were reported in Kota Kinabalu, Sabah in 2013 and this resulted in four deaths intoxicated from eating contaminated shellfish (Suleiman et al. 2017). This incident has alarmed relevant authorities regarding the importance of HABs monitoring. The HABs' monitoring, prevention and mitigation strategies need to be strengthened for food safety and public health. Due to this problem, rapid, simple and sensitive devices are needed for real-time and in-situ analysis of saxitoxin detection. Currently, biosensor devices can offer a very attractive alternative technology for contaminant detection since they can be rapid, sensitive and simple to perform as well as can provide real-time and on-site analysis (Cossettini et al. 2022). Therefore, this paper presents research work on the development of an electrochemical biosensor for the detection of saxitoxin, that can be applied as portable devices for HABs monitoring activity.

MATERIALS AND METHODS

Sensor-surface modification

The carbon screen-printed electrode was used for the sensor-surface modification analysis by electro-deposition with polypyrrole (ppy) layer, ppy with nanogold (ppy/Au), two different immobilization steps (ppy/Au-STX Ab and ppy/Au/STX Ab) and two-step immobilization without polypyrrole (Au/STX Ab and Au-STX Ab). The current responses of the modifications were measured using differential pulse voltammetry (DPV) in 5 mM

ferricyanide/ferrocyanide (redox solution) in 0.1M KCl. The scan rate was applied at 2 mV/s and the potential range was set up in a range of -250 mV to +250 mV.

Electrochemical measurement for the standard calibration curve

Autolab PGSTAT 20 potentiostat (Eco Chemie, Netherlands) was used for the sensor analysis. A standard calibration plot in the buffer system was carried out by using an optimized modified electrode (ppy/Au/STX Ab). The modified SPCE (ppy/Au) was incubated for 1 hour at room temperature with STX Ab. Then, unbound Ab was washed with 0.01M PBS, rinsed with deionized water and left dried at room temperature. The remained active site of AuNP was blocked with 0.5 % BSA to prevent unspecified binding when standard/sample applied. After that, Saxitoxin standards at different concentrations (0 ppb – 20 ppb) in the buffer system and (0 ppb -12.5 ppb) in the matrix system were incubated on the SPCE for 1 hour and unbound antigen was washed out with PBS and rinsed with distilled water. The Electrochemical measurements for different concentrations of STX standard were performed in a redox solution of $K_3Fe(CN)_6/K_4Fe(CN)_6$. The electrodes were analyzed using Autolab using NOVA 1.11 with differential pulse voltammetry (DPV) measurement with potential range -0.25 to 0.25 V and scan rate at 2 mV/s

RESULTS AND DISCUSSION

Figure 1(a) shows the current changes in the determination of set potential (mV) with multiple sensor-surface modifications with polypyrrole (ppy) layer, ppy with nano gold (ppy/Au), two different immobilization steps (ppy/Au-STX Ab and ppy/Au/STX Ab) and two-step immobilization without polypyrrole (Au/STX Ab and Au-STX Ab). The peak potential of the bare electrode was obtained at 38 mV and left-shifted to 4 mV when electro-deposited with a polypyrrole layer. The immobilization of ppy-nano gold slightly shifted the peak potential to 2 mV and increased the current up to 35 µA. Gold nanoparticles (Au) are biocompatibility and has high conductivity to transfer electron rapidly between electrolytic solution and transducer. This enhances the amplification of the current signal. (Faridah et al. 2017; Vu et al. 2021). The use of AuNPs in electrochemical biosensor development is established in many studies for toxin and bacterial detection (Cossettini et al. 2022; Habibi et al. 2021). The addition of antibodies on the ppy/Au sensor surface changed the peak potential to -40 mV and reduced the signal background.

Meanwhile, the comparison of peak currents due to sensor surface modification is presented in Figure 1(b). The immobilization steps with polypyrrole polymer, gold and antibodies (ppy/Au/STX-Ab) showed a higher current signal (5.36 µA) as compared to 3.61 µA of ppy/Au-STX-Ab that immobilized with gold conjugated antibodies. However, the antibody immobilization without polypyrrole showed an average current of 3.3 µA and 3.8 µA for the Au/Ab and Au-Ab respectively. Polypyrrole is among the most-used organic polymers that have superior electrical conductivity and redox properties (Divakaran, Kale & Wu 2022). Therefore, the combination of staged immobilization of ppy/Au/STX-Ab was chosen as the optimum modified sensor electrode for the electrochemical biosensor development.

A standard calibration graph in a buffer system was developed using the modified sensor electrodes for the STX concentration in a range of 0 to 20 ppb (Figure 2). The differential pulse voltammetry (DPV) was measured at a set potential of -0.25 V to +0.25 V. The peak currents were obtained in a range of 2 to 7 µA (Figure 2a). The calibration plot shows an inverse proportion between the current signal and the STX concentration. The current signal was gradually decreased with the increase of STX concentration. This describes the binding interaction of antibodies with target analytes on the sensor surface had affected the electron transfer of redox response. The linear correlation coefficient of the curve was 0.967 (Figure 2b). The limit of detection (LOD) and quantification (LOQ) were calculated to be 5.55 ppb and 16.81 ppb respectively.

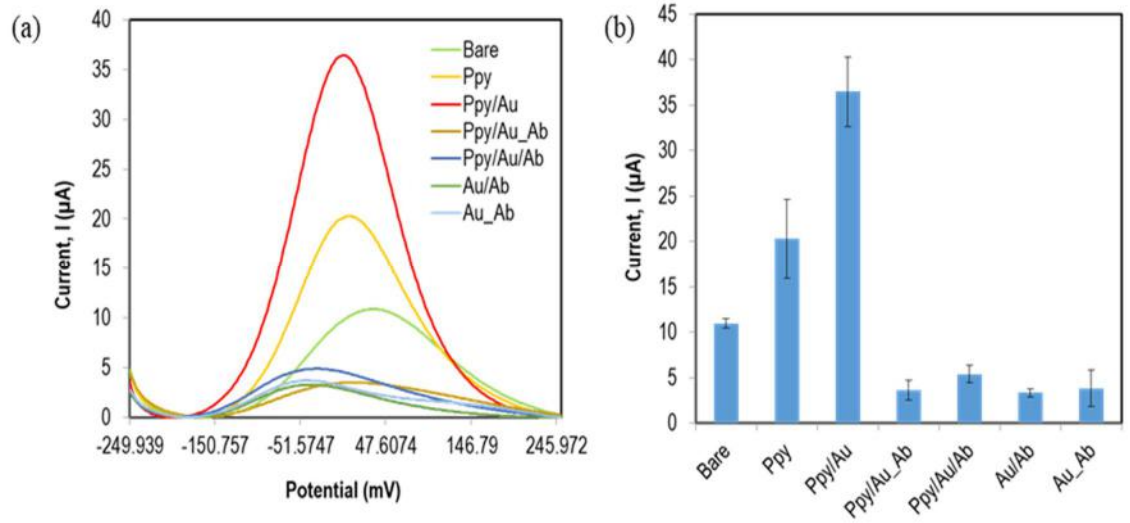

Figure 1. Sensor-surface modification using differential pulse voltammetry (DPV) analysis with a potential range of -250 mV to +250 mV

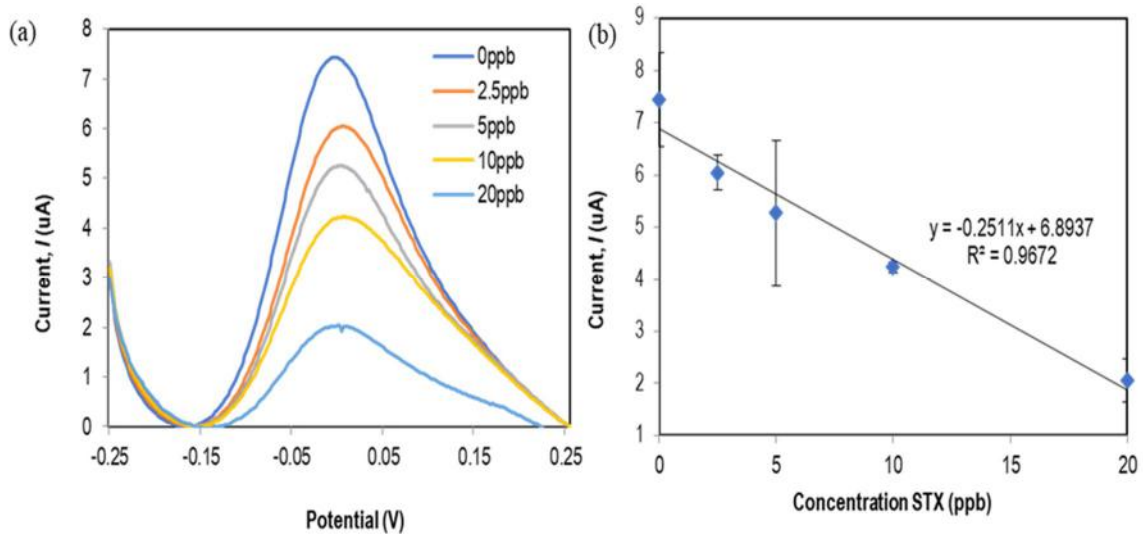

Figure 2. Calibration plot of saxitoxin in buffer system using differential pulse voltammetry (DPV) analysis

CONCLUSIONS

A good response in sensor-surface modification using DPV analysis shows the potential of free-label electrochemical immunosensors for biotoxin detection of harmful algae blooms. The combination of polypyrrole, nano gold and saxitoxin-specific antibodies was optimized as bioactive recognition elements of the surface-modified sensor for the high reactivity towards saxitoxin. The calibration curve in the buffer system was successfully developed and will be expanded in the shellfish matrix system for the HABs IoT biosensor development.

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EFFECT OF DIFFERENT CARRIER-AGENT COMBINATIONS ON THE MOISTURE CONTENT AND WATER ACTIVITY OF SPRAY-DRIED PHILIPPINE WILD RASPBERRY (*Rubus rosifolius* LINN.)

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Abstract: It has become an interest to efficiently valorize seasonal Philippine wild raspberry or '*Sapinit*' (*Rubus rosifolius* Linn.) to produce a shelf-stable product. In this study, the effect of different carrier-agent combinations of gum arabic (GA), maltodextrin (MD), and soy protein isolate (SPI) on moisture content (MC) and water activity (Aw) of spray dried *sapinit* was evaluated. The MC and Aw of resulting powders produced from ten (10) combinations of varying 0-25% GA-MD-SPI concentrations were analyzed. Carrier-agent combinations significantly ($p<0.05$) affected the MC and Aw of *sapinit* powder. Results showed that MC ranged from 5.17 to 7.27% while Aw ranged from 0.18 to 0.38, which were within the usual values for food powders. Powders made from combinations with greater than 12.50% GA or MD concentrations had lower mean MC and mean Aw compared to other samples. *Sapinit* powder using 12.50% GA, 12.50% MD, and 0% SPI had the lowest mean MC (5.17%) and lowest mean Aw (0.18). The results of this study showed that GA, MD, SPI, or their combinations can be used to produce spray-dried *sapinit* powder with low MC and low Aw, indicating a shelf-stable product.
***Keywords*:** Spray drying, *Sapinit*, food powder, moisture content, water activity

INTRODUCTION

Philippine wild raspberry, locally known as *Sapinit* (*Rubus rosifolius* Linn.), is a perennial shrub that typically grows on Mt. Banahaw in Lucban, Quezon (Negosentro, 2012). It was recently discovered in 2009, and studies revealed that *sapinit* contains a promising variety of phytochemicals, including good quantities of phenolics and flavonoids, and possesses antioxidant and antibacterial species (QAES, 2011; Recuenco et al., 2020; Alvarez et al., 2013). However, the *sapinit* fruit only lasts for three to four days if stored at room temperature. As a seasonal fruit, its availability is limited and dependent on production volume within a particular cropping season. Consequently, there is a need to convert *sapinit* into shelf-stable products.

One of the promising technologies that can be utilized to make *sapinit* available throughout the whole year is microencapsulation by spray drying. It converts liquid material into powder continuously in just one single step (Moreira et al., 2009; Ahmed et al., 2010). During the process, the feed is atomized into the drying chamber, where the resulting spray mixes with hot gas and turns into dried particles (Caparino et al., 2012). Stickiness due to the high amounts of soluble solids in fruit juices causes operational problems during spray drying (Obon et al., 2009; Tan et al., 2011). To address this, carrier agents such as GA, MD, SPI are used to prevent the deposition of powder onto the drying chamber in order to improve the drying process and increase product yield (Tan et al., 2011; Souza et al., 2015). These carrier agents have been widely used due to their low viscosity, high solubility, good film-forming properties, subtle taste, and high oxidative stability (Shishir and Chen, 2017; Phisut, 2012). However, the high cost and limited availability of GA and the low emulsifying capacity of MD limit the utilization of these carrier agents (Lee et al., 2018). Hence, the need to use combinations of these carrier agents.

There is, so far, few literatures on the efficient valorization of this endemic variety of raspberry to produce a more shelf-stable product. Information on the evaluation of different carrier-agent combinations in spray drying to produce a more stable form of *sapinit* powder with desirable physicochemical properties adds to scientific knowledge and will greatly help in increasing *sapinit* production and marketability. The aim of this study was to evaluate the effect of different carrier-agent combinations of gum arabic, maltodextrin, and soy protein isolate on the moisture content and water activity of spray-dried *sapinit*.

MATERIALS AND METHODS

Collection and preparation of raw materials

Sapinit fruit samples with the same maturity were handpicked randomly at Bangkong Kahoy Valley in Mt. Banahaw, Quezon Province. It was washed with potable water and subjected to primary processing. Primary processing included pulping and freeze drying to ensure its availability throughout all experiments. Freeze drying was carried out at a heater temperature of 25–30 °C, a chilling temperature of -30°C, and an operating vacuum pressure of 100–300 Pa for 40 hours (Zubia et al., 2023). Freeze-dried *sapinit* pulp was then stored in foil laminates with oxygen absorbers and placed in a relative humidity (RH)-controlled chamber prior to subsequent use.

Preparation of feed mixture and spray drying

Spray drying was employed using the methods of Bhusari et al. (2014) and Flores et al. (2014), with modifications. Aqueous solutions (25% w/v, 3.5 L) of the different combinations of gum arabic, maltodextrin DE 11.20, and soy protein isolate were prepared using distilled water at room temperature according to the carrier agent ratio generated from simplex lattice mixture design using Design-Expert Version 13.0 software (Table 1).

Table 1. Carrier agent combinations and actual weight used in spray drying.

Sample	Gum Arabic (GA)		Maltodextrin (MD)		Soy Protein Isolate (SPI)	
	Concentration, %	Weight, g	Concentration, %	Weight, g	Concentration, %	Weight, g
A	0.00	0.00	12.50	437.50	12.50	437.50
B	12.50	437.50	12.50	437.50	0.00	0.00
C	12.50	437.50	0.00	0.00	12.50	437.50
D	8.33	291.55	8.33	291.55	8.33	291.55
E	4.17	145.95	4.17	145.95	16.67	583.45
F	25.00	875.00	0.00	0.00	0.00	0.00
G	4.17	145.95	16.67	583.45	4.17	145.95
H	0.00	0.00	0.00	0.00	25.00	875.00
I	16.67	583.45	4.17	145.95	4.17	145.95
J	0.00	0.00	25.00	875.00	0.00	0.00

Rehydration of the freeze-dried *sapinit* was done by adding freeze-dried *sapinit* pulp into distilled water until 10° Brix, which is the same brix content as the fresh *sapini*t puree, was achieved to adhere to the minimum 8° Brix level of reconstituted puree for red raspberry (CODEX STAN 247-2005). Approximately 3.3 liters of aqueous solutions of each carrier agent combination were mixed into the 2.7-L rehydrated freeze-dried *sapinit* pulp to obtain the 6-L feed mixture. Each feed mixture was mixed well and filtered using a 60-mesh filter. These filtered feed mixtures were then pumped at 20 mL/min speed via a peristaltic pump to the atomizer of a spray dryer at 150°C inlet air temperature and 80°C outlet air temperature, following the parameters of Flores et al. (2014). The temperature of the feed mixture was maintained at 25 ± 2°C. The produced powders, packed in foil laminates containing oxygen absorbers, were stored in a refrigerator until subsequent analyses.

Determination of moisture content and water activity

The moisture content and water activity of the spray-dried *sapinit* powder samples were determined using the services of Food Processing Division-Industrial Technology Development Institute of the Department of Science and Technology. The analyses were carried out in triplicates using a vacuum drying oven (specs: VD-23, Binder GmbH, Germany) and an Aw meter (specs: AWC503-C, Novasina, Switzerland). The results of both analyses were statistically analyzed using variance analysis (p-value < 0.05). If the mean results showed a significant minimum difference, Tukey's honest significance difference (HSD) test was applied at a probability level of 5% (p-value < 0.05) using R-4.0.4 statistical software.

RESULTS AND DISCUSSION

The moisture content and water activity of powder samples affect their stability, storage properties, and other technical properties. Moisture content is a measurement of the total water contained in a food product, usually expressed as a percentage by weight on a wet or dry basis (Zambrano et al., 2019). It is an important powder property that is related to drying efficiency, powder texture, flowability, stickiness, and storage stability due to its effect on glass transition and crystallization behavior (Shrestha et al., 2007). On the other hand, water activity is defined as the vapor pressure of water in a sample divided by the vapor pressure of pure water at the sample temperature (Nielsen et al., 2012). It measures the availability of free water in a food system that is responsible for biochemical reactions during storage. Moisture content and water activity together provide valuable information about microbial spoilage, chemical stability, and physical stability of the spray-dried sapinit powder. The mean moisture content (wet basis) and mean water activity of spray-dried sapinit powders using different carrier agent combinations ranged from 5.17 to 7.27%

and 0.18 to 0.38, respectively. Among the produced powders, samples (B, F, G, and J) with high GA and/or MD concentrations showed lower moisture contents and water activities compared to the samples with high SPI concentrations or SPI alone (Figure 1).

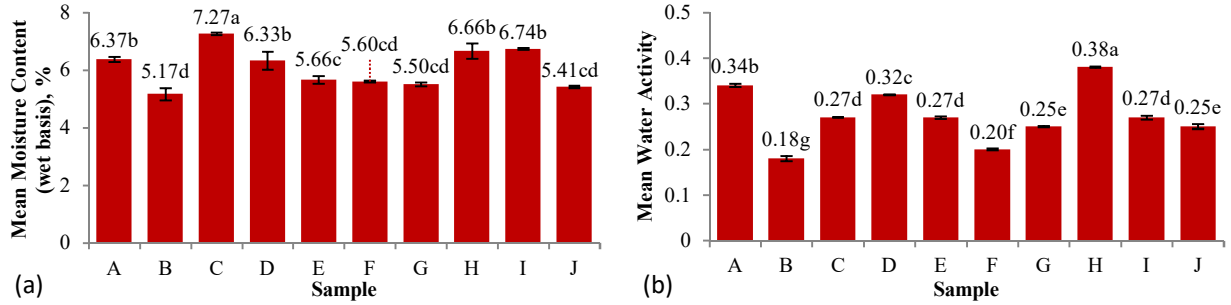

Figure 1. (a) Mean (n = 3) % moisture content (wet basis) and (b) mean (n = 3) water activity of sapinit powders from spray drying using different carrier agent combinations.

Where sample A – 0:12.50:12.50, B – 12.50:12.50:0, C – 12.50:0:12.50, D – 8.33:8.33:8.33, E – 4.17:4.17:16.67, F – 25:0:0, G – 4.17:16.67:4.17, H – 0:0:25, I – 16.67:4.17:4.17, J – 0:25:0 of gum arabic: maltodextrin: soy protein isolate (%) combination used in spray drying. Error bars represent the ± standard deviation for each sample. Means with same lowercase letter are not significantly different at $p \leq 0.05$ using Tukey's HSD test.

Food products generally formed by a method of rapid heat exchange, such as spray drying, are not at thermodynamic equilibrium. Depending on the initial moisture content of the feed material prior to spray drying and the molecular weight of the carrier agent used, they will undergo a shift from a glassy crystal to a rubbery state (Lee et al., 2018). In this study, the high molecular weight of GA and MD helped to increase the glass transition temperature of the feed material, which contributed to lower moisture content and water activity, better powder stability, and reduced stickiness problems. This was evident in the *sapinit* powder produced using 12.50% GA, 12.50% MD, and 0% SPI, which had the lowest mean MC (5.17%) and lowest mean Aw (0.18).

Moisture content generally decreases with high GA concentrations, high MD concentrations, and extremely low and high SPI concentrations. In addition, samples B and F showed the least water activity compared to other samples (Figure 1). Having moderate to high GA and MD concentrations as well as very low SPI concentrations will most likely result in lower water activity. This was in contrast to the findings of Bazaria and Kumar (2016), wherein treatments containing GA as a carrier agent showed higher water activity and moisture content owing to the higher water retention capacity of hydrocolloids compared to starch derivatives and protein isolates. However, samples A and H obtained using the MD:SPI combination or SPI alone showed an increase in water activity, which can be attributed to the high water retention capability of soy protein in its amorphous state.

The moisture content of food powders should be <10% and the water activity in the range of 0.1–0.4, depending on the product type (Ezzat et al., 2020). In this study, results obtained using all carrier agent combinations achieved these desirable limits and are close to the amounts reported by Santhalakshymy et al. (2015), and Bazaria and Kumar (2016) on their studies on the effect of temperature and different carrier agents on spray-dried jamun fruit juice and beet root juice concentrate, respectively. Therefore, the use of GA, MD, SPI, or their combinations was effective in decreasing both the moisture content and water activity of the produced sapinit powders.

CONCLUSION AND RECOMMENDATIONS

Utilization and processing of *sapinit* can solve the problems of seasonality and production fluctuations. One of the promising technologies by which *sapinit* may be processed is by making it into fruit powders through microencapsulation by spray drying. The study aimed to evaluate the effect of different carrier-agent combinations of gum arabic, maltodextrin, and soy protein isolate on the moisture content and water activity of spray-dried *sapinit*. The moisture contents of *sapinit* powders were close to the usual 4-8% moisture content for sugar-rich products, while their water activities were less than 0.4. The molecular weight and concentration of the carrier agent used significantly affected its corresponding moisture contents and water activities. *Sapinit* powder produced using 12.50% gum arabic, 12.50% maltodextrin, and 0% soy protein isolate had the lowest mean moisture content of 5.17% and the lowest mean water activity of 0.18. These may indicate that the spray-dried *sapinit* powder was shelf-stable. However, the storage conditions should also be considered for the *sapinit* powders with maltodextrin, which are highly hygroscopic, and should be stored properly in airtight containers with low water vapor transmission and kept in a cool, dry place.

For studies concerning the utilization of *sapinit* into a high-value food product, it is recommended to use other carrier agent options and their combinations to obtain the most cost-efficient and desirable powder properties. Processing and storage stability studies and degradation kinetics in terms of color, microbial, and phytochemical

properties can also be done to determine the actual shelf-stability, appropriate storage and packaging conditions, and consumer acceptance of the powder.

ACKNOWLEDGEMENTS

This study would not have been possible without the financial assistance from the Department of Science and Technology – Science Education Institute through their Accelerated Science and Technology Human Resource Development Program (ASTHRDP) and the University of the Philippines Diliman – College of Home Economics through their Graduate Research Grant (CHE – GRG).

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
Borneo Convention Centre, Kuching, Sarawak

IMPACT OF FOOD SECURITY ON HUMAN DEVELOPMENT IN MALAYSIA: AN AUTOREGRESSIVE DISTRIBUTED LAG (ARDL)

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Abstract: Food security became a crucial issue in Malaysia due to changes in the global environment climate and economic slowdown which have a huge impact on production in the agricultural sector. In addition, the growing income gap between the richest and the poorest has affected the country's development. Thus, the link between food security and human development cannot be denied as it is indirectly related to food and human beings. Therefore, this study aims to examine the relationship between food security and human development in Malaysia. The ARDL bound test was applied which makes extensive use of secondary databases from 1990 to 2019. The result shows that food security has a strong and significant positive effect on human development in the long run but has a negative relationship between the variables in the short run. These findings justify strengthening food security policies and development strategies.
Keywords: Food security, human development, temperature, income inequality, ARDL

INTRODUCTION

Changes in the global climate and unstable economic conditions have affected food supplies and production in most countries. The effects of climate change have had a significant impact on both the physical and mental aspects of human activities, including economic and agricultural practices. Human development plays a crucial and multifaceted role in driving economic growth and prosperity. It refers to the overall well-being and capabilities of a population, encompassing factors such as education, healthcare, income, and quality of life. Low levels of human development might contribute to income disparity and a decline in living standards, among other direct repercussions on a nation's long-term growth. In addition, it threatens human security by causing accidents, criminality, and violence as well as political instability (Gani and Prasad, 2007). For a country to achieve effective human development, it is crucial to ensure all citizens have access to sufficient quantities of high-quality food. Food security, according to the World Food Summit definition from 1996, is the condition in which all people, at all times, have physical and financial access to enough food that is safe, nourishing, and fits their dietary needs and food choices for an active and healthy life. The issue of food insecurity and scarcity would have an impact on human growth and well-being. Malaysia's performance in terms of food security still has to be improved. Despite moving up in the Global Food Security Index (GFSI) from position 44 in 2019 to position 41 in 2022 among 113 nations, there is still room for improvement due to factors like agricultural production volatility, reliance on food imports, dependence on natural resources, etc. (The Economist Intelligence Unit, 2022). Malaysia still depends on imported food to feed its population, particularly in the agro-food industry. According to the Department of Statistics Malaysia (2022), 19 out of 45 commodities in Malaysia had a self-sufficiency ratio (SSR) of more than 100% in general. Limited production output results in an insufficient supply. Thus, according to the Ministry of Agriculture and Food Industries Malaysia (2020), the overall value of food imports climbed from RM34 billion in 2011 to RM63 billion in 2021.

Higher levels of human development equate to better human capital, which can directly enhance lifetime productivity and hence boost economic growth and social development. Basic needs must be met to advance human growth. Human development and the availability of food, calories, and proteins are positively correlated, claim by Gani & Chand Prasad (2007). According to Sow, Berete, and Uche's (2015) research, there is a beneficial relationship between food security and human development. It has been stated that human development and food security are closely related, and that significant advancement in one area is impossible without advancement in the other (Conceiço et al., 2011). Therefore, the current study discusses the relationship between food security and human development, both of which are chosen as Sustainable Development Goals (SDG). To the best of our knowledge, there are still just a few empirical studies connecting food security with human development, particularly in Malaysia. Therefore, this study seeks to bridge the gap by examining the connection between food security and human development in Malaysia

in the short- and long-term. The Autoregressive Distributed Lag (ARDL) bounds testing method was used in this investigation. The ARDL test can be used to apply cointegration analysis to empirically determine the relationship between economic variables, regardless of whether the regressors are stationary at their level, integrated of order one, or a combination of the two, according to Pesaran and Shin (1999) and Pesaran et al. (2001). Furthermore, the ARDL technique performs better with a small sample, according to Haug (2002). The conclusion of this research demonstrates that, in the long run, food security has a substantial and considerable positive impact on human development, but that there is a short-run negative association between the variables.

MATERIALS AND METHODS

Time series data from Malaysia from 1990 to 2019 were used in this study. The information was obtained from the Human Nations Development Programme (UNDP), the Food and Agriculture Organization (FAO), the World Bank (WB), and the Standardized World Income Inequality Database (SWIID). This study has four independent variables; food production per capita (FPP) proxy for food security, change in temperature (TEMP) proxy for climate change, gross domestic product in agriculture (GDPAG) proxy for market size or output in agriculture, and gini index (GINI) proxy for income inequality which the ranges from 0 (or 0%) to 1 (or 100%), with 0 representing perfect equality and 1 representing perfect inequality. Human development index (HDI), a composite measure that gauges average performance in three fundamental areas of human development a long and healthy life, knowledge, and a decent standard of living is the dependent variable. Three fundamental elements of human growth are measured by an index, with scores ranging from low (less than 0.550), medium (0.550–0.699), high (0.700–0.799), and very high (greater than 0.800).

Model estimation and specification

The model converted all the variables into logarithms (L) to analyse the relationship between the dependent variable and independent factors for the short- and long-term. Equation 1 represents the model's empirical form as follows:

$$LHDI_t = \alpha + \beta_1 LFPP_t + \beta_2 LTEMP_t + \beta_3 LGDPAG_t + \beta_4 LGINI_t + \varepsilon_t \quad (1)$$

Where LHDI is the human development index, LFPP is food production per capita, LTEMP is temperature change, LGDPAG is a gross domestic product in agriculture, and LGINI is the Gini index. In addition, α explains the slope of coefficient, t is time period, ε is error term and $\beta 1,\ldots\ldots,4$ are associated with the coefficients of independent variables to be estimated. Next is to develop equation into an unrestricted error correction model once cointegration is found in the ARDL bounds test, next step is to estimate the long-run coefficients of variables in the equation using ARDL (s,r1,r2,r3,r4) specification which is defined as:

$$LHDI_t = \alpha_0 + \sum_{i=3}^{s} \alpha_{1i}\, \Delta LHDI_{t-i} + \sum_{i=3}^{r_1} \alpha_{2i}\, \Delta LFPP_{t-i} + \sum_{i=3}^{r_2} \alpha_{3i}\, \Delta LTEMP_{t-i} + \sum_{i=3}^{r_3} \alpha_{4i}\, \Delta LGDPAG_{t-i} + \sum_{i=2}^{r_4} \alpha_{5i}\, \Delta LGINI_{t-i} + \mu_t \quad (2)$$

The optimal lag lengths s,r1,r2,r3,r4 in equation (2) are selected by the lowest value of Akaike Selection Criteria (AIC). To estimate the short run dynamic coefficients of the variables, error correction model specifies that takes the following form:

$$\Delta LHDI_t = \alpha_0 + \sum_{i=3}^{s} \alpha_{1i}\, \Delta LHDI_{t-i} + \sum_{i=3}^{r_1} \alpha_{2i}\, \Delta LFPP_{t-i} + \sum_{i=3}^{r_2} \alpha_{3i}\, \Delta LTEMP_{t-i} + \sum_{i=3}^{r_3} \alpha_{4i}\, \Delta LGDPAG_{t-i} + \sum_{i=2}^{r_4} \alpha_{5i}\, \Delta LGINI_{t-i} + \emptyset ECM_{t-1} \quad (3)$$

Where ECM is a lagged error correction term with its coefficient of speed of adjustment ∅. The coefficient of the lagged ECM is expected to has a negative sign and is statistically significant to confirm that variables are adjusting back to long-run equilibrium following a disturbance in the model. This also examines the goodness of fit of the ARDL framework by performing diagnostic check of serial correlation test, normality test, heteroscedasticity test and stability test.

RESULTS AND DISCUSSION

Table 1 displays a summary of the testing findings that need to be performed before performing the short- and long-run ARDL estimate analysis.

Table 1. Summary of the testing results

Test	Results
The Variance inflation factor (VIF)	There is no multicollinearity problem in this analysis since the VIF is less than 5.
The Stationary test	A combination of integration order and stationary at level I(0) and first difference I(1).
The lag length selection	A maximum lag of 3 the optimal lag order by Akaike information criteria (AIC) are selected.
The ARDL Bound test for co-integration	F-statistics (8.433) greater than the upper bound critical value (5.06) which indicates the existence of a long-run co-integration relationship between its determinants and HDI in Malaysia.

ARDL Estimation Result

The different models' long-run and short-run estimates are shown in Table 2. In Malaysia, the contribution of food production per capita (LFPP) to the human development index (HDI) is positive and substantial at 5% level. It demonstrates that a 1% increase in FPP will improve Malaysia's human development by 0.84%. This conclusion supports the findings of studies by Gani & Chand Prasad (2007) and Berete, & Uche (2015) showing food production has a positive and substantial relationship with HDI. The coefficient of error correction term (ECT) value for speed of adjustment is negative (-0.080) and statistically significant at the 1% level. This validates the long-run relationship between the factors mentioned above, which suggests that human development is adjusted from the short to the long run in order to attain long-term equilibrium at 8% in the future. On the other hand, the short-run conclusion shows that human development in Malaysia will decline by 0.005% if food output increases by 1% per capita.

Table 2. Long-and Short-run result

Long-run analysis			
Variable	**Coefficient**	**Std. error**	**Prob.**
$LFPP_t$	0.836542	0.334819	0.0370**
$LTEMP_t$	-0.166481	0.174644	0.3684
$LGDPAG_t$	-0.159603	0.126121	0.2413
$LGINI_t$	-0.298893	0.228238	0.2267
Short-run analysis			
C	-0.272335	0.035350	0.0001***
$\Delta LHDI_{t-2}$	-0.565436	0.130067	0.0025***
$\Delta LFPP$	-0.068863	0.017715	0.0046***
$\Delta LTEMP$	-0.005404	0.002180	0.0381**
$\Delta LGDPAG_{t-2}$	0.020841	0.005842	0.0073***
$\Delta LGINI_{t-1}$	0.038606	0.015247	0.0351**
ECT_{t-1}	-0.080217	0.010087	0.0000***

Notes: *** Statistical significance at 1% level; ** Statistical significance at 5% level; * Statistical significance at 10% level.

CONCLUSION AND RECOMMENDATIONS

According to the empirical findings of this study, Malaysia's long-term human development tends to increase when there is enough access to food. However, there is an inverse relationship between them in the short run. It is necessary to address the problem of food security (quantity and nutritious) to reach better levels of human development. Growing access to unhealthy food would retard human growth and reduce lifespans. Government actions to improve food security are required in order to promote human development and maintain a healthy lifestyle. Increasing agricultural output with more nutrient-dense food is therefore crucial.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
Borneo Convention Centre, Kuching, Sarawak

HIGH CALCIUM KALE EFFECT ON DOUGH AND BREAD QUALITY

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Abstract: Bread is an ideal vehicle for addition of ingredients that increase nutrient density and add health benefits. Malaysian are reported to have calcium intake below the RNI value. In recent years, kale vegetable has gained a great popularity as a 'superfood' with its macronutrient, phytochemical and biological activity. MARDI has developed Our study focus on evaluation the effect of kale puree when added into bread dough. Response Surface with 1 numerical and 1 categorical factor was employed and generated 16 experimental units. 11 quality assessments (3 dough quality assessments + 8 bread quality assessments) were studied. Analyzed results showed, only 1 response has an interaction effect, bread crumb color. Remaining 10 responses yielded with no interaction. Optimization with 40% kale puree was successful to embedded nutritional claim (source of calcium and high in dietary fiber) and yielded satisfactory bread quality (good handling and 30% water absorption). Economic evaluation showed it is a viable business with IRR at 36%, NPV at RM 204303.00, ROI at 65%, PBP at 2.7 years, and BCR at 1.13.
***Keywords*:** kale, bread, dietary fiber.

INTRODUCTION

Bread, a frequently consumed food, is an ideal vehicle for addition of ingredients that increase nutrient density and add health benefits. The baking industries is trying to catch up with the tremendous demand on healthy food (Pycia, Pawłowska, Kaszuba, & Żurek, 2022). Malaysian were reported to have calcium intake below the RNI value (Zainuddin, 2015).

Kale vegetables is grown for its edible leaves and some as ornamentals. Kale has been extensively studied for its nutritional value (Šamec, Urlić, & Salopek-Sondi, 2019) attributed to the high content of bioactive components, including phenolic compounds (Abellán, Domínguez-Perles, Moreno, & García-Viguera, 2019). Kale is also a very good source of dietary fiber, the consumption of which reduces the risk of cancer and cardiovascular diseases (Barber, Kabisch, Pfeiffer, & Weickert, 2020). Planting kale with enriched fertilizer has made its high in calcium.

A strong association between the consumption of vegetables and lower incidence of diet-related non communicable diseases including hypertension and type 2 diabetes mellitus has been established in two independent meta-analyses of prospective cohort studies (Hong & Gruda, 2020). Kale holds its texture well when cooked where it can be steamed, stir-fried, roasted, or eaten raw. Kale can be blended into smoothies, roasted to make chips, mashed with potatoes, or used as a side dish. This research is meant to incorporate high-calcium kale, promote vegetable consumption as well as to diversified (value-add) local bread products.

MATERIALS AND METHODS

Materials. The materials were high protein wheat flour (protein content 13.0% - 13.4%, wet gluten min. 36.0%) (Prestasi Flour Mill (M) Sdn Bhd), salt, vegetable shortening (Delima Oil Products Sdn Bhd), sugar, lyophilized instant yeast (Lesaffre, France), and water. Kale leaves was obtained from MARDI's plant factory, with the following

composition (in dry basis weight): protein 23.2 g/100g, ash 16.01 g/100g, calcium 3.6 g/100g, and total dietary fiber 28.0 g/100g. Freshly harvested kale was steamed at 98ºC for 10-20 minutes (depending on total weight), grounded, blast freezed and stored frozen until used. Kale puree with the following composition (in dry basis) was used in bread making: protein 12.0 g/100g, ash 18.5 g/100g, calcium 3.8 g/100g, and total dietary fiber 28.7 g/100g.

Dough and Bread Making. Dough was made using straight-dough method with 1kg of flour. Other ingredients added as follow (baker's percent): 60% water, 20% shortening, 10% fine sugar, 11% instant yeast, and 1.5% salt. In enriched bread, kale puree was added from 10% - 60%, based on preliminary study. The amount of water added was optimized by the technician baker at our laboratory.

Experimental Design. A response surface with 1 numerical and 1 categorical factor was adopted. Sixteen (16) experimental units were generated via Design Expert program (9.0 Stat-Ease, Inc. Minneapolis, MN). It was employed to visualize and identify the effect of independent factors on the response's traits. There were eleven (11) quality assessments (3 dough quality and 8 bread quality assessments) measured before the best two (2) experimental units been chosen for sensory evaluation.

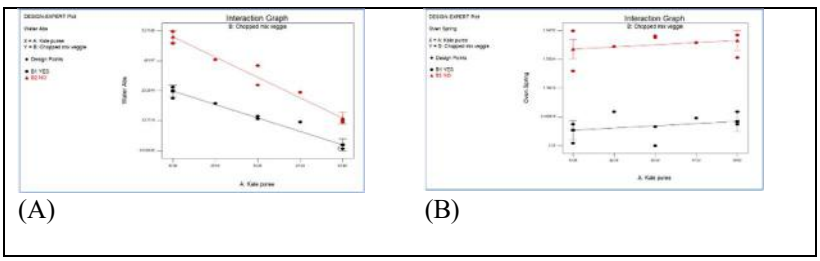
Quality Assessments and Sensory Evaluation. Dough quality assessments: water absorption, oven spring, and dough expansion, were measured according to Khalid et al. (2022). Bread quality assessments: loaf volume, specific volume, and loaf weight were measured according to Khalid et al. (2022). Crumb and crust color were measured via Minolta Chroma Meter CR300 (Konica Minolta, Inc, Tokyo, Japan); water activity and moisture content measured by Sartorius MA37, and bread crumb firmness measured by TA.XTPlus texture analyzer (Stable Micro system) with the aluminum radiused AACC (P/36R) (test speed 1.70 mm/sec; puncture distance 5.00mm; 5kg load cell). Sensory evaluation. Two (2) coded bread were randomly numbered and scored by 60 untrained consumer panel using 7-point hedonic rating scale and paired-preferences.

RESULTS AND DISCUSSION

There was no interaction effect between numerical factor (kale puree percentage) and categorical factor (chopped mixed kale) for all dough quality assessments (water absorption, oven spring, dough expansion, and dough handling) (Figure 1(A-D). However, presents of chopped mixed kale always had lower readings when compared with without chopped mixed kale in the dough system. Adding fiber in big particle size (compared to kale's puree particle size in general) had disturbed the gluten network formed in the dough system (Khalid, Manthey, & Simsek, 2017, 2018), hence the dough handling was poor and sticky.

Same phenomena were seen for loaf volume and loaf weight (Figure 2 (E-F). Absent of big particle size (chopped kale) resulted in higher loaf volume, and extrapolated graph figure may lead to interaction effect. Crumb color had shown an interaction effect between kale puree percentage and chopped mixed kale (Figure 2(G)). An opposite trend was shown for moisture content, calcium content and dietary fiber content. Whereby, all treatments containing chopped mixed kale had lower reading when compared to all treatments without it. There was still no interaction effect between kale puree percentage and chopped mixed kale in the dough system for these three responses (Figure 2(I, J, K)).

Optimization was done with configuration as follow: kale puree (10% - 60%), water absorption (max), loaf volume (987cc – 1812cc), dough handling (5-9), calcium content (600mg-1,000mg), dietary fiber content (3g-6g). The system (Stat-Ease) has suggested only one solution, with this configuration: kale puree at 40% and without chopped mixed kale. Verification was executed and technical specification of kale loaf with source of calcium and high in dietary fiber was shown in Table 1. Feasibility study was calculated and the results were shown at Table 2.



(A) (B)

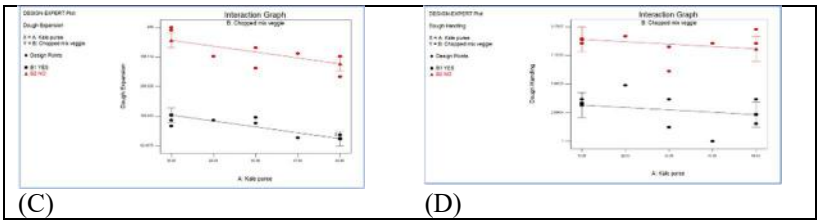


Figure 1: Interaction graph for (A) water absorption, (B) oven spring, (C) dough expansion, and (D) dough handling.

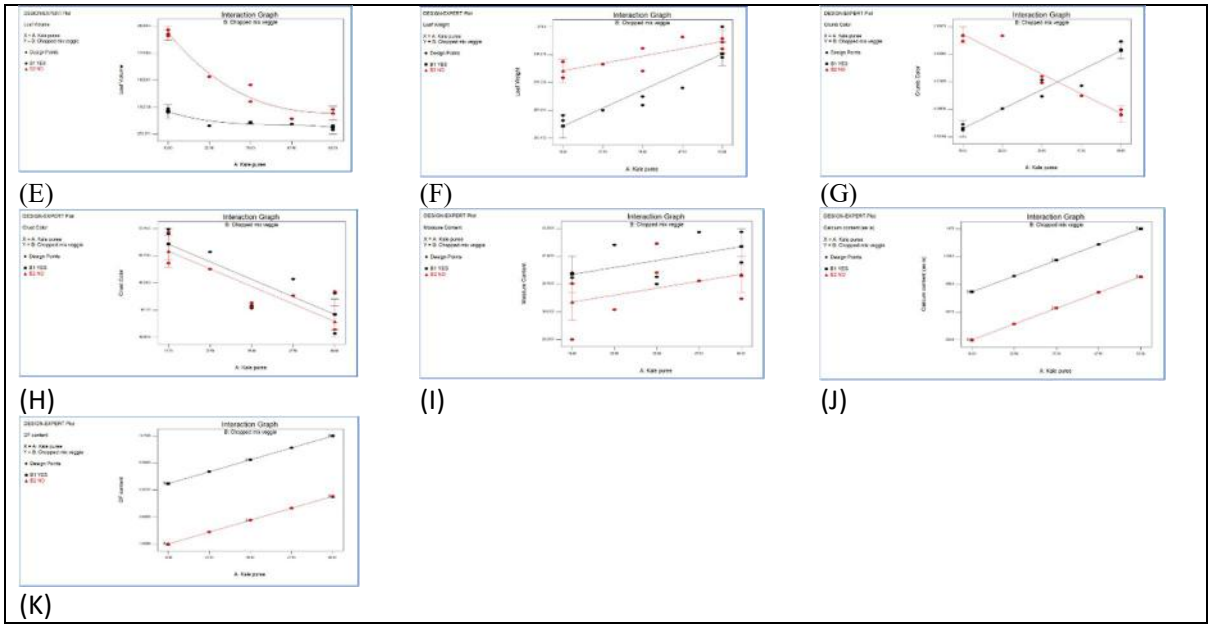


Figure 2: Interaction graph for (E) loaf volume, (F) loaf weight, (G) crumb color, (H) crust color, (I) moisture content, (J) calcium content, and (K) dietary fiber content.

Table 1: Technical Specification of Kale Loaf with Source of Calcium and High in Dietary Fiber

Product Name: Kale Loaf

Product Description: A loaf made from yeast-raised dough; naturally light green crumb color; added with 35% kale puree (baker's percent) and permissible preservatives.
Current retail: 240g

Control and Characteristics

Technical characteristics: (check lead on raw material of every batch received)

Ingredient(s)	High protein flour, kale puree, sugar, shortening, eggs, skim milk, salt, instant yeast, permissible food conditioner.
Origin(s)	Malaysia
Thermic process	Hot air oven
Date of minimum durability	6 days from the production date

No artificial colorings.

Physico-chemical characteristics: (check all along the production) (depending on methods and equipment used)

Protein, %by mass, min	9.6
Moisture content, %by mass, max	31.4
Dietary fiber, % by mass, max	7.5
Calcium content, mg/100g, as is, min	150.0

Storage

Store in room temperature (25-32^{o}C); pack in LDPE or PP plastic bag with close opening. The product must be stored away from substances with strong odors and shall be kept in close plastic/container in all time.

Table 2: Feasibility Study on Kale Loaf, Source of Calcium and High in Dietary Fiber.

Financial Projections	
Cost price	RM 11.53/loaf
Retail price	RM 13.50/loaf
Internal Rate of Return (IRR)	36%
Net Present Value (NPV)	RM 204,303
Return of Investment (ROI)	65%
Payback Period (PBP)	2.67 years
Benefit-Cost Ratio (BCR)	1.13

CONCLUSIONS

Adding kale puree does gave an impact towards dough and bread quality. A maximum of 40% kale puree (baker's percent) can be added into dough system to achieve a nutritional claiming 'source of calcium and high in dietary fiber' and yielded a satisfactory bread quality (good handling and 30% water absorption).

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OPTIMISATION OF THE RHEOLOGICAL PROPERTIES, EMULSIFYING AND ENCAPSULATION CAPABILITIES OF SONICATED K-CARRAGEENAN HYDROGEL BEADS

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Abstract: Several combinations of sonication amplitude and duration were used to treat κ-carrageenan hydrogel to optimise the physical property of the gel in terms of rheological behaviour, emulsifying and encapsulation capabilities as a flavouring encapsulant and a beverage conditioner. K-carrageenan hydrogel was first prepared by dissolving 2% w/w κ-carrageenan in water at 60°C before undergoing sonication at combinations of different amplitudes (20% - 40%) and duration (8s – 30s). Sweet-smelling flavouring compounds (ethyl vanillin and ethyl maltol) were then dissolved at 0.2% w/w in the sonicated hydrogel before being spherified in a cold (5°C) 1Mol potassium chloride bath to form hydrogel beads. The beads were then tested for flow behaviour, emulsifying properties and encapsulation capabilities via rotational test, turbidity test, and flavouring compound extraction respectively. Sonicating the hydrogel at amplitude of 20% for 10s resulted in a shear thickening system with a higher yield stress, σ_0 (σ_0 = 0.042875 Pa); smaller emulsion destabilisation rate, n (n = -0.034); and higher encapsulation efficiency, %Encap (%Encap= 26.5%). The improved rheological, emulsifying, and encapsulation properties of the hydrogel could potentially be used to engineer a sweetness-and-creaminess-enhancer, which could ultimately be applied into dairy beverages to reduce sugar and fat content.
Keywords: *sonication, κ-carrageenan, rheological behaviour, emulsifying properties, encapsulation efficiency*

INTRODUCTION

Sonication is gaining popularity in sustainable food processing due to its cost-effectiveness, low energy consumption, and environmental friendliness. Tellez-Morales et al. (2020) identified two key functions of sonication in the food industry: 1) altering chemical composition, especially in macromolecules, and 2) modifying techno-functional properties like foam capacity, emulsification, water absorption, and gel formation. K-carrageenan is a hydrocolloid commonly used for stabilising dairy products, due to its superior emulsifying and suspension properties. Recent research (Berton et al., 2020) even suggests its potential use as a soil conditioner due to its superabsorbent characteristic. In addition, sonication enhances the cold gelation properties of κ-carrageenan (Farahnaky et al., 2013), sparking interest in its application as a flavour encapsulant.

Our preliminary research (data not shown) revealed that sonicated κ-carrageenan develops a fibrous microstructure, facilitating aroma compound retention. Dynamic viscosity and yield stress of the sonicated gel solution has also increased by 87%. Emulsion stability has also improved. Therefore, to obtain the optimal parameters (amplitude & duration) for the most desired outcome, the effect of sonication amplitude and duration on the encapsulation capabilities, emulsion stability, and yield stress were analysed using response surface methodology (RSM).

In this study, 2% w/w κ-carrageenan hydrogel was prepared at 60°C, sonicated at varying amplitudes (20%-40%) and durations (8s-30s), and used as a matrix for flavour encapsulation (ethyl vanillin and ethyl maltol). The resulting hydrogel beads were freeze-dried for subsequent analyses, including flow behaviour, emulsifying properties, and encapsulation efficiency.

From the study, it was found that sonicating κ-carrageenan gel at 20% amplitude for 10s resulted in the most desired outcome, with a desirability index of 0.709. At usage level of 0.1%w/w, a shear-thickening solution with yield stress of 43mPa, average encapsulation efficiency of 34% and most stable emulsion could be produced. These results highlighted the potential of the material to be used as a sweetness-and-creaminess-enhancer, where the improved emulsifying stability and yield stress could enhance the perception of creaminess, and the increased encapsulation efficiency allows more sweetness enhancement flavouring compound to be entrapped. Ultimately, this material could be potentially applied to dairy beverages to reduce sugar and fat content.

MATERIALS AND METHODS

Preparation of κ-carrageenan hydrogel beads

The hydrogel was prepared according to the modified methods described by Azizi & Farahnaky, (2016). K-carrageenan (2%) was dissolved in deionised water at 55°C for 15 minutes. The gel (30 ml) was sonicated at different amplitudes and durations, with aroma compounds added at 0.2%. The hydrogel beads were formed in a 1M potassium chloride bath (5°C), rinsed, and freeze-dried according to the methods described by Zhao et al., (2020) for 48 hours (0.05mbar, -45°C).

Encapsulation capabilities

A standard curve was created with ethanol solutions of ethyl maltol and ethyl vanillin at various concentrations (10ppm-50ppm). Aroma compounds were extracted from 16.8mg hydrogel beads using ethanol for 2 hours at 25°C. Encapsulation efficiency was determined by comparing extract absorbance to the standard curve based on the methods described by Yang, et al(2014).

$$\%\ Encapsulation\ efficiency,\ \%\ \text{Encap} = \frac{mass\ of\ aroma\ compound\ encapsulated\ (g)}{mass\ of\ aroma\ compound\ added\ (g)} \times 100\%$$

Emulsion stability

Lyophilised hydrogel samples (0.1%) were dissolved in 60°C deionised water, mixed with RBD palm kernel oil (1.5%) and skimmed milk powder (0.5%), and homogenised (18,000rpm; 1 min). Turbidity of the emulsion was measured using the modified method described by (Aizawa, 2014). Emulsion (50µl) was diluted in 3ml of 0.1%w/v sodium dodecyl sulphate (SDS) solution dissolved in phosphate buffer. Samples are read at 550nm in triplicates at intervals of 5 min, 15 min, 30 min, 60 min, 90 min and 120 min. Graph of Absorbance (A) against Time (min) was plotted and fitted into a power function.

Flow behaviour

Flow behaviour of 0.1% lyophilised hydrogel solutions in deionised water was measured using a rheometer (Haake Mars rheometer; Thermo Scientific) based on the method described by Hadnadev et al (2014). Measurement of the samples (1ml) were performed at 25°C using 35mm diameter parallel plate geometry with 1mm gap under controlled stress of 1 Pa and increasing shear rate from 0/s to 100/s for 180s.Yield stress was determined using the Herschel-Bulkley model.

Experimental design and Statistical analysis

Response surface methodology was employed to optimise sonication parameters with amplitude (A) and duration (B) as the independent variables. A total of 15 runs were conducted with three repeats of central points. Linear and polynomial regressions were used to analyse the relationships between variables (Saravana et al., 2019).

RESULTS AND DISCUSSION

The optimal sonication condition was found to be 20% amplitude for 10 seconds, yielding a desirability index of 0.709. At a 0.1%w/w usage level, this condition produced a shear-thickening solution with a yield stress of 43mPa and an average encapsulation efficiency of 34%, resulting in a stable emulsion.

The results acquired from the confirmation experiments showed agreement with the predicted values to a certain extent, where the empirical data were better than the predicted values. This would be discussed further in the coming sections. Optimisation with a narrower range could be repeated to improve the accuracy of the models.

Encapsulation capabilities

In terms of encapsulation efficiency (%Encap), ethyl vanillin could be encapsulated significantly more than Ethyl maltol. Compared to the predicted values, the results were in agreement in all the samples, except for the $\%Encap_{EM}$ of 20A10s, which was significantly higher. This higher efficiency is favourable because it enabled more sweetness enhancing aroma compound to be kept in the system, thus potentially increase the perception of sweetness when being consumed.

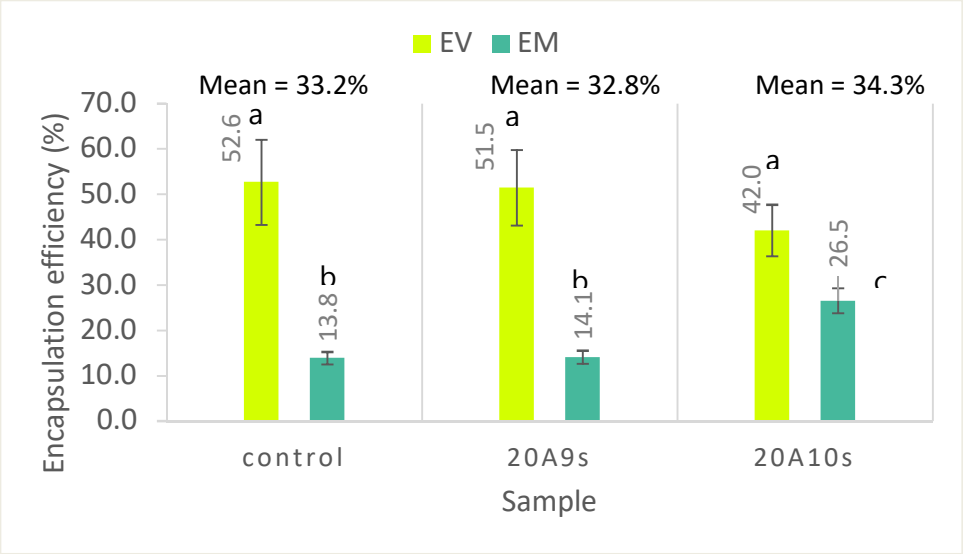

Figure 1. Encapsulation efficiency of ethyl vanillin (EV) and ethyl maltol (EM)

Emulsion stability

20A10s had the highest mean turbidity (0.239A) and the lowest rate of change (n = -0.034) showing that the emulsion remained the most stable within the 2 hours. Although the results deviate from the predicted values, it was proven that sonication improves emulsion stability, thus potentially enhancing the creamy mouthfeel. Optimisation conditions could be refined so the data would fit the model better.

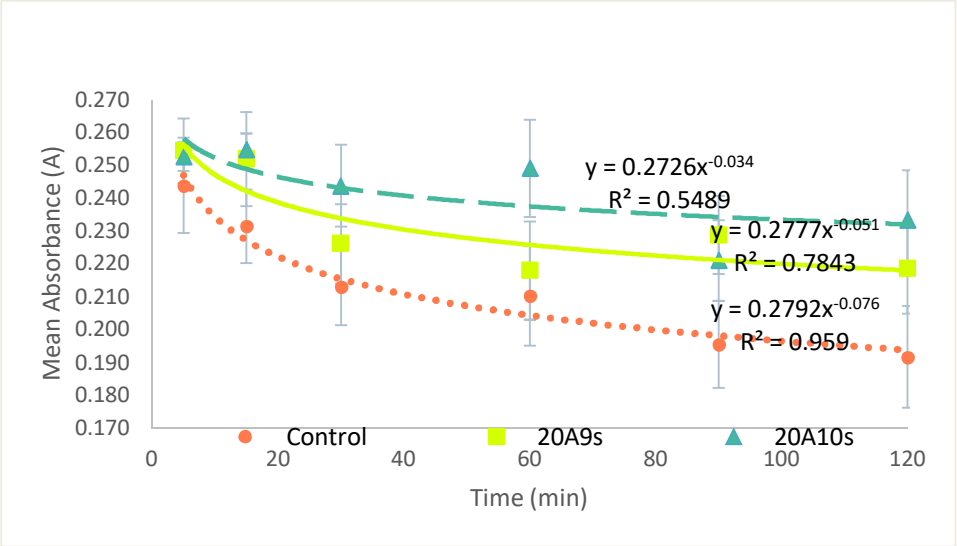

Figure 2. Emulsion stabilities of control, 20A9s and 20A10s across 120 min

Flow behaviour

From the results (Table 1), all samples were found to be shear-thickening, with 20A10s exhibited the most shear-thickening behaviour with the highest yield stress. It was hypothesised when the yield stress is higher, more effort was required to masticate, creating a perception that the sample was richer and having a higher satiation capacity. Therefore, 20A10s was the most desired condition to produce a hydrogel with a high satiation capacity. Sensory tests would be carried out to confirm this hypothesis.

Table 1. Flow behaviour of control, 20A9s and 20A10s; and their corresponding yield stress

Sample	Amplitude (%)	Duration (s)	Herschel-Bulkley $\tau = \tau_0 + k\dot{\gamma}^n$*			
			yield stress, τ_0 (mPa)	n	R^2	**Flow behaviour**
Control	0	0	24.22	1.031	0.9996	Shear thickening
20A9s	20	9	27.53	1.046	0.9972	Shear thickening
20A10s	20	10	42.88	1.115	0.9871	Shear thickening

**Herschel-Bulkley model, where τ is shear stress; τ_0 is yield stress; k is the flow consistency index; $\dot{\gamma}$ is the shear rate; and n is the flow behaviour index*

CONCLUSION

Sonication of κ-carrageenan gel at 20% amplitude for 10 seconds enhances encapsulation efficiency, emulsion stability, and yield stress. This material holds potential as a sweetness-and-creaminess-enhancer in dairy beverages, offering improved emulsifying stability and sweetness enhancement while reducing sugar and fat content.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
Borneo Convention Centre, Kuching, Sarawak

IMPROVING NUTRITIONAL QUALTY OF CURLY KALE: RED-BLUE LIGHTING SPECTRUM AND CALCIUM ENRICHMENT IN PLANT FACTORY

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Abstract: Curly kale is known to have high calcium content compared to other green leafy vegetables. In this study, curly kale was cultivated in a plant factory using a hydroponic system with a range of red-blue lighting spectra. Fifteen different spectra and six different high-calcium fertilizers were employed. The curly kale plants were harvested at day 35 of plantation. The study results indicate a significant increase in calcium content in curly kale under the combination spectrum of five red and one blue (5R1B), using a calcium-enriched fertilizer with a concentration of 200 ppm (Ca 200 ppm). Furthermore, the antioxidant content demonstrated high values under the same combination of spectrum and fertilizer. Based on these findings, it can be concluded that calcium-rich curly kale grown in a plant factory with specific lighting spectra is more nutritious compared to commercially available curly kale.
Keywords: Spectrum, LED light, calcium, curly kale

INTRODUCTION

Curly kale (Brassica oleracea var. sabellica) has long been recognized for its exceptional nutritional profile, with a particular emphasis on its high calcium content in comparison to other green leafy vegetables. Calcium is an essential mineral for human health, playing a pivotal role in bone health, muscle function, nerve transmission, and numerous enzymatic processes. Therefore, increasing the calcium content in curly kale could have significant implications for improving its nutritional value and enhancing its potential health benefits.

The cultivation of curly kale in controlled environments, such as plant factories utilizing hydroponic systems, offers an exciting avenue for optimizing its nutrient content. Hydroponics provides precise control over nutrient delivery, environmental conditions, and lighting, allowing for the customization of growth parameters to maximize nutrient accumulation (Li et al. 2021).

Lighting, in particular, plays a crucial role in the growth and development of plants. Different spectra of light can influence various physiological processes, including photosynthesis and the accumulation of bioactive compounds (Lin et al. 2013). Among the various lighting spectra available, red-blue lighting combinations have shown promise in enhancing plant growth and nutrient content. According to Matysiak et al. (2021), Different spectra of light have been found to significantly affect the growth and development of plants. Studies have shown that red light (600-700 nm) in combination with blue light (400-500 nm) has the greatest influence on plant growth and photosynthesis. A high proportion of red light stimulates biomass production in green-leaf lettuce, while blue light increases the levels of bioactive compounds such as flavonoids. Additionally, green light (500-600 nm) has been shown to drive photosynthesis efficiently. Overall, a red-blue lighting combination has shown promise in enhancing plant growth and nutrient content.

The study's objectives included evaluating the impact of different lighting spectra and calcium-enriched fertilizers on the calcium content of curly kale. Additionally, the investigation extended to the assessment of antioxidant levels in response to these growth conditions, as antioxidants are crucial for combating oxidative stress and promoting human health.

MATERIALS AND METHODS

Nutrient content

Nutrient content such natrium, potassium, kalium, kalsium and magnesium were analysed using AOAC (2023) method.

Ascorbic acid content

Ascorbic acid content was determined by extracting 10 g of the sample with 100 mL of 3% metaphosphoric acid. Then, 10 mL of extraction was titrated immediately with a standard dye solution to the first permanent pink endpoint.

Total Phenolic Content

Total phenolic content was determined using the Folin-Ciocalteu method which is based on a colourimetric oxidation and reduction reaction with some modification (Sunita and Dhananjay, 2010). Samples (0.3 mL) were introduced into test tubes followed by 1.5 mL of Folin-Ciocalteu's reagent (diluted 10 times with water) and 1.2 mL of sodium carbonate (7.5%w/v). The tubes were vortexed; covered with parafilm and allowed to stand for 30 min. Absorption at 765 nm was measured. Total phenolic contents were expressed in Gallic Acid Equivalents (mg per 100 g fresh weight).

Free Radical-Scavenging Capacity (DPPH Assay)

Antioxidant activity for lettuce was studied through the evaluation of the free radical scavenging effect on the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical (Yen and Hsieh, 1997). A 0.5 mL sample of the extract was added to 1 mL methanolic solution of DPPH radical (0.2 mM). The mixture was shaken vigorously and left for 30 min. The absorbance was then measured at 517 nm. The antioxidant activity was reported as the percentage of radical scavenging as follows: % radical scavenging = (1-Asample/Acontrol) x 100 where Asample is the absorbance of the mixture of the sample extract and DPPH, Acontrol is the absorbance of the mixture of DPPH and acidified methanol.

Ferric-Reducing Ability of Plasma (FRAP) Assay

The FRAC of the samples was determined by using the potassium ferricyanide-ferric chloride method (Oyaizu, 1986). 1 mL of different dilutions of the sample (50, 20, 10, and 5 g/L) was added to 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%). The mixtures were incubated at 50°C for 20 min, after which 2.5 mL of trichloroacetic acid (10%) was added. An aliquot of the mixture (2.5 mL) was taken and mixed with 2.5 mL of water and 0.5 mL of 1% $FeCl_3$. The absorbance at 700 nm was measured after the solution had been allowed to stand for 30 min. The FRAC of a sample is estimated in terms of Trolox equivalent antioxidant capacity (TEAC) in millimoles per liter Trolox. Each assay was carried out in triplicate.

RESUITS AND DISCUSSION

The study began by investigating the influence of different lighting spectra on the calcium content of curly kale. Curly kale was grown under fifteen distinct lighting conditions, each with varying combinations of red and blue light wavelengths. The results in Table 1 demonstrated that the combination spectrum of five red lights and one blue light (5R1B) significantly increased the calcium content of curly kale compared to other spectra. This finding aligns with previous research highlighting the importance of red and blue light in photosynthesis. Red light is absorbed by chlorophyll and contributes to energy production, while blue light plays a vital role in chlorophyll formation (Rahman et al. 2021). The 5R1B spectrum appears to optimize these processes, leading to enhanced calcium uptake and accumulation in curly kale leaves.

In addition to lighting spectra, the study explored the impact of six different high-calcium fertilizers on calcium enrichment in curly kale. Among these fertilizers, one with a calcium concentration of 200 ppm (Ca 200 ppm) demonstrated remarkable results in increasing the calcium content of the plants. This outcome underscores the importance of nutrient management in hydroponic systems. The use of calcium-enriched fertilizers directly supplied the essential mineral to the plants, promoting calcium uptake and incorporation into the plant's tissues. The choice of an appropriate fertilizer formulation is crucial for achieving the desired nutrient enrichment in hydroponic cultivation. Beyond calcium content, the study examined the antioxidant levels in curly kale under the specific conditions of the 5R1B lighting spectrum and the Ca 200 ppm fertilizer. Antioxidants play a pivotal role in human health, as they help combat oxidative stress and reduce the risk of chronic diseases. The study revealed that the combination of the 5R1B lighting spectrum and the Ca 200 ppm fertilizer not only increased calcium content but also led to a significant elevation in antioxidant levels. This finding suggests a potential synergy between the growth conditions that promote calcium enrichment and the accumulation of bioactive compounds with antioxidant properties. LED light spectrum has been found to have a significant impact on the antioxidant content of lettuce (Promratrak 2017). Studies have shown that different ratios of blue and red LED light can modulate the nutritive and phytochemical composition of lettuce, including its antioxidant content. The specific wavelengths of light emitted by LEDs can influence the synthesis of antioxidants in lettuce, leading to variations in their levels (Matysiak et al. 2021), Therefore, by manipulating the LED light spectrum, it is possible to enhance the antioxidant content of lettuce and potentially improve its nutritional value.

The findings of this study hold significant nutritional implications. Calcium is an essential mineral for bone health and various physiological processes in the human body. Increasing the calcium content in curly kale through controlled cultivation methods has the potential to provide consumers with a more nutritious source of this vital

mineral. Furthermore, the concurrent increase in antioxidant levels in response to specific growth conditions could enhance the overall health benefits of curly kale consumption. Antioxidants, such as vitamins C and E, carotenoids, and polyphenols, have been associated with reducing the risk of chronic diseases, including heart disease and certain types of cancer.

Table 1. The effect of LED lighting spectrum on curly kale nutrient using fertilizer with Ca 200 ppm

Lighting spectrum	N (%)	P (mg/100g)	K (mg/100g)	Ca (mg/100g)	Mg (mg/100g)
100R	2.92 c	54 b	367 f	283 d	19 c
100B	4.19 a	52 b	24 g	19 e	1 d
100G	4.24 a	54 b	415 d	370 b	23 b
2R1B	3.65 b	47 c	372 f	28 e	17 c
1R2B	2.91 c	52 b	468 c	361 b	29 ab
1B1G	3.36 b	46 c	414 d	369 b	23 b
1R1W	3.01 bc	56 b	480 c	310c	21 b
1B1W	4.54 a	46 c	476 c	364 b	25 b
RGB	3.15 b	53 b	522 b	344 bc	25 b
RG	4.15 a	47 c	480 c	308 c	22 b
RB	3.03 bc	46 c	431 cd	352 b	24 b
5R1B	3.35 b	61 a	696 a	533 a	35 a
4R1B1G	4.35 a	53 b	485 c	366 b	24 b
Full spectrum new	4.08 ab	52 b	401 e	305 c	21 b
Full spectrum old	4.18 a	47 c	425 d	354 b	21 b

[abcdefgh] Different alphabet in the same column show significant difference ($p<0.05$)

Table 2. The effect of LED lighting and calcium-rich fertilizer towards antioxidants and fenolic content in curly kale

Lighting Spectrum	Ascorbic Acid Content (mg/100g FW)	Total phenolic (mg GAE Eq/100 g FW)	% DPPH inhibition	FRAP (g FeSO4 Eq /100g)
100R	10.19±5.71bc	99.79±1.68i	79.17±0.21h	263.82±13.99^{ef}
100B	13.86±1.19ab	127.76±3.75bc	85.37±0.36e	331.55±7.07^{b}
100G	13.83±2.69ab	102.79±3.09hi	77.21±0.32i	270.21±7.44^{e}
2R1B	8.41±2.17c	108.67±0.76gh	86.01±0.45cde	253.24±2.30^{fg}
1R2B	8.82±1 75c	133.66±1.43ab	88.63±0.55b	309.49±7.87^{c}
1B1G	8.61±2.22c	108.17±2.18gh	86.67±0.09c	270.57±5.65^{e}
1R1W	9.65±1.21bc	135.14±1.69a	80.10±0.48g	274.30±20.17^{e}
1B1R	7.16±1.38c	116.36±1.60ef	89.457±0.20a	242.50±7.13^{g}
RGB	6.89±1.54c	112.07±0.75fg	80.05±0.48g	271.60±7.62^{e}
RG	6.90±1.13c	98.02±1.53i	77.213±0.39j	201.79±1.44^{h}
RB	8.46±1.28c	139.09±3.07a	85.88±0.28de	371.88±4.17^{a}
5R1B	7.14±0.61c	121.25±3.55de	85.48±0.48e	273.87±6.97^{e}
4R1B1G	9.84±1.91bc	108.80±1.01gh	83.76±0.36f	268.85±4.86^{ef}
Full spectrum new	16.47±4.03a	123.59±11.54cd	86.33±0.24cd	291.57±11.00^{d}
Full spectrum old	16.40±0.67a	100.16±2.17i	80.68±0.16g	278.67±12.22^{de}

[abcdefgh] Different alphabet in the same column show significant difference ($p<0.05$)

CONCLUSION AND RECOMMENDATIONS

In conclusion, this study demonstrates that the cultivation of curly kale in a controlled environment, utilizing a specific lighting spectrum (5R1B) and a calcium-enriched fertilizer (Ca 200 ppm), leads to a substantial increase in calcium

content and elevated antioxidant levels. These findings suggest that calcium-rich curly kale grown under these optimized conditions in a plant factory may offer a more nutritious and health-promoting option compared to commercially available curly kale. Future research could further explore the practicality and scalability of these cultivation methods to meet the increasing demand for nutrient-rich vegetables in a controlled environment.

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The 17th ASEAN Food Conference 2023 (AFC2023)
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DEGRADATION KINETICS OF CHLORPYRIFOS IN FRUITY AND LEAFY VEGETABLES BY DISSOLVED OZONE

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Abstract: Ozonated water is a potential sanitizer to eliminate pesticide residues in raw vegetables. This work aimed to evaluate the influence of ozone dissolution on the mechanism of pesticide residue degradation in vegetables during ozone washing. Ozone dissolution rate at different pH and temperatures were quantified using validated methods. Degradation kinetics of chlorpyrifos residue in spiked leafy (red cabbage) and fruity (cherry tomato) vegetables at different pH (3, 7, 8) and temperatures (5, 25 °C) were analyzed using gas chromatography-electron capture detection (GC-ECD). The effects of ozone treatment on total phenolic content (TPC) of vegetables were also evaluated. Ozone dissolution rate at different pH and temperatures within 60 minutes followed zero-order kinetics which increased with pH and temperature decrease attributed to higher ozone stability. Degradation kinetics of chlorpyrifos residue followed first order with increasing rate of degradation at higher pH and temperature due to ozone instability and potent oxidation. Low concentration of ozonated water (2 mg/L) significantly reduced chlorpyrifos residues in leafy and fruity vegetables during 30 min washing but the efficacy of ozone treatment varied with type of vegetables. No significant changes in color and TPC of ozonated cherry tomatoes and red cabbage. In sum, ozone is potential for green postharvest treatment of vegetables in both industry and households.
***Keywords*:** pesticide residue, total phenolic content, ozone dissolution, kinetics degradation

INTRODUCTION

Pesticides are widely used to improve the quality as well as quantity of crops. Organophosphorus pesticides are highly toxic to human and animals. Despite the surveillance carried out by the competent authorities, recent research has shown significant incidence of pesticide residues at levels above the maximum residue limits (MRLs) in various vegetables (Fatunsin et al., 2020). According to Malaysia Food Regulation 1985, the MRLs for leafy and fruity vegetable are different due to different physical structure and properties that would affect the amount of pesticide retained (Legal Research Board, 2015). When the recommended application doses and harvest intervals are not followed, pesticide residues may remain in postharvest food crops. Therefore, mitigation strategies of pesticide residues before consumption is important, especially in freshly eaten vegetables.

Ozone, which has qualities like fast disintegration and few repercussions during fruits, vegetables, and grains preservation, is an appealing approach of pesticide residue decontamination. Ozone quickly auto decomposes into molecular oxygen leaving no hazardous halogenated compounds in the food products (Pandiselvam et al., 2018). It is a commercially viable strategy because it works in both gaseous and aqueous states and is economically practical (Wang et al., 2018). Currently, most of the kinetic study of ozone focuses on its decomposition behaviour instead of its dissolution behaviour. However, study of kinetic behaviour of dissolution of ozone in water is important to satisfactory predict the concentration of ozone in water which greatly affected by water pH and temperature. Moreover, quality of the vegetables treated with ozone is also concerned due to its strong oxidizing properties which could affect colour and antioxidant compounds in vegetables. This present study aimed to quantify the rate of ozone dissolution in water at different pH and temperature in relation to the kinetic degradation of pesticide residues in selected leafy and fruity vegetables. Also the effect of ozone treatments on the quality attributes of these vegetables during storage.

MATERIALS AND METHODS

Production and Quantification of Dissolved Ozone

Ozonated water was prepared by purging 250 mL distilled water at different pH (3, 7, 8) prepared by addition of citric acid or sodium bicarbonate solution and different temperatures (5, 15, 25 °C) for 60 minutes with domestic ozone generator (Beyond Food Junction, Zhulian, Penang, Malaysia) at ozone flow rate of 300 mg/hr. This experiment was carried out in fume hood with excess ozone being captured in 2 % potassium iodine (KI) solution. The concentration of ozone dissolved in distilled water of different pH and temperature were monitored every 15 minutes, determined

using indigo colorimetric method according to (Tarabová et al., 2018) and verified using potassium iodide wet-chemistry method (Ruffino & Zanetti, 2019).

Spiking Vegetables with Chlorpyrifos and Ozone Decontamination

Fresh and unblemished chlorpyrifos-free cherry tomatoes and red cabbages were immersed in chlorpyrifos solution at 4 mg/L in acetonitrile for 4 minutes with gentle rotation by gloved hand separately. A triplicate sample of vegetables were analyzed to ensure they were free from chlorpyrifos residues. Vegetables with chlorpyrifos on their surface were then air-dried in fume hood in dark for 12 hours at room temperature.

Kinetic degradation of chlorpyrifos in the spiked cherry tomatoes and red cabbages samples with ozonated water at 2 mg/L was conducted by immersing 40 g vegetables for 5, 10, 20 and 30 minutes. Control sample was prepared by immersing the spiked vegetable in distilled water at different pH and temperature combinations: pH 3 at 25 °C, pH 3 at 5 °C and pH 7 at 5 °C. Ozone treated vegetables were let air dried in fume hood for 15 minutes and stored in polyethylene (PE) bag at 4 °C prior to quality determination.

Quantification of Chlorpyrifos

Chlorpyrifos residues were extracted from ozone treated vegetables samples following method prescribed by (Cong et al., 2020). Samples of vegetables were taken in accordance with the prior report. Each lettuce sample was first homogenised in a blender, and then 10 g of the resulting homogenate was combined with 10 mL of acetonitrile. Finally, 5 mL of the organic phase was then passed through a Florisil cartridge (WH-12 YOONING Inc. Hangzhou, China) that had been previously activated with acetone (1:9 volume) and 5 mL n-hexane. The active ingredient was recovered in a centrifuge tube after the Florisil cartridge (SPE ANPLE Laboratory Technologies Inc. Shanghai, China) was eluted three times with acetone (1:9). Utilising a nitrogen evaporator, the collected solution was dried, and the residue was then dissolved in 5 mL of n-hexane for GC analysis. Analysis of chlorpyrifos residues in ozone treated vegetables samples were carried out using gas chromatography (GC-2010 Plus, Shimadzu, Japan) with electron capture detector (GC-ECD).

Quality Evaluation of Vegetables During Storage

Storage analysis was performed on cherry tomatoes and red cabbages samples after completion of 30 minutes ozone treatments. The vegetables samples were let air dried in fume hood for 15 minutes and stored in PE bags at 4 °C for 5 days. Analysis of weight loss, colour change and TPC were carried out for 5 days. Comparison between mean were analyzed using two-way ANOVA using Minitab (Minitab Inc. USA).

RESULTS AND DISCUSSION

Figure 1shows (a) ozone dissolution rate, (b) final ozone concentrations and (c) Arrhenius plot at different pH and temperatures range between 0.5 to 4.8 mg/L. Ozone can dissolve at a faster rate at lower temperature (5 °C) for all water pH. Increase in water temperature significantly reduced concentration of dissolved ozone by 1-fold. Increase in water pH from 3 to 7 and 8 further reduced dissolution rate of ozone and final ozone concentrations. At alkaline condition, ozone only slightly dissolved in the water yet maintaining linear trend. This is because ozone will react with water and OH- ion to produce hydroxyl free radicals which possess very high oxidation potential to initiate a series of chain reactions in water, thus the decomposition rate of ozone increased with increasing water pH resulting in lower range of ozone concentration (Sullivan & Roth, 1980). Ozone has a higher stability in water of lower pH and temperature. The highest dissolution rate was achieved when the water temperature was 5 °C and pH 3 with k value of 0.0514 mg/L/min. No significant interaction effect ($p>0.05$) between water pH and temperature which indicated that the effect of water temperature on k value was not depended on the water pH and vice versa. Linear observation suggests that the k value of ozone dissolution in water of different pH obeyed Arrhenius equation. This shows that increase in water temperature reduced the rate of ozone dissolution in water applied at different pH.

Figure 2 shows kinetic degradation of chlorpyrifos residues in cherry tomato and red cabbage at different pH and temperature combinations. Initial chlorpyrifos residues in red cabbage (13.8 mg/kg) was twice higher than cherry tomato (6.7 mg/kg) due to higher surface area to volume ratio that took up more chlorpyrifos. This is in argreement with the study conducted by Khaled et al. (2017) on eight vegetables that showed generally leafy vegetables has a higher initial level of chlorpyrifos residues compared to fruity vegetables. The chlorpyrifos residues degradation from ozone treatment was pH and temperature-dependent with almost similar rate of degardation for both vegetables. Higher rate of degradation at high pH and temperature due to higher ozone instability. Figure 3 shows the percentage of chlorpyrifos degradation in cherry tomato and red cabbage at different pH and temperature combinations. Higher percentage of degradation at higher temperature with higher chlorpyrifos degradation in cherry tomato.

Table 1 shows weight loss, color change and total phenolic content (TPC) of vegetables after 5 days of storage. Regardless of ozone treatment, there was no significant of treatments groups on the weight loss, color change and TPC. However, higher losses was recorded in red cabbage compared to cherry tomato.

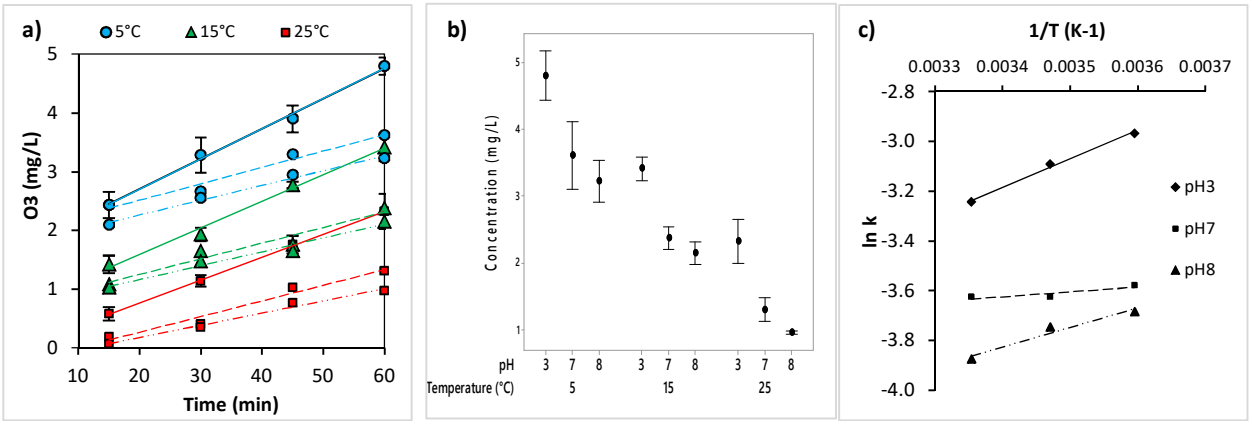

Figure 1: (a) Ozone dissolution rate, (b) final ozone concentration, and (c) Arrhenius plot of ozone dissolution rate in water at pH 3 (——), pH 7 (- - -), and pH 8 (··—··) and different temperatures.

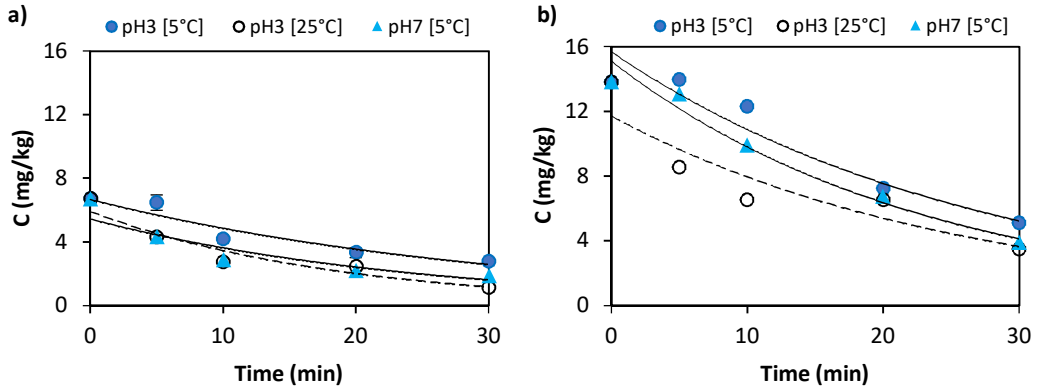

Figure 2 . Chlorpyrifos, C degradation in (a) cherry tomato and (b) red cabbage during ozone treatments at different pH and temperatures.

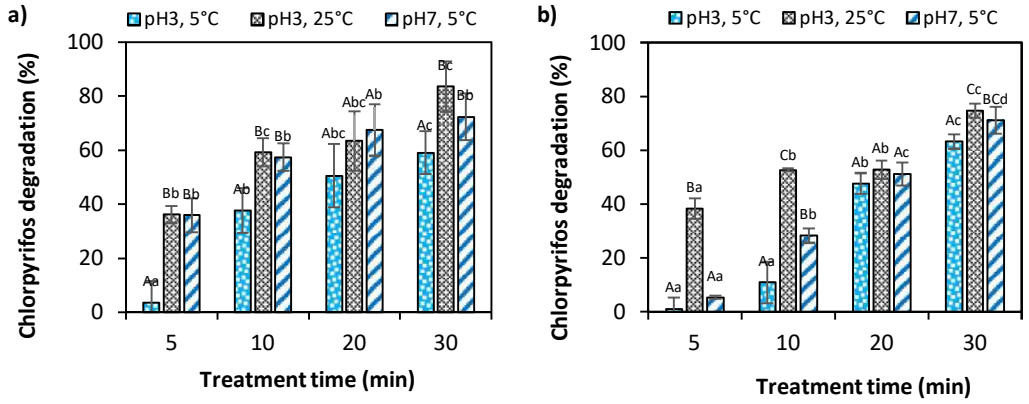

Figure 3. Percentage of degradation of chlorpyrifos residue in (a) cherry tomato and (b) red cabbage after ozone treatments at different pH and temperatures

Table 1. Weight lost, total colour change, and total phenolic content (TPC) loss of cherry tomato and red cabbage washed with ozonated water at different pH and temperature after 5 days storage compared to control.

Treatment conditions	Cherry tomato			Red cabbage		
	Weight loss (g)	Colour change (ΔE)	TPC loss (mg GAE/100 g)	Weight loss (g)	Colour change (ΔE)	TPC loss (mg GAE/100 g)
Control	0.18 ± 0.04^{Aa}	3.9 ± 0.6^{Aa}	3.9 ± 1.0^{ABa}	0.53 ± 0.1^{Ab}	2.8 ± 1.0^{Ab}	6.7 ± 3.0^{Cb}
pH 3, 5 °C	0.15 ± 0.03^{Aa}	2.4 ± 1.0^{Aa}	1.3 ± 0.2^{Ba}	0.60 ± 0.2^{Ab}	3.3 ± 0.4^{Ab}	10 ± 0.6^{Bb}
pH 3, 25 °C	0.24 ± 0.05^{Aa}	1.6 ± 0.8^{Aa}	5.5 ± 0.5^{Aa}	0.85 ± 0.4^{Ab}	2.6 ± 1.7^{Ab}	18 ± 1.8^{Ab}
pH 7, 5 °C	0.14 ± 0.02^{Aa}	3.1 ± 1.0^{Aa}	2.0 ± 0.4^{ABa}	0.53 ± 0.2^{Ab}	3.0 ± 0.3^{Ab}	13 ± 1.0^{Bb}

CONCLUSIONS

Present study successfully developed a method to quantify the concentration of dissolved ozone in water. This method was developed by the combination and modification of the established indigo colorimetric method and KI wet chemistry method. The k value of degradation of chlorpyrifos residues was significantly ($p<0.05$) affected by the pH and temperature of ozonated water. Increase in pH and temperature of water resulting in a higher k value which can be explained from high degree of ozone instability. It can be concluded that current condition of ozone treatment does not affect the quality attributes of vegetables. In the future, it might be worthwhile to investigate the impact of agitation during purging. Since farm growth of vegetables typically entails the application of more than one type of pesticide, study on the effectiveness of ozone treatment to degrade more than one type of pesticide in vegetables is necessary.

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24-27th October 2023
Borneo Convention Centre, Kuching, Sarawak

EFFECTS OF DIRECT OZONE DEPURATION ON MICROBIAL SAFETY AND SURVIVAL OF TROPICAL OYSTERS (*CRASSOSTREA IREDALEI*)

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Abstract: Depuration is an effective method to eliminate pathogens in oysters but high initial load increases risk of inadequate elimination. The effects of direct ozone depuration concentration (0.2-0.5 mg/L) and time (2-6 hours) generated using venturi injector on microbial reduction, physicochemical changes (pH and TVB-N) and oysters' survival were evaluated on tropical oysters (*Crassostrea iredalei*) collected from Sungai Merbok estuary. Total coliform (14-1103 MPN/g), faecal coliform (14-210 MPN/g), *Vibrio cholerae* (23- 1100 MPN/g) were initially present in raw oysters. Oysters were depurated at 6:7 ratio of oysters: artificial seawater at salinity of 21 ppt. Total plate count (TPC) reduced significantly after 2 hours depuration regardless of initial microbial load. Total coliform and faecal coliform decreased significantly to 2.5 MPN/g after 2 hours ozone depuration compared to 6 hours for conventional depuration. *Vibrio cholerae* count significantly reduced after 4 hours ozone depuration at 0.3 ppm ozone whereas 6 hours depuration without ozone only able to reach 22 MPN/g. TVB-N value greatly decreased with ozone concentration above 0.3 ppm. Freshness of tropical oysters maintain at pH 6.6-6.5 with survival rate (9.5-15.5 days) varied regardless of depuration parameters. Direct ozone depuration enables fresh tropical oysters to be safe for consumption whilst preserving their freshness and quality.

***Keywords*:** Tropical oyster, depuration, microbial safety, crassostrea iredalei, survival rate

INTRODUCTION

Crassostrea iredalei, known as the black scar oyster, is a sought-after oyster species in tropical countries due to its fast growth, appealing appearance, and delicious flesh. In Malaysia, it ranks third in mollusc aquaculture production after the blood cockle and green mussel (Doinsing & Ransangan, 2023). Oysters are mainly sold as fresh live oysters to the hotels and restaurants to be eaten raw. As fresh seafood, oysters are more perishable than other high-protein foods and have a short shelf-life (Pardío-Sedas, 2015). The raw consumption of tropical oysters is potential to cause foodborne illness due to contamination of seafood-borne pathogens, *Vibrio* sp. which is frequently found in mollusc like oyster (Wright et al., 2009). The tropical climate contributes to the warm water which is favorable to the *Vibrio* species (Froelich & Noble, 2016). Bivalve mollusks like oysters are filter feeders and their immobility in coastal estuaries make them susceptible to accumulating pathogens, fecal contamination.

Post-harvest processing methods such as depuration are viable food processing methods employed to reduce microbial load in bivalve by utilizing natural physiological functions of the live animal. Depuration leverages the animals' natural gastrointestinal functions, with its success dependent on animal well-being, health status, physiological activity, pumping rate, and behavioral responses (Schneider et al., 2009). However, it is not effective in eliminating other health risks, such as viruses, toxins, and heavy metals and requires long hours up to 48 hours that may affect the animal's viability. Thus, sustainable post-harvest processing for effective elimination of pathogen and heavy metal is paramount in the growth of this industry to meet regulatory requirements.

Ozone depuration is a frequent treatment utilized by breeders, consist of flow through, closed environment, recirculate with ozone to remove impurities, eliminate pathogens and spoilage microorganisms from live oysters. Ozone is a very powerful oxidizing agent that could destroy bacteria and viruses directly (Powell & Scolding, 2018)

resulting in purging of intestinal contents (Martinez-Albores et al., 2020). Venturi injectors can produce dissolved ozone effectively in the form of nanobubbles when ozone gas mixes with water under vacuum (Yang & Chen, 2022).

This research aims to evaluate the effects of direct ozone depuration using venturi system on the microbial safety and survival of tropical oysters (*Crassostrea iredalei*).

MATERIALS AND METHODS

Oyster Collection and Handling

A total of 200 naturally polluted medium-size (6–7 cm long) tropical oysters (*Crassostrea iredalei*) were collected on the same day of experiment from Sungai Merbok estuary, Kedah Malaysia with recorded water salinity of 21± 3 ppt. Oysters were transported directly to laboratory at 25°C and cleaned before subjected to depuration. Duplicate samples of 10 raw oysters were tested for *Escherichia coli*, *Vibrio parahaemolyticus*, total coliform and fecal coliform within 2 h of collection.

Direct Ozone Depuration Trials

Each depuration trial was carried out with 35 oysters in 30 L artificial seawater (ASW) at 21 ppt salinity, pH (8±1) at room temperature in layered baskets suspended 10 cm above the bottom tank to prevent contamination of sediments. ASW at was prepared by mixing a synthetic sea salt (Instant Ocean Inc., Blacksburg, VA, USA) with reverse osmosis water and tested for fecal coliforms and *E. coli* prior to each depuration trial. Ozonated ASW at four levels of concentration (0.2, 0.3, 0.4, 0.5 ppm) was produced using venturi injector in a closed chamber and then transferred directly into treatment tank recirculated with a pump (JP- 450G, Sunsun Co. Ltd., China) and passed through a filter and UV light (UV-36W, Atman Co. Ltd., China), primarily to avoid debris and dirt from accumulating during depuration process. Ozone concentration was monitored using dissolved ozone meter (Hach, Loveland, CO, USA). Oyster samples from each tank were taken out at 2, 4, and 6 hours to conduct microbial, pH, and total volatile basic nitrogen (TVB-N) analysis. The depurated oysters were kept at room temperature in a container wrap with wet cloth.

Evaluation of Depurated Oyster

Total plate count (TPC), total coliform, fecal coliform, *Escherichia coli*, and *Vibrio parahaemolyticus* were tested on the depurated oysters in triplicate. The homogenized oyster's pH taken at each interval was determined according to (Tsai et al., 2023). The total volatile basic nitrogen (TVB-N) of oyster's samples were determined using semi micro Kjeldahl method according to Chen et al. (2019). The survival rate of the depurated oysters was determined daily according to opening and closing gaps and the death of the samples was recorded. The results were expressed in the average of the number of survival days for each sample.

Statistical Analysis

Comparison between means were analyzed using One-Way ANOVA using Minitab (Minitab Inc. USA).

RESULTS AND DISCUSSION

Figure 1 shows the total plate count (TPC), total coliform, feacal coliform, and *Vibrio cholera* counts of oyster subjected to depuration at different time interval and ozone concentration. TPC gradually decreased with increase in depuration time. Increase in ozone concentration beyond 0.4 ppm drastically reduced TPC especially after 2 hours depuration. Total coliform and fecal coliform count significantly decreased after 2 hours ozone depuration regardless of concentration while control sample reached similar reduction after 6 hours. Initial load of *Vibrio cholera* at 53 MPN/g significantly reduced <10 MPN/g at 0.4 ppm ozone after 4 hours. Similar study by Pardío-Sedas (2015) and Hernández et al. (2018) reported low reduction rates of *Vibrio cholerae* even under ozone depuration. Pruzzo et al. (2003) proposed firm attachment of the pathogen in the water column and tissues of oyster that reduced removal efficacy.

The effects of ozone depuration on the pH, protein degradation and survival rate of the tropical oysters is shown in Figure 2. pH increased for control sample but gradually reduced with increase in the duration of ozone treatment. Reduction in pH may suggest that the oysters experienced stress forcing them to close the shell and undergo anaerobic metabolism (Jeong et al., 2021). pH is a useful indicator to determine the freshness of oysters. The pH of oysters between 6.3-6.5 reflecting very good condition, between 5.9 to 6.2 is good, 5.8 is identified as off, 5.5 - 5.7 is smelly or musty and <5.2 as putrid (Pardío-Sedas, 2015). Overall, it can be inferred that pH of the ozonated oysters did not vary much from the control sample. The TVB-N value ranged between 20.84 - 28.23 mg/100 g in control which was higher compared to all treated samples. High TVB-N value in fresh tropical oysters defined by the less glycogen content and greater nitrogen content present in the flesh as compared with the treated samples. High ozone concentration and longer depuration resulted in lower TVB-N value. Reduction in TVB-N may also suggest reduction in microbial count, thus lower degradation of protein and non-protein nitrogenous compound in which primarily was caused by microbial activity (Moosavi-Nasab et al, 2021). Ozone at 0.3 ppm seems to be sufficient in reducing TVB-

N value. The survival rate was comparable between 9.5 to 11 days especially at ozone concentration above 0.4 ppm. Six hours depuration significantly increased the oyster's survival to 14 days due to lower microbial load.

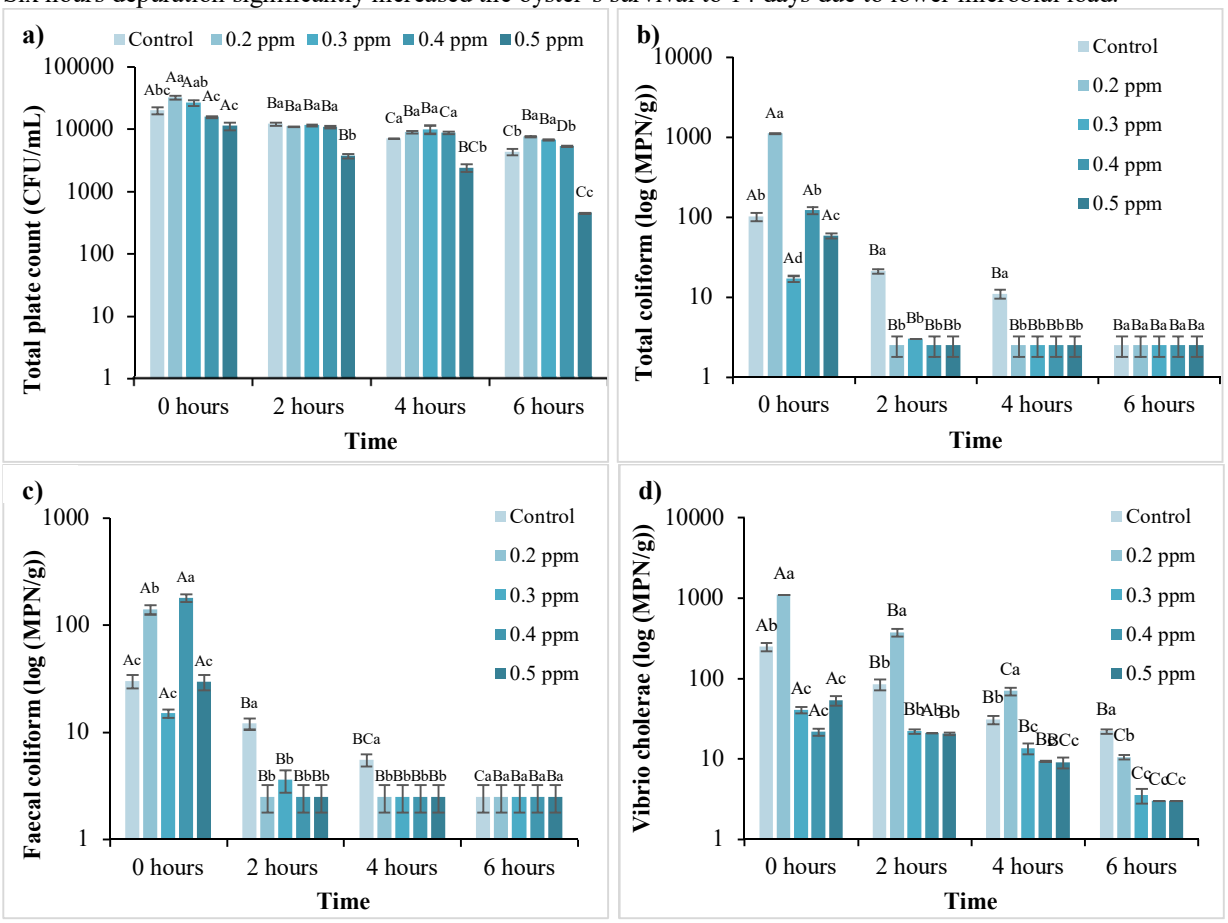

Figure 1: Total plate count (a), total coliform (b), feacal coliform (c), and *Vibrio cholera* (d) of tropical oyster depurated at different ozone concentrations.

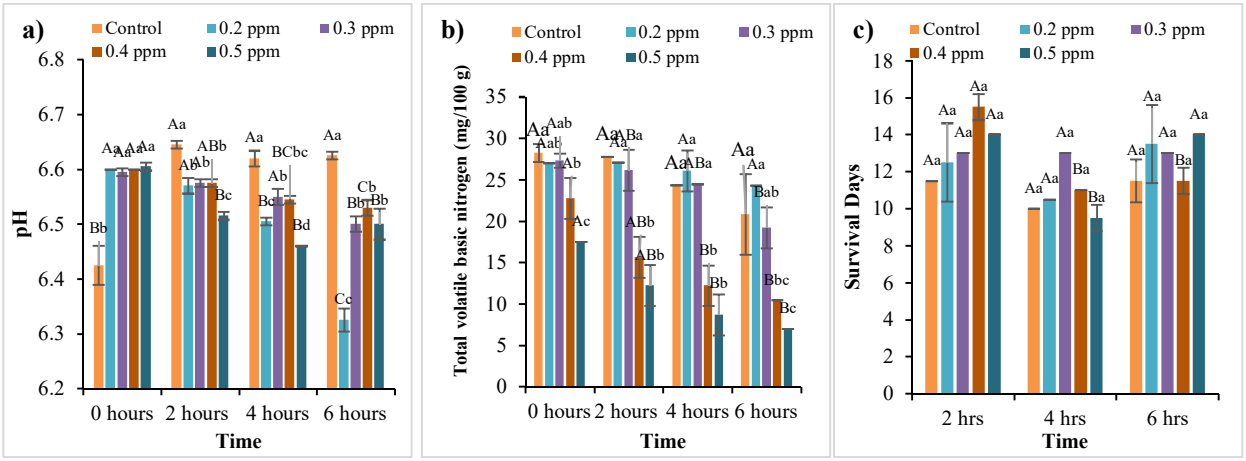

Figure 2: Changes in pH (a) and (TVB-N) of tropical oysters during depuration at different ozone concentration and their survival rate (c).

CONCLUSIONS

Direct ozone depuration has been shown to eliminate pathogenic microorganisms (*Vibrio spp., E.coli*) effectively in a short time at high loading capacity (triple oysters load per volume seawater), increasing survival rate and minimal

protein degradation of live oysters. The dissolved ozone also helps treated the water from organic compounds of the loaded oysters reducing the necessity of water treatment and usage.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
Borneo Convention Centre, Kuching, Sarawak

PHYSICOCHEMICAL PROPERTIES AND SENSORY ACCEPTANCE OF WATERMELON ROLL-UP

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Abstract: Watermelon biomass can be categorised into flesh, seed, and rind. Although discarded, the rind is edible and nutritious. The study aimed to optimise watermelon fruit rolls-up formulation combining the flesh and rind purees. F1 (80:0), F2 (70:10), F3 (60:20), F4 (50:30), F5 (40:40), F6 (30:50), and F7 (20:60) were formulated from watermelon flesh-to-rind purees. Samples were prepared by pasteurising the watermelon flesh and rind purees with a predetermined amount of ingredients at 90 °C for 5 min, then dried at 60°C for 11 hours, cut, rolled and packed in an aluminium polyethene bag until evaluation. There were significant differences in pH, total soluble solids (TSS), moisture content (MC), water activity (A_w) and thickness of the samples. It showed that the increase of watermelon flesh puree in the formulations reduced the pH value and thickness of the samples. By contrast, the increase in watermelon rind puree decreases the samples' TSS, MC and A_w values. For sensory acceptance, the F2 sample had the highest mean scores for colour, texture (chewiness), sweetness, sourness and overall acceptability, indicating it was the most preferred formulation among panellists. Thus, watermelon rind may be employed in watermelon fruit roll-up formulations to improve their physicochemical and sensory properties.

Keywords: watermelon, fruit roll-up, roll-up, physicochemical, sensory acceptance

INTRODUCTION

Watermelon (*Citrullus lanatus*) is a tropical fruit that grows in almost all parts of Africa and Southeast Asia. Watermelon contains vitamins C, A, B, amino acids, and lycopene. Watermelon biomass can be categorised into three main components: flesh, seed, and rind. The flesh constitutes approximately 68% of the total weight, the rind approximately 30%, and the seeds approximately 2%. Besides being eaten fresh, watermelon fruit can also be made into juice. Watermelon rind can also be eaten, but it is usually discarded due to its bland taste and lack of taste. Watermelon rind also contains various nutrients, including high in potassium, dietary fibre, and citrulline, a type of amino acid that smooths the blood circulation in the body and helps to relax the blood vessels. Citrulline is very good for the heart to prevent cardiovascular disease. Based on previous research reports, watermelon is high in citrulline, where citrulline content (based on dry weight) in the rind was higher (24.7 mg/g) compared with watermelon flesh (16.7 mg/g).

Watermelon has about 92% water content, thus making it susceptible to deterioration. Hence, reducing the moisture content to produce shelf-stable watermelon products, such as fruit roll-up, is crucial. Fruit roll-ups or fruit leathers are dried sheets of fruit pulp with a soft, rubbery texture and a sweet taste. Fruit roll-ups also can be produced from a mixture of fruits and eaten as a snack. It is chewy and flavourful, naturally low in fat and high in fibre and carbohydrates, lightweight and easily stored and packed. Consuming fruit roll-up is an economical and convenient value-added substitute for natural fruits as a source of various nutritional elements. Fruit roll-ups are healthy and organoleptically acceptable to customers. They are produced by dehydrating fruit puree into a leather sheet and rolled-up. Mainly, fruit pulps are mixed with appropriate quantities of sugar, pectin, acid, and colour and then dried into sheet-shaped products with added sugars and pectin to the roll-up. The sugar gave the product a sweeter taste and increased the solids content; then, pectin was used to thicken the pulp, modify the flexible texture, and ensure the retention of the shapes of the dried product. Fruit roll-up contains substantial amounts of dietary fibre, vitamins, and antioxidants. Therefore, the study aimed to develop a watermelon fruit roll-up, combining watermelon flesh and rind.

MATERIALS AND METHODS

Preparation of watermelon fruit roll-up

Watermelons were purchased from Pasar Borong Selangor, Seri Kembangan, Selangor, Malaysia. Watermelons were washed thoroughly, and the outer green rind was peeled. The red flesh and rind were segregated. Then, the flesh and rind were blended until they became slurry-like purees. The flesh and rind puree were stored in the aluminium polyethene bags at freezing temperature (-18°C) until further use. Seven formulations of watermelon fruit roll-up were developed based on the proportion of watermelon flesh and rind purees, respectively: F1 (80:0), F2 (70:10), F3 (60:20), F4 (50:30), F5 (40:40), F6 (30:50), and F7 (20:60) (Table 1). Samples were prepared by pasteurising the watermelon flesh and rind purees with a predetermined amount of ingredients, namely sugar, glucose syrup, maltodextrin, citric acid, vegetable oil, pectin, water, flavour and potassium sorbates at 90 °C for 5 min, then dried at 60°C for 11 hours, cut, rolled and packed in an aluminium polyethene bag until evaluation.

Table 1. Formulations of watermelon roll-up

Sample	Flesh puree (%)	Rind puree (%)	Other ingredients (%)
F1	80	0	20
F2	70	10	20
F3	60	20	20
F4	50	30	20
F5	40	40	20
F6	30	50	20
F7	20	60	20

Determination of total soluble solids and pH

The total soluble solid (TSS) contents were measured using a pocket refractometer (Atago, Tokyo, Japan) with a scale of 0–53°Brix. The pH of the samples was measured using a pH meter (FE20, Mettler Toledo, Switzerland).

Determination of moisture content

The moisture content was determined using the air oven method (AOAC 2000). The samples were dried in the oven at 105 °C for 24 h. The moisture content was calculated from the weight difference between the original and dried sample and expressed in percentage. The observation was done in duplicates for each sample and the average was reported.

Determination of water activity

The water activity (Aw) of fruit-roll samples was determined using a Labswift-aw hygrometer (Novasina, Switzerland). The dehydrated candies were cut into small pieces, loaded into a sample dish, and put in the measurement chamber. The equilibrium of the air humidity over a sample (water-vapour pressure), which is proportional to the Aw value, was measured. For each sample, duplicates were obtained and the mean was reported.

Determination of colour intensity

The colour intensity of the fruit-roll samples was measured using Chroma Meter Minolta CR- 400/410 (Minolta Co., Osaka, Japan) based on $L^* a^* b^*$ colour system. L^* denotes the lightness on a 0 – 100 scale from black to white, while a^* and b^* denote the redness (+) or greenness (–) and yellowness (+) or blueness (–) hues, respectively.

Determination of thickness

Fruit-roll sample thickness was determined using a digital micrometre (Mitutoyo, Japan). Five readings were taken randomly and averaged.

Sensory acceptability test

The sensory evaluation was carried out at the Food Science and Technology Research Centre, MARDI Serdang Selangor, Malaysia by 40 untrained panellists. Sensory attributes were evaluated according to the degree of liking in sweetness, sourness, taste, colour and overall acceptability. All samples were served and coded with random three-digit numbers. The sensory attributes of the samples were evaluated using a 7-point category hedonic scale (1 = dislike very much; 4 = neither like nor dislike; 7 = like very much) as described by Meilgaard *et al*. (1999).

Statistical analysis

All analyses were done in triplicate. Experimental data were subjected to the analysis of variance (ANOVA), and the significant differences among means were determined by the Least Significant Difference (LSD) at $p \leq 0.05$ using SAS software (Ver. 9.4., SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

Total soluble solid and pH

There were significant differences in total soluble solid (TSS) contents of the watermelon rolls-up samples, as shown in Table 2. Among the seven formulations, F1 significantly yielded the highest TSS (10.60 ± 0.17 °brix) while F7 yielded the lowest (8.13 ± 0.06 °brix). The result also showed that TSS significantly increased in formulations with a

higher ratio of watermelon flesh puree as the watermelon flesh puree showed significantly higher TSS (5.10 ± 0.01) than the rind puree (5.27 ± 0.01) (Table 1).

The pH of the formulated watermelon rolls-up in the present work ranged from 3.41 to 4.01, which could be classified as high-acid food (pH < 4.6) (Babajide *et al*., 2013), thus rendering the samples resistant to microbial spoilage. Table 2 shows significant differences in the pH of the rolls-up samples. F1 yielded the lowest pH (3.41 ±0.01), whereas F7 yielded the highest (4.01 ± 0.01). The result revealed that pH values significantly decreased in formulations with a higher ratio of watermelon flesh puree. This result could be due to the low pH of flesh puree (5.10 ± 0.01) as compared to rind puree (5.27 ± 0.01) (Table 1).

Table 2. Physicochemical properties of watermelon rolls-up formulations

Sample	pH	TSS (°brix)	MC (%)	A_W	Thickness (mm)
F1	3.41 ± 0.01d	10.60 ± 0.17a	16.90 ± 0.16c	0.54 ± 0.01e	0.58 ± 0.01e
F2	3.78 ± 0.01c	8.57 ± 0.23b	16.23 ± 0.06d	0.57 ± 0.01d	0.99 ± 0.09b
F3	3.83 ± 0.06c	8.70 ± 0.01b	21.63 ± 0.12a	0.67 ± 0.00a	0.70 ± 0.05d
F4	3.79 ± 0.04c	8.53 ± 1.27b	18.44 ± 0.49b	0.61 ± 0.01b	0.88 ± 0.05c
F5	4.00 ± 0.01a	8.77 ± 0.12b	14.67 ± 0.20e	0.53 ± 0.01e	0.95 ± 0.03b
F6	3.90 ± 0.01b	8.23 ± 1.79b	15.94 ± 0.20d	0.59 ± 0.01c	1.09 ± 0.08a
F7	4.01 ± 0.01a	8.13 ± 0.06b	12.90 ± 0.15f	0.52 ± 0.00f	1.06 ± 0.04a

Table 3. Physicochemical properties of watermelon flesh and rind purees

Sample	pH	TSS (°brix)	*L** (lightness)	*a** (redness)	*b** (yellowness)
Flesh puree	5.10 ± 0.01b	8.80 ± 0.01a	14.61 ± 0.07	6.91 ± 0.06	5.10 ± 0.05
Rind puree	5.27 ± 0.01a	4.40 ± 0.01b	22.69 ± 1.10	-0.88 ± 0.09	5.10 ± 0.41

Moisture content and water activity

Drying is the critical process of removing moisture from the fruit roll-up. The presence of higher moisture content results in an increase in microbial activity, thus spoilage of the product. According to FAO (2007), the moisture content for fruit leather or fruit roll-up should be in the range of 15- 25% so that the product can be kept longer without any deterioration caused by microorganisms. There were significant differences in the moisture content of the watermelon fruit roll-up samples, ranging between 12.90 - 21.63% (Table 2). The F7 sample had the lowest moisture content, while the F3 sample showed the highest moisture content after drying for 11 hours in the cabinet dryer. It can be seen that the samples' moisture content decreased with the increase of rind puree in the formulations. It may be due to the watermelon rind puree containing more pulp than flesh puree, which helped to speed up the drying process.

Water activity (A_w) is the amount of free water in food materials that the microorganisms can utilise for their growth. The decrease in the water activity value was associated with the reduction in water content, indicating the decrease in free water required for microorganism activity (Fachriah and Rahmawati, 2021). There were significant differences in the samples' water activity, ranging between 0.52 - 0.67, as the F7 sample recorded the lowest A_w value and the F3 sample showed the highest A_w value (Table 2). According to Jangam et al. (2008), food with water activity between 0.4 and 0.6 is considered a dry product, while food with water activity between 0.65 and 0.75 is regarded as an intermediate moisture-content food (IMF). All the samples were within the range of IMF since their A_w values were below 0.75. Food stability and safety are improved when the product's water activity is decreased.

Thickness

There were significant differences in the thickness of watermelon roll-up samples after drying. The thickness ranged between 0.58 - 1.06mm (Table 2). The result showed that the watermelon flesh and rind purees influence the samples' thickness in the formulations. The increase of rind puree in the formulations resulted in increased thickness of the samples after drying and vice versa. It may be due to the watermelon rind puree containing more pulp than flesh puree, which was watery as it contains more juice.

Colour intensity

Colour is important in product appearance, influencing consumers' acceptance of a food product (Duangmal *et al*., 2008). There were significant differences among the rolls-up samples for *L**, *a** and *b** values (Table 4). The F7 sample significantly yielded the highest *L** value (lightness), *b** value (yellowness) and the lowest *a** (redness) compared to other samples. The increase in *L* and b** values and decrease of *a** value in the samples was contributed by the increase of watermelon rind puree in the formulations since the rind puree had a higher *L** value and a lower

*a** value than the flesh puree (Table 3). The result indicated that adding higher rind puree concentration in the formulations produced lighter, less red and yellower colours of the watermelon roll-up samples.

Table 4. Colour intensity of watermelon rolls-up formulations

Sample	*L** (lightness)	*a** (redness)	*b** (yellowness)
F1	31.86 ± 0.04d	6.19 ± 0.04b	8.92 ± 0.02e
F2	31.12 ± 0.62d	5.99 ± 0.22b	10.24 ± 0.42d
F3	33.48 ± 1.49c	8.41 ± 1.18a	12.23 ± 1.30c
F4	33.19 ± 0.88c	3.24 ± 0.13c	16.81 ± 1.52a
F5	25.23 ± 0.51e	3.42 ± 0.05c	9.14 ± 0.12de
F6	38.96 ± 1.20b	8.04 ± 0.34a	15.55 ± 0.92b
F7	43.77 ± 0.30a	3.19 ± 0.05c	17.50 ± 0.06a

Sensory acceptance

The mean scores given by the panellists for sensory attributes of the watermelon roll-up samples are presented in Table 5. Significant differences were observed in all attributes, namely colour, aroma, texture (chewiness), sweetness, sourness and overall acceptance. Panellists gave F6 the lowest mean score for almost all attributes, including overall acceptance. On the other hand, the F2 sample received significantly highest score for colour (6.37), texture (5.74), sweetness (5.86), sourness (5.57) and overall acceptability (5.80) as compared to the other samples, indicating it was the most preferred formulation among panellists. A minimum sensory score of 5.0 for all attributes would be considered essential and acceptable to establish the best formulation of the watermelon roll-up. Therefore, F2 was chosen as the best formulation for watermelon roll-up.

Table 5. Mean score for colour, aroma, texture, sweetness, sourness and overall acceptability of watermelon roll-up formulations

Sample	Colour	Aroma	Texture	Sweetness	Sourness	Overall acceptance
F1	6.09 ± 1.29a	5.43 ± 1.20a	5.06 ± 1.33b	5.74 ± 1.09ab	5.57 ± 1.29ab	5.54 ± 1.15ab
F2	6.37 ± 0.88a	5.37 ± 1.35a	5.74 ± 1.34a	5.86 ± 1.24a	5.77 ± 1.14a	5.80 ± 1.13a
F3	5.91 ± 1.42a	5.37 ± 1.19a	5.80 ± 1.13a	5.77 ± 0.97ab	5.54 ± 1.12ab	5.77 ± 1.06ab
F4	5.91 ± 0.92a	5.14 ± 1.31ab	5.06 ±1.11b	5.37 ± 1.11ab	5.26 ± 1.17ab	5.23 ± 1.06bc
F5	4.77 ± 1.14b	5.03 ±1.01ab	5.34 ± 1.08ab	5.46 ± 1.01ab	5.23 ± 1.19ab	5.23 ± 1.00bc
F6	4.57 ± 1.36b	4.66 ± 1.33b	4.20 ± 1.51c	4.69 ± 1.39c	4.43 ± 1.44c	4.51 ± 1.44d
F7	4.23 ± 1.46b	4.60 ± 1.31b	4.77 ± 1.37bc	5.29 ± 1.18b	5.00 ± 1.14cb	4.94 ± 1.21cd

CONCLUSIONS

The physicochemical properties and sensory acceptance of watermelon roll-up formulated from flesh and rind purees have been evaluated. The results showed that the different ratios of flesh and rind purees in the watermelon roll-up formulations significantly affected the total soluble solids, pH, moisture content, water activity, colour intensity and sensory acceptance of the formulated samples. The increase of flesh puree in the formulations significantly reduced the samples' pH values and thickness after drying. On the contrary, increased rind puree in the formulations significantly resulted in lower total soluble solids, moisture content and water activity values. Panellists preferred the F2 formulation for sensory acceptance, as evidenced by the highest mean score received for all attributes compared to the other samples. Therefore, it can be concluded that the F2 formulation was the best formulation for watermelon roll-up.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
Borneo Convention Centre, Kuching, Sarawak

STABLE ISOTOPE AND MULTI-ELEMENTAL ANALYSES OF PHILIPPINE CACAO BEANS *(Theobroma cacao L.)* USING IRMS AND XRF FOR GEOGRAPHICAL ORIGIN IDENTIFICATION

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Abstract: Origin mislabeling has been an alarming issue in many industries concerning products of high value and those possessing qualities associated to its origin, like cacao. Philippines, given its strategic location and climatic conditions, has several regions producing cacao with distinctive qualities which are attributable to the farm location, specific agricultural practices, and other factors. To establish the profiles and to prevent origin mislabeling, the isotopic ratios ($\delta^{13}C$ and $\delta^{15}N$) and multi-elemental compositions of 31 dried fermented cacao bean samples sourced from 3 regions were analyzed using IRMS and portable XRF, respectively. Random Forest (RF) was trained to predict the region of origin of cacao samples using the obtained data. Isotopic analysis yielded $\delta^{13}C$ values ranging from -53.75‰ to -30.01‰, while $\delta^{15}N$ values ranged from 0.64‰ to 5.73‰. Additionally, out of 24 elements detected, 11 elements (K, Mg, P, S, Cl, Cu, Cr, Mn, Rb, Sr, Zn) were consistently present in all regions, with K being the most abundant element. Integrating both datasets to RF generated a model with 77.42% training accuracy which indicated that the geographical origin of cacao beans may be differentiated using their isotopic and multi-elemental profiles, thus making this an innovative tool to address mislabeling among products.

Keywords: Philippine cacao, geographical origin, stable isotope ratio, multi-elemental analysis, product mislabeling

INTRODUCTION

Philippines is among the tropical countries within the "cacao belt", a region between 10 to 20 degrees north and south of the equator, where cacao trees grow typically due to the suitable climatic conditions in these areas. It ranked 24th among the top countries producing cacao beans. Latest report showed that Philippines produced 9,340.73 MT from the 31,285.36 ha of cacao plantation sites (DA-HVCDP, 2022). From 2013 to 2020, the average annual increase in area planted with cacao was 2,743 ha which may be attributed to the seedling dispersal programs conducted by the Department of Agriculture (DA), Department of Environment and Natural Resources (DENR), and the Philippine Coconut Authority (PCA).

The strategic location and climatic condition in the Philippines give rise to multiple growing areas of cacao, most of which are located in Mindanao. The Davao region alone produced 78% of the national cacao production, with a collective yield of 7,257.85 MT from 19,975 ha of land in 2020. The rest of the regions in Mindanao represented 11% of the total cacao production, while certain regions in Luzon and Visayas shared the remaining 11% wherein Aurora and Iloilo are the top producing provinces (DA-HVCDP, 2022).

The geographical origin of cacao, including all the factors associated with it such as the localized weather patterns, soil and water composition, and agricultural practices, influences the composition profile and unique qualities of cacao beans. Identical varieties from different origins may taste differently and bear distinctive characteristics which may be attributed to the location where they originated from. Given that there are several plantation farms in the Philippines for cacao, establishing the identities of cacao beans based on their respective origin is instrumental to assess product authenticity through disclosure of commercial frauds such as mislabeling. Moreover, it protects consumers by guaranteeing food safety and quality and benefits producers, especially smallholder cacao farmers, who are interested in protecting their brands and in improving their products' marketability. In Europe, there is a growing trend among consumers to associate a particular product origin with quality (Perez *et al*., 2020). As the higher quality cacao beans are more expensive, the application of food authenticity techniques for origin identification of Philippine cacao beans could be an effective scientific means to devise more refined regulatory and governance actions for quality control, trade, marketing, and innovation.

In agrarian food traceability, the distribution of stable isotopes is studied since isotopes reflect both meteorological events (precipitation, condensation, and evaporation) and geographical location (altitude, latitude, and continent). Additionally, multi-elemental profiling is another useful origin traceability technique as edaphic and environmental factors such as fertilization, soil

type, climate and temperature easily change metallic elements (Perez *et al*., 2020). In the Philippines, stable carbon isotope ratio analysis had only been employed in detecting honey adulteration using C4 sugars (Lao *et al*., 2021). Application of the same technique, coupled with elemental determination, has not been explored in geographical origin identification of Philippine cacao beans. Hence, the aim of this work was to analyze the stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) and multi-elemental compositions of 31 dried fermented cacao bean samples from three growing regions in the country using IRMS and a portable X-ray Fluorescence (pXRF), respectively. A machine learning tool, Random Forest (RF), was also utilized to determine whether the specific isotopic and elemental composition profiles of cacao beans may be used to differentiate their region of origin.

MATERIALS AND METHODS

Sample Collection and Preparation

A total of 31 samples of dried fermented cacao beans were collected from three regions in Mindanao. Five samples were sourced from Zamboanga Peninsula (Region IX), 18 from the Davao region (Region XI), and 8 from the SOCCSKSARGEN region (Region XII).

All cacao samples were oven-dried for 24 hrs at 60℃ using the BOV-V230F IOB SE electrothermal blast drying oven. The shells of the cacao beans were removed manually before grinding with an agate mortar and pestle. The ground samples were further subjected to particle size reduction using Krups coffee blade grinder F203 to produce fine, powdered samples. Representative portions of ground samples were obtained by coning and quartering. These were placed in resealable plastic pouches and stored in a desiccator until analyses.

Stable Isotope Analysis

About 3-4 mg of ground cacao samples was weighed, added to 6x4 mm tin capsules, folded securely, and then placed in a 96-well microplate. These were analyzed for $\delta^{13}C$ and $\delta^{15}N$ in triplicates using IRMS (Sercon 20-22 IRMS) following total combustion in an elemental analyzer (Sercon SL and GSL Elemental Analyser). To ensure the accuracy of the instrument, the following IPE standards were used in the analysis: IPE-2019-2 (willow wood), IPE-2017-3 (banana leaf), and IPE-2014-3 (tobacco leaf mixture).

The concentrations of C and N were expressed in %, while $\delta^{13}C$ and $\delta^{15}N$ were expressed in ‰. The stable isotope ratio (δ) was calculated against the reference standards:

Equation 1

$$\delta\ (‰) = (R_{sample}\text{-}R_{standard})/R_{standard} * 1000$$

Where R is the isotope ratio of an element (e.g., $^{13}C/^{12}C$)

Multi-Elemental Analysis

About 2 g of each sample was pelletized using 3636 X-Press hydraulic laboratory pellet press. The pellet press was set to 5 tons pressure, 15 sec dwell time, and 30 sec release time to obtain 31 mm in diameter and 3.5 mm thick pellets, which were then stored in cups.

A pXRF (Bruker 2020 S1 TITAN Model 800) with a rhodium target x-ray tube and graphene window silicon drift detector (50 kV, 5-100μA) was used for the semi-quantitative elemental analysis. The equipment was set to the calibration GeoExploration and the method Automatic Calibration Selection. Each pellet was measured for 90 sec for every reading and in triplicate by varying its position within the surface of the pellet.

To measure the reliability of the instrument, reference values of four plant standards from the National Institute of Standards and Technology (NIST) were obtained. The plant standards used were IPE-2018-2-2 (poplar leaf), IPE 2018-2-4 (tomato), IPE-2018-3-2 (cacao leaf), and IPE-2018-3-4 (lucerne).

Statistical Analysis

A machine learning algorithm, specifically RF, was trained to predict the region of origin of cacao samples using the isotopic and elemental data. The analysis involved the use of the open-source software R version 4.2.2 as well as RStudio. Generating the RF model required the setting of the following parameters: n_{trees}, which pertains to the number of decision trees, and m_{try}. The decision trees are grown by looking for the best variables from a subset having randomly selected features. The RF automatically divided the data into train and test sets to generate an out-of-bag (OOB) error rate. Based on the initial default model and OOB error rate, the parameters n_{tree} and m_{try} were set to 1100 and 6, respectively. A multidimensional scaling (MDS) plot was then generated to visualize the distribution of the samples according to their isotopic and multi-elemental profiles.

RESULTS AND DISCUSSION

The $\delta^{13}C$ ratios of 31 dried fermented cacao beans from three growing regions in Mindanao ranged from -53.75‰ to -30.01‰, while $\delta^{15}N$ values ranged from 0.64‰ to 5.73‰. Figure 1 shows the distribution of $\delta^{13}C$ and $\delta^{15}N$ isotopes data wherein it can be observed that there is a larger spread among $\delta^{15}N$ values compared to $\delta^{13}C$ data. A sample from Region XI exhibited a $\delta^{13}C$ ratio of -53.75‰ which was the only data point that deviated from the observed -33.17‰ to -30.01‰ $\delta^{13}C$ values from the rest of the samples. Similarly, a sample from Region XII gave a 1.15‰ $\delta^{15}N$ which differs relative to the other samples within the same region.

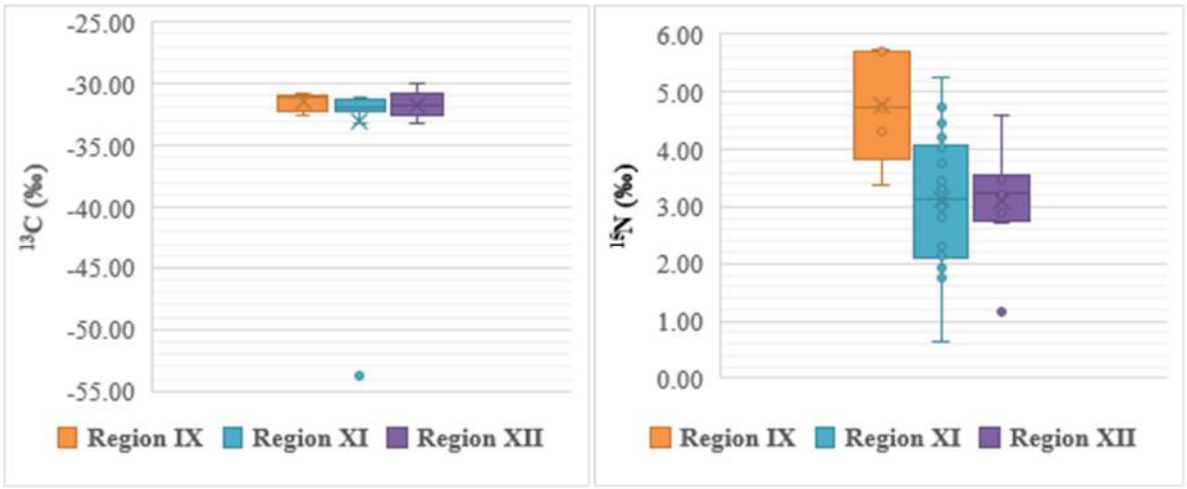

Figure 1. Box and whisker plots of δ^{13}C and δ^{15}N from cacao bean samples from three regions in the Philippines.

Comparison of the variations between δ^{13}C and δ^{15}N ratios shows that the latter is a better discriminating variable for geographical origin identification in terms of stable isotope ratio. Carbon isotope ratio (δ^{13}C) is highly dependent on the environment (Perez *et al.*, 2020). C3 plants, like cacao, exhibit carbon isotope compositions ranging from −20‰ to−37‰, generally reflecting a physiological response to aridity (anomalously high δ^{13}C) and a combination of low light levels plus leaf litter recycling (anomalously low δ^{13}C) (Kohn, 2010). Given that all cacao samples analyzed in this study came from Mindanao island, the regions where they were collected from share fairly similar weather patterns resulting to narrow range of δ^{13}C ratios and small variations among regions. On the other hand, nitrogen can be acquired from both the atmosphere through biological fixation and through the soil from application of synthetic or organic fertilizers. The unique agricultural practices done in each farm give rise to variability of δ^{15}N ratios that is useful for geographical origin differentiation.

For multi-elemental analysis, 24 elements were detected by the pXRF which were as follows: Mg, P, S, Cl, K, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Y, Nb, Pd, Cd, Ba, Pt, Au, and U. Of these elements, only 11 were consistently present in all the cacao samples, the average concentration of which are listed in Table 1. The remaining elements consisted of transition and few rare earth metals and had concentrations below the limit of detection. The major elements analyzed from the cacao bean samples were K, Mg, and P, with K being the most abundant. This was in accordance with the study of Bertoldi *et al.* (2016) in which K was also found to be the most abundant macro-element in cacao using ICP-MS. Sulfur was also measured in minor concentrations less than 1% in all three regions. Trace elements include Cl, Cu, Cr, Mn, Rb, Sr, and Zn with concentrations expressed in ppm.

Table 1. Elemental content present in all cacao bean samples from Regions IX, XI, and XII. K, Mg, P, S are expressed in %, while Cl, Cu, Cr, Mn, Rb, Sr, Zn are expressed in mg/kg.

Element	Mean ± SD		
	Region IX (N = 5)	Region XI (N = 18)	Region XII (N = 8)
K	2.44 ± 0.15	3.37 ± 2.23	2.64 ± 0.47
Mg	1.01 ± 0.05	1.13 ± 0.53	1.02 ± 0.12
P	1.38 ± 0.11	1.44 ± 0.26	1.64 ± 0.34
S	0.56 ± 0.03	0.72 ± 0.44	0.56 ± 0.03
Cl	732.60 ± 67.55	850.20 ± 831.58	857.38 ± 240.19
Cu	39.60 ± 5.41	125.31 ± 203.43	38.17 ± 16.48
Cr	51.27 ± 3.49	69.37 ± 58.71	51.04 ± 5.72
Mn	293.07 ± 84.57	244.81 ± 118.99	195.25 ± 82.29
Rb	20.73 ± 4.85	12.46 ± 6.36	18.79 ± 7.60
Sr	29.13 ± 2.30	68.69 ± 43.96	51.00 ± 14.48
Zn	51.47 ± 2.80	126.04 ± 185.64	56.88 ± 9.54

Differences based on the isotopic and elemental data of cacao beans were assessed using RF in order to determine whether the region of origin of the samples is distinguishable with respect to their profiles. RF is a classifier that utilizes decision trees that were trained using the bootstrap method and randomization to make an aggregated prediction. The decision trees are grown by

looking for the best variables from a subset having randomly selected features (Sukanya *et al.*, 2021). Integration of both stable isotope ratios and multi-elemental compositions of cacao samples from the three growing regions generated a RF of 1100 decision trees (n_{tree}) with 6 variables at each split (m_{try}). Such parameters resulted to OOB estimate of error rate at 22.58%. The confusion matrix from this model (Table 2) shows that 3 out of 5 samples from Region IX were correctly classified, only 1 sample from Region XI was misclassified, and half of the samples from Region XII was correctly predicted.

Table 2. Confusion matrix of RF (n_{tree}=1100, m_{try}=6).

Predicted Values	Actual Values			
	Region	IX	XI	XII	Class Error
	IX	3	1	1	0.40000000
	XI	0	17	1	0.05555556
	XII	0	4	4	0.50000000

The generated model had an accuracy of 77.42%. The MDS plot in Figure 2 provides a visualization of the multivariate data wherein each point corresponds to a single sample and the regions of origin are differentiated by color. From the plot, it can be observed that data points are scattered and lack regional clustering. Such observation may be attributed to small sample size per region and may require fine tuning of the model for better differentiation.

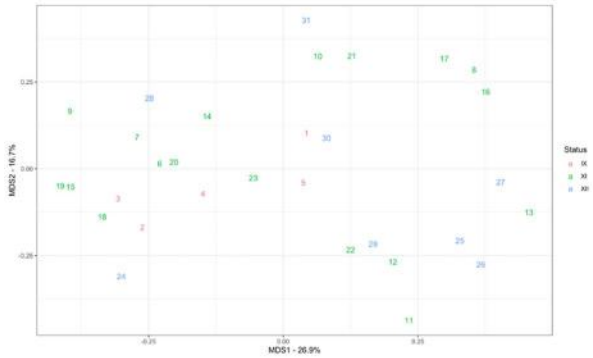

Figure 2. Multidimensional Scaling (MDS) plot differentiating cacao bean samples based on region of origin.

CONCLUSION

Analysis of the stable isotope ratios and multi-elemental composition allowed profiling of Philippine cacao beans for geographical origin identification. In this study, 31 dried fermented cacao beans from three growing regions in the Philippines were analyzed for their respective isotopic and elemental profiles. Comparing the data obtained for $\delta^{13}C$ and $\delta^{15}N$ showed a larger distribution of $\delta^{15}N$ ratios, which may be attributed to the dissimilarity of agricultural practices among farms, as compared to $\delta^{13}C$ that is highly dependent on weather and environment. On the other hand, determination of multi-elemental content using pXRF resulted to detection of 24 elements wherein K, Mg, and P were measured in major amounts. These datasets were subjected to RF, a machine learning tool, that can be trained to predict the region of origin of cacao samples using isotopic and elemental data. It generated a model with 77.42% accuracy which can be further improved by fine tuning and addition of more samples per region.

In general, the application of IRMS and XRF for food authenticity and traceability are useful techniques in potentially addressing commercial frauds like mislabeling to protect both producers and consumers. The isotopic and elemental profiles of Philippine cacao beans may serve as signatures in establishing their national identities and in geographical origin identification.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
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DISASTER RESILIENCY: THE DEVELOPMENT OF READY-TO-EAT FOOD PRODUCTS FOR CHILDREN AGED ONE TO FIVE YEARS OLD

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Abstract: The Philippines is third on the World Risk Report's list of countries with the highest potential for damages from natural disasters due to its location along the Pacific Ring of Fire and Typhoon belt. The Philippine Women's College of Davao conducted research on disaster resilience with funding from the Department of Science and Technology – Philippine Council for Health Research and Development (DOST-PCHRD). The study aimed to contribute to the development and production of safe and nutritious emergency foods for children aged one to five. The results showed that children prefer processed foods, fruits, and vegetables, except okra and ampalaya, due to their appearance and taste. Some respondents do not consume pork dishes due to religious beliefs. The survey revealed a lack of preparation in disaster-prone areas, with the majority relying heavily on emergency food supplies. The Ready-To-Eat (RTE) products developed from the research were All-Veggie Monggo Meal, All-Veggie Cornmeal Porridge, Fruit-Veggie Leather with Peanuts and Puffed Rice, and Seed-Enriched Whole Grain Cookies. These RTE products were compliant with microbiological standards and achieved a "liked" rating in sensory evaluations. Future studies may include using other commodities to make these emergency foods. The outcomes of this project can be incorporated into regional feeding programs for children aged one to five years old.
***Keywords*:** survey, disaster resiliency, emergency foods, Philippine Council for Health Research and Development

INTRODUCTION

The Philippines ranks third in the World Risk Report (2018) for natural disaster damage, with 74% of its population at risk. Climate change has worsened the effects of both natural and man-made disasters, affecting farming and food availability. Natural disasters and floods are becoming more frequent and intense, compromising food and water safety. Man-made disasters and pandemics also pose challenges in food safety and security, as food can become contaminated with pollutants, leading to foodborne illnesses. Food preparation becomes difficult due to a lack of fuel, water, and power.

This research project generally aimed to develop safe and nutritious food products for children aged one (1) to five (5) years old that can be used during calamities or disasters when access to potable water and safe food is limited. Specifically, this research aimed to obtain information about the current food security practices in the flood-prone areas in Davao City as provided by the households and/or supplemented by the Davao City's City Disaster Risk Reduction Management Office (CDRRMO). It also aimed to create four (4) shelf-stable food products that meet the nutritional requirements of children in this age group.

The study utilized both qualitative and quantitative research methods to develop safe and nutritious emergency foods for children aged one to five. The research involved interviews with mothers or legal guardians and

Barangay Health Workers (BHW) to gather baseline data. The findings were used to develop four Ready-To-Eat (RTE) foods, divided into snack foods and meal foods. The development process involved screening raw materials based on food preferences, considering nutritional content, affordability, and availability. The sensory evaluations were conducted with mothers and main respondents to determine the best formulation. The final product formulations and packaging designs were standardized, and laboratory analysis was conducted to ensure the product's safety.

The results showed that children prefer processed foods, fruits, and vegetables, and rely heavily on emergency food supplies. The Ready-To-Eat (RTE) products developed from this research were All-Veggie Monggo Meal, All-Veggie Cornmeal Porridge, Fruit-Veggie Leather with Peanuts and Puffed Rice, and Seed-Enriched Whole Grain Cookies. These RTE products were compliant with microbiological standards and achieved a "liked" rating in sensory evaluations. Future studies may include using other commodities to make these emergency foods. The outcomes of this project can be incorporated into regional feeding programs for children aged one to five years old.

MATERIALS AND METHODS

This research was conducted in three phases which are as follows:

Phase 1. The researchers conducted a focus group discussion (FGD) with community residents, barangay officials, local government units, and government offices to discuss disaster management procedures and challenges. The initial process involved selecting raw ingredients based on nutritional requirements and combining them with suitable packaging materials. The study standardized the process and evaluated the nutritional content, commercial sterility, and shelf life of ready-to-eat (RTE) formulations. However, the COVID-19 pandemic changed the procedure, and the focus shifted to research and consulting with Davao City's City Disaster Risk Reduction Management Office. The team identified four barangays as disaster-prone, considering previous disasters, recurrence frequency, and population density. Data was collected through one-on-one interviews using a mixed-method approach, with a multi-method randomization strategy to reduce potential bias. The team collaborated with local barangay units and health workers to ensure successful research. Thirty (30) parents or guardians were interviewed in each barangay, and a preliminary survey was completed by 120 respondents.

Phase 2. The survey results prompted the development of four Ready-To-Eat (RTE) foods, divided into snack and meal foods. The development process involved screening raw materials based on food preferences, considering nutritional content, affordability, and availability. Formulations were developed based on these ingredients, with an initial evaluation by a registered nutritionist and dietitian. Sensory evaluations with mothers and respondents were conducted to determine the best formulation. Process parameters were established, and suitable packaging material was selected. Laboratory analysis was conducted, and microbiological results were ensured before sensory evaluations with children. The second phase focused on consumer acceptability of the final products, with twenty children interviewed in each barangay. Households that participated in the initial survey were prioritized for the subsequent survey on the acceptability of the developed RTE products. After determining the product's acceptability, the researchers created the packaging label by commissioning skilled labeling artists from the institution.

Phase 3. Making the product known to the target market as well as other potential clients was the final stage of the research process. An audio-visual presentation, which had been developed by the researchers through the service of a professional videographer, was aimed to be included among the resources that would be shown to each potential customer. A product launch was also scheduled to take place in conjunction with the opening of the newly installed toll packing center in the Food Processing and Innovation Center. In addition, an application for the utility model registration for the RTE products was submitted to the Philippine Intellectual Property Office.

RESULTS AND DISCUSSION

Food scientists play a crucial role in the creation of novel products, although their involvement varies by industry (Aramouni & Deschenes, 2015). It is crucial to remain current on market trends and consumer preferences, taking into account factors such as religion, ethnicity, age, and personal experiences. Scientists and technologists in the food industry must be intimately familiar with their target markets in order to design products that meet consumer expectations.

The preferences of consumers change over time due to their life experiences and the prevailing scientific theories during their formative years (Aramouni & Deschenes, 2015). The first part of this research was conducted to determine the food preferences of children based on their age as shown in Table 1. The results showed that 4 and 5-year-olds preferred processed foods like hotdogs, while 3-year-olds preferred vegetables, 2-year-olds preferred chicken meat, and 1-year-olds had no specific preference. The research also found that households where mothers are both the purchaser and the cook tend to consume more convenience foods. Most respondents did not provide examples of meals they dislike, but when asked about their preferred food type or appearance, the majority answered soup.

Table 1. Summary of food preference of children per category according to age.

Age	Favorite Foods	Disliked Foods	Preferred Food Type	Consumption of Convenience Foods	Purchaser in the Family	Cook in the Family
1	Nothing in particular	Nothing in particular	Soup	No	Mother	Mother
2	Chicken	Nothing in particular	Soup	Yes	Mother	Mother
3	Vegetables	Nothing in particular	Soup	Yes	Mother	Mother
4	Hotdog	Nothing in particular	Soup	Yes	Mother	Mother
5	Hotdog	Nothing in particular	Soup	No	Mother	Mother

The influence of mothers on their children's food preferences and nutrition is a crucial factor that can have long-term effects on their diet quality and overall health (Scaglioni et al., 2018; Beckerman et al., 2017; Paroche et al., 2017). Mothers play a dual role as both consumers and preparers of food within households. Research has demonstrated a correlation between the food preferences of mothers and the dietary habits of their children (Beckerman et al., 2017; Scaglioni et al., 2018). According to Kähkönen et al. (2021), the eating attitudes of fathers have a greater impact on children's preferences, and extensive research has demonstrated the significance of cultivating a preference for fruits and vegetables in early childhood. This preference is crucial in the prevention of chronic diseases, such as cardiovascular disease and obesity. Therefore, it is crucial to recognize the significance of the mother's role in ensuring their children's vegetable intake and addressing the obstacles that hinder the improvement of vegetable consumption. Furthermore, Paroche et al. (2017) emphasize the importance of understanding children's learning processes, including imitation, statistical learning, causal learning, and causal explanations of events. Different cultures have different norms and preferences regarding vegetable consumption, so it is crucial to consider the cultural context of children and their parents' educational levels. Food beliefs are closely linked to cultural and religious values, making sociocultural analysis crucial for policy planning and design (Monterrosa et al., 2020; Looby et al., 2020). While no significant association was found between parental religious behaviors and children's dietary behaviors, parental involvement in religious activities may influence children's dietary behavior, potentially influencing their future health habits (Wu et al., 2020). Religious beliefs and practices are transmitted intergenerationally through the family, and religious traditions and communities must endure (Vermeer, 2014).

During disasters, the majority of the population, including children, the elderly, the poor, and people with disabilities, are most vulnerable. Food and water security are compromised during emergencies, with limited options for preparing food. In the Philippines, emergency food supplies include canned foods, noodles, coffee, rice, and crackers, which require water and heat and may contain excessive salt, sugar, or preservatives. A survey found that 77% of the population prepared food for a disaster at the start of the disaster, and 75% of it was shared with children aged one to five.

After the survey was conducted, the research team developed four semi-solid and two dry food products based on the dietary preferences of children aged one to five years old in the study areas. The formulations were determined by sensory qualities, cost, and nutritional content. The team aimed to produce nourishing, affordable, and convenient foods for children during emergencies. The final decision was made with the participation of target communities and some mothers on campus. Food insecurity in the Philippines is a significant issue, with limited availability of safe and nutritious foods and uncertainty in availability. Research shows that households struggling with food insecurity tend to consume less diverse diets. The team aimed to contribute to the nutritional needs of children aged one to five affected by natural disasters.

The research produced the Ready-To-Eat (RTE) products, namely the All-Veggie Monggo Meal, All-Veggie Cornmeal Porridge, Fruit-Veggie Leather with Peanuts and Puffed Rice, and Seed-Enriched Whole Grain Cookies. The RTE products demonstrated promising characteristics as emergency food items. The products met microbiological standards and received a favorable rating in the sensory evaluation for color, aroma, taste, texture, appearance, and general acceptability. The shelf-life studies conducted using the direct method also received a passing remark. Potential future research endeavors could involve the utilization of alternative commodities for the production of these emergency food products. The project's outcomes can be integrated into the regional feeding programs targeting children aged one to five years old.

CONCLUSIONS

In the Philippines, households struggle with food shortages, especially during natural disasters. Addressing food security and nutrition is crucial during such times. The development of market-driven emergency foods is essential to address these concerns. Research is needed to develop these products. Children prefer processed foods, while religion influences their consumption. Ready-to-eat foods with high nutrients are on the rise, with ready-to-eat products like All-Veggie Monggo Meal, All-Veggie Cornmeal Porridge, Fruit-Veggie Leather with Peanuts and Puffed Rice, and Seed-Enriched Whole Grain Cookies showing potential as emergency food products.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
Borneo Convention Centre, Kuching, Sarawak

IN SILICO STUDY ON THE ALLERGENICITY OF UNDERUTILIZED LEGUMES IN THE PHILIPPINES

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Abstract: With the current global challenges such as food insecurity, poverty, and climate change, threats to food availability, production, and nutritional security are continuously arising. To effectively address the increasing global food demands, it is imperative to prioritize the cultivation and utilization of underutilized legumes, as they hold immense potential for enhancing food and nutrition security. However, the successful exploitation and utilization of these legumes heavily rely on the existing knowledge regarding them. Therefore, this study aimed to investigate the potential allergenicity of underutilized legumes in the Philippines, specifically Psophocarpus tetragonolobus (winged bean), Lablab purpureus (hyacinth bean), and Phaseolus lunatus (lima bean), through in silico studies. The protein sequences of the storage proteins of the specified legumes were obtained from UniProt, and subsequently analyzed for their potential allergenicity through AlgPred 2.0 and Allermatch. The analysis revealed that all three legumes were potential allergens as they were found to be cross-reactive with other known allergens. Furthermore, only the IgE epitope of Phaseolin was found to be digestible by the enzymes pepsin (with pH 1.3 and >2), chymotrypsin (low and high specificity), and trypsin. Other allergens were found to either have no epitopes or indigestible to the specific gastric enzymes. These underutilized legumes, although they have tremendous potential as food sources, should undergo characterization, specifically regarding their potential allergenicity, before promoting their utilization and commercialization.
***Keywords*:** in silico, legumes, allergenicity, bioinformatics, food insecurity

INTRODUCTION

The continuous and rapid increase of the global population has led to the emergence of numerous problems such as environmental, economic, and health (Ericksen 2008). Among the challenges currently being faced by the global community is food insecurity, wherein providing sufficient, nutritious, and safe food for every individual becomes uncertain. Multiple attempts have been made to address this issue such as intensification of food production and distribution. However, most processes being used were reported to bring negative environmental impacts and greatly contribute to climate change. Due to these concerns, a call to increase agricultural productivity and output, reduce environmental impact, alter systems of governance and consumption patterns, and switch to more sustainable methods of food production has been prompted (Godfray & Garnett 2014). Adopting cultivation of future food crops and exploiting underutilized crops were among the proposed strategies to improve food security (Cheng 2018; Nayak et al. 2022). This refers to the need for both major and minor underutilized crops to be utilized and commercialized more (Nayak et al. 2022). Underutilized crops are those that are grown and utilized locally, but are less used elsewhere due to agronomic, economic, or cultural reasons (Cheng et al. 2019).

Legumes are plants that come from the Leguminosae or Fabaceae family, which are known to be highly diverse and rich sources of biomolecules. Among the 19,000+ species, only a few of them are much utilized, and many are still left for improvement and domestication. Their wide distribution around the world suggests that they are able to withstand different and changing seasonal and temperature changes (Nayak et al. 2022). Aside from this, legumes are known to be the best plant-based source of proteins, containing up to 20-45% essential amino acids. They also contain complex carbohydrates, dietary fiber, vitamins, minerals, and other components with health benefits, which make legumes an ideal food crop (Maphosa & Jideani 2017; Nayak et al. 2022). In the Philippines, indigenous food legumes such as mung bean (*Vigna radiata*), jack bean (*Canavalia ensiformis*), and rice bean (*Vigna umbellate*), are among the most utilized and studied for their biochemical and nutritional qualities. However, other legumes such as *Psophocarpus tetragonolobus* (winged bean or sigarilyas), *Lablab purpureus* (hyacinth bean or bataw), and *Phaseolus lunatus* (lima bean or patani) are among the underutilized and understudied (Nayak et al. 2022; Tecson-Mendoza 2007). Research and journal publications on the characterization of these legumes, particularly the identification and

profiling of the allergens, were found to be very limited. It is important to note that characterizing the allergenicity of legumes is necessary due to their high possibility of cross-reactivity, making their high protein content accountable for their high potential allergenicity and high risk of inducing symptoms to atopic individuals.

Allergenicity is defined as the ability of an antigen to induce an abnormal immune response that can cause physiological function disorder or tissue damage. It exhibits antigen specificity, meaning it can react on different levels and to different individuals (Zhang & Tao 2015). In the study of Riascos et al. (2009), they were able to identify 13 allergen-containing protein families among legume species which includes cupins, cereal prolamin family, profilins, and others. Among the reported common symptoms of allergies on legumes are oropharyngeal, acute urticaria, anaphylaxis (Vitaliti et al. 2015), and respiratory allergies through cross-sensitization with other proteins (Riascos et al. 2009). One of the new methodological processes of assessing potential allergenicity of species or substances is through in silico studies – a tool used to identify potential allergens in a quick and straightforward way (Deocaris et al. 2020).

Due to the lack of research on the identification, profiling, and inhibition of allergens in local underutilized legumes, there is a need to conduct a characterization on their potential allergenicity. Thus, this study was conducted to assess the potential allergenicity of local underutilized legumes. Specifically, the study aimed to (1) determine the specific allergens present in the legumes through in silico approach, and (2) determine the effect of proteolytic digestion in the allergenicity of legumes. However, this study only focused on assessing the potential allergenicity of the local underutilized legumes winged bean, hyacinth bean, and lima bean. Other types and species of legumes were excluded from this study. Moreover, no additional approaches other than in silico analysis were used to complete the assessment.

MATERIALS AND METHODS

The protein sequences of winged bean, hyacinth bean, and lima bean were retrieved from the UniProt (Universal Protein Resource) database (https://www.uniprot.org/), a public functional genomics data repository. The protein sequences retrieved were only limited to their storage proteins, as it was reported to contain most of the allergenic proteins (Martínez San Ireneo et al. 2008). All sequences retrieved were in their FASTA format, following the 80-amino-acid sliding window. The retrieved protein sequences were then subjected to allergenicity screening using AlgPred 2.0 (https://webs.iiitd.edu.in/raghava/algpred2/index.html) and Allermatch (https://www.allermatch.org/). These tools compare the query amino acid sequence with all known allergens from protein databases by using a sliding window of 80 amino acids with more than 35%. As advised by the FAO/WHO to obtain more accurate results (FAO & WHO, 2001), both tools were used to obtain comparative data on the cross-reactivity and IgE binding properties of the retrieved protein sequences.

The prediction of cleavage sites of proteases in the retrieved protein sequences was performed using the software PeptideCutter from ExPASy Server. The protein sequences were first subjected to IgE epitope mapping to locate the IgE epitopes and then submitted in the PeptideCutter to predict potential cleavage sites by specific proteases. Pepsin (pH 1.3 and pH >2), chymotrypsin (high and low specificity), and trypsin were the only selected proteases since they were reported to be the most effective in reducing the induction of allergic reaction.

RESULTS AND DISCUSSION

Winged beans have only one storage protein, which was identified as albumin-1 (P15465). Similarly, hyacinth beans were found to have only one storage protein known as Flt3 receptor-interacting lectin (Q9ZTA9). On the other hand, lima beans were found to have two storage proteins, namely Zeatin O-glucosyltransferase (Q9ZSK5), and Phaseolin with two varying protein sequences (P80463 and Q43617). Following the acquisition of the protein sequences of the storage proteins, their preliminary screening with AlgPred and Allermatch resulted in at least one allergenic protein for each legume. However, some storage proteins obtained were identified to be susceptible to proteolytic digestion.

The storage protein of winged beans, known as albumin-1, was identified as a potential allergen by both AlgPred and Allermatch. It has homologous sequences with potato (*Solanum tuberosum*) cysteine protease inhibitor 10 with about 8.33% similarity and soybean (*Glycine max*) Kunitz trypsin inhibitors with about 6.67–26.04%. These proteins were identified to be primary allergens causing food-induced anaphylaxis (Quirce et al., 2002) and distinct wheal-and-flare responses during skin prick tests (Seppala et al., 2001). The confirmed allergenicity of these proteins from soybean and potato may result in similar allergic reactions to albumin-1. However, the homology of the proteins did not reach the 35% standard set by the FAO/WHO, indicating that it is unlikely to result in a high-affinity cross-reaction (Aalberse et al., 2001).

The Flt3 receptor-interacting lectin from the hyacinth bean was identified as a potential allergen upon the analysis of both AlgPred and Allermatch with homologous sequences from the allergenic proteins of peanut (*Arachis hypogaea*) and Malassezia sympodialis (a type of fungi). However, only the proteins from the peanut resulted in a

homology greater than 35%. Hence, hyacinth beans are likely to be cross-reactive with peanuts. Specifically, the Flt3 receptor-interacting lectin of hyacinth beans is 50.78% homologous to the galactose-binding lectin of peanuts. Its sequence was identified as homologous to other IgE-binding epitopes of other legume lectins, resulting in similar characteristics to other known major allergens belonging to the group of seed storage proteins such as Ara h 1 (7S globulin/vicilin), Ara h 2 and Ara h 6 (2S albumin), and Ara h 3 (11S globulin/legumin). However, the galactose-binding lectin of peanuts is only identified as a minor peanut allergen and is not officially recognized as a peanut allergen by the WHO/IUIS committee (Barre et al., 2020).

The storage proteins identified for lima beans are the non-allergen Zeatin O-glucosyltransferase and two varying protein sequences of the potential allergen Phaseolin. The two varying sequences of Phaseolin protein were identified to be homologous to a number of allergenic proteins such as in mung bean (*Vignia radiata*), soybean (*Glycine Max*), pea (*Pisum sativum*), narrowleaf lupin (*Lupinus angustifolius*), white lupin (*Lupinus albus*), lentil (*Lens culinaris*), peanut (*Arachis hypogaea*), common hazel (*Corylus avellana*), Macadamia nut (*Macadamia integrifolia*), English walnut (*Juglans regia*), pecan (*Carya illinoinensis*), and sesame (*Sesamum indicum*). The identified allergens from these other legumes are capable of inducing symptoms including anaphylaxis and inflammation in the perivascular and peribronchial regions of the lungs (Misra et al., 2011), manifestations in the skin, digestive tract, and ventilatory system (Salgado Castro et al., 2022), and bronchial asthma (López-Torrejón et al., 2003).

The reduction in the risk of inducing allergic reactions in patients by the underutilized legumes was determined through in silico study of their resistance to proteases. Albumin-1 from the winged bean was found to have no IgE-binding epitopes, indicating it as non-allergen as there were no similar proteins found against the software's database of IgE epitopes (Saha & Raghava, 2006). However, it was still identified as a potential allergen under the hybrid approach of AlgPred 2.0. This is possible since mapping epitopes is a different approach from the hybrid approach. The mapping of epitopes is only one component of the hybrid approach. Therefore, albumin-1 is still identified as a potential allergen. The protein sequences of Flt3 receptor-interacting lectin, Zeatin Oglucosyltransferase, and Phaseolin were determined to have IgE epitopes. Therefore, they were further assessed for their digestibility by specific proteases, as summarized in Table 1. Both Flt3 receptor-interacting lectin and Zeatin O-glucosyltransferase remained stable to proteolytic digestion as there was no cleavage in the IgE epitopes on their protein sequences. This indicates that the risks of their potential allergenicity cannot be reduced during digestion. The allergens entering the digestive system, therefore, remain undigested, increasing the possibility of allergic reactions (Misra et al., 2011). The use of other proteases or methods of hydrolysis should be employed to further reduce their potential allergenicity. On the contrary, the IgE epitope of phaseolin was cleaved by all proteases except trypsin. This indicates that its allergenicity and risks can be reduced upon digestion. However, it is still important to determine the severity of the symptoms of atopic individuals to determine their immune response.

Table 1. IgE epitopes of the identified seed storage proteins and its digestibility to specfic proteases.

Protein Name	IgE Epitope	Pepsin (pH 1.3)	Pepsin (pH > 2)	Chymotrypsin (high specificity)	Chymotrypsin (low specificity)	Trypsin
Albumin-1	No epitope found					
Flt3 receptor-interacting lectin	IAT	✕	✕	✕	✕	✕
Zeatin O-glucosyltransferase	IAT	✕	✕	✕	✕	✕
Phaseolin	FFLSSTEAQ	✓	✓	✓	✓	✕
Phaseolin	FFLSSTEAQ	✓	✓	✓	✓	✕

✕ - not digestible

✓ - digestible

CONCLUSIONS

Local underutilized legumes, specifically winged bean, hyacinth bean, and lima bean were all identified to have potential allergenicity as they were found to be cross-reactive to other known allergens. However, not all identified

allergens are capable of resisting enzyme hydrolysis upon digestion. Only the IgE epitope of phaseolin was found to be digestible by the enzymes pepsin (with pH 1.3 and >2), chymotrypsin (low and high specificity), and trypsin. Other allergens were found to either have no epitopes or indigestible to the specific gastric enzymes mentioned. It is important to acknowledge that the findings of this study are based solely on in silico research, and it is crucial to recognize the associated limitations. Some of these limitations include the potential existence of storage proteins in the studied legumes investigated that have not yet been characterized or elucidated to date, as well as the need to assess allergen digestibility by other enzymes. Subsequent analysis of the digestibility of the proteins is needed to determine the possibility of allergenicity reduction. This study also recommends conducting an in vivo assessment of the allergenicity of the storage proteins, along with other allergenicity tests such as specific and targeted serum screening, as recommended by the FAO/WHO. Furthermore, it is recommended to assess the allergenicity of other underutilized legumes to obtain more information and complete profiling of the allergens they contain.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
Borneo Convention Centre, Kuching, Sarawak

PHYSICAL AND CHEMICAL PROPERTIES OF *Saccharum edule* JUICE POWDER

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Abstract: *Saccharum edule* (tebu telor) is commonly used in the production of sugarcane juice which is commercialized as an acceptable beverage with a low shelf-life under room temperature or at refrigerated / frozen condition. In order to prolong the shelf-life, dehydration by spray drying was carried out to preserve the flavour, nutritional and nutraceutical qualities of sugarcane juice. The physical and chemical properties of spray dried sugarcane juice powder with anticaking (ASJ) and without anticaking (XSJ) were analysed including hygroscopicity, wettability, colour measurement, bulk density, viscosity, total solid content, total soluble solid content, moisture content, water activity and pH. The results showed that there were no significant differences between ASJ and XSJ for the wettability (4.19s and 4.54s), colour measurement L*a*b* (90.91* -2.10* 10.90 and 90.00* -1.77* 10.73), bulk density (0.62 and 0.67 g/cm^3), hygroscopicity (11.61 and 13.75%), viscosity at 25 °C (3.15 and 3.40 mPa.s), total solid content (12.70 and 13.13 %), total soluble solid content (12.9 and 13.6 °Brix), moisture content (2.20 and 2.97 %), water activity (0.32 and 0.31) and pH (5.38 and 5.43).
Keywords**:** *Saccharum edule* , sugarcane juice powder, spray dry, physical chemical properties

INTRODUCTION

Sugarcane (*Saccharum* sp.), from the grass family of Poaceae is well known for its refreshing and nutritious juice. In Malaysia, tebu kuning (Saccharum *officinarum)* and tebu telor (*Saccharum edule)* are grown as cash crops with the statistic of planted area of 1,678 ha and production of 25,591 Mt in 2021. *Saccharum edule* is commonly commercialized as a natural thirst-quenching drink with a low shelf-life under room temperature or at refrigerated / frozen condition. Nutrient content and high amount of sugar in sugarcane juice favour microbial growth (Yusof et al., 2000) and the presence of polyphenols, organic acid which coupled with oxidase activity could stimulate fermentation within a few hours of extraction and affected the natural refreshing properties of the juice (Nishad et al., 2017). Pasteurization, drying or evaporation in high temperature could cause degradation due to high sugar content in the juice – would affect the sensory attributes of the juice. Adding preservatives to maintain the refreshing properties of sugarcane juice at room temperature and in liquid form would affect the organoleptic qualities of the juice. Thus, one possible step is to remove the water content in order to prolong the shelf-life and preserve the nutritional, nutraceutical qualities and flavour of the sugarcane juice. Dehydration by spray drying was carried out to preserve the flavour, nutritional and nutraceutical qualities of sugarcane juice.

Spray drying is a rapid and economic drying technology which transform liquid state of sample to dry particulate form as powders by spraying the liquid through a hot drying chamber with addition of carrier agents,

such as maltodextrin or arabic gum. Spray drying involves atomization, mixing of spray and air, evaporation and product separation which produce granular form or powder without substantial loss in nutraceutical and nutritional substances (Hari et al., 2013). Spray dried powder has good reconstitution characteristics, preserve the flavour and sensory quality of the juice. The aim of this study was to analysed the physical and chemical properties of spray dried sugarcane (*Saccharum edule)* juice powder with anticaking (ASJ) and without anticaking (XSJ) including hygroscopicity, wettability, colour measurement, bulk density, viscosity, total solid content, total soluble solid content, moisture content, water activity and pH.

MATERIALS AND METHODS

The sugarcane juice of *Saccharum edule* was extracted using commercial extractor and filtered through double layer of muslin cloth. The juice was blended with carrier agent (with anticaking agent and without anticaking agent) and spray dried using Mini Spray Dryer B-290 (Buchi, Switzerland). The powders collected were stored at room temperature in a sealed aluminium pouch until further analysis.

Physical and rheological analysis

Physical and rheological characteristics including hygroscopicity, colour measurement, wettability, and viscosity were carried out according to the method of Hari et al. (2013) while water solubility and water absorption index were carried out according to the method of Nishad et al. (2017).

Chemical analysis

Chemical analysis including total solid content (TS), total soluble solid content (TSS), moisture content, ash, total sugar, water activity and pH.

Microbiological analysis

Microbiological analysis was carried out according to Bacteriological Analytical Manual (BAM) standard methods (Feng et al., 2002)

Statistical analysis

The data for physical and chemical characteristics were tested by conducting a one-way analysis of variance (ANOVA) using the SAS System, ver. 9.0 statistical software. When statistically significant differences were indicated, the Duncan New Multiple Range Test (DMRT) was employed for comparisons between powders with anticaking and without anticaking. All values are expressed as mean ± standard deviation (SD) and a difference was considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

The highest powder yield of 200.5 g was obtained after spray drying 1 L of sugarnane juice. The powder obtained recorded a moisture content of 2.20 - 2.97%, water activity of 0.31 - 0.32 , bulk density of 0.62 - 0.67 g/cm^3 and wettability of 4.19 - 4.54 s. Table 1 showed the physical and chemical characteristics of sugarcane juice powder with and without anticaking agent. There were no significant differences between ASJ and XSJ for the physical characteristics including wettability, water solubility, water absorption index, colour measurement, bulk density and viscosity except for hygroscopicity ($p<0.05$). Presence of anticaking agent is estimated in reducing the moisture content and hygroscopicity of the powder due to its ability that can take water up 10% of its weight. While the chemical properties of ASJ and XSJ showed that there were no significant differences for total solid content, total soluble solid content, moisture content, water activity, pH, ash and total sugar ($p>0.05$). These results showed that anticaking agent did not show any effect on the sugarcane juice powder except for hygroscopicity. The shelf-life study shall be continued to study the characteristics of both ASJ and XSJ during storage.

Table 2 showed the microbiological results of both sugarcane juice powder with anticaking (ASJ) and without anticaking (XSJ). According to Yusof et al. (2000), it is difficult to preserve sugarcane juice due to its pH and nutrient content which favor microbial growth to spoilage. The results in Table 2 supported this statement which both ASJ and XSJ showed high total plate count. But according to Compendium of Microbiological Criteria for Food (Food Standards Australia – New Zealand, 2018), the total plate count of ASJ and XSJ still in satisfactory category. Both ASJ and XSJ showed a satisfactory result for Yeast & Mould Count and Coliform

while the analysis of *Escherichia coli* and *Staphylococcus aureus* showed that the microorganisms tested were not detected in both analysed samples. The results showed there were no significant differences of microbiological analysis between ASJ and XSJ.

Table 1. Physical and chemical characteristics of sugarcane juice powder with and without anticaking agent

	Sugarcane juice powder with anticaking (ASJ)	Sugarcane juice powder without anticaking (XSJ)
Wettability (1g/sec)	4.19 ± 0.06 [a]	4.54 ± 0.35 [a]
Colour L*a*b*	90.91* -2.10* 10.90	90.66* -1.76* 11.04
Bulk density (g/cm^3)	0.62 ± 0.02 [a]	0.67 ± 0.01 [a]
Viscosity at 25 °C (mPa.s)	3.15 ± 0.17 [a]	3.40 ± 0.19 [a]
Hygroscopicity (%)	11.61 ± 0.54[a]	13.75 ± 1.15[b]
Water solubility (%)	6.51 ± 0.44 [a]	5.05 ± 0.44 [a]
Water absorption index, WAI (w/w)	0.07 ± 0.00 [a]	0.06 ± 0.01 [a]
Total soluble solid content, TSS (°Brix)	12.97 ± 0.21 [a]	13.60 ± 0.17 [a]
Total solid content, TS (%)	12.70 ± 0.04 [a]	13.13 ± 0.05 [a]
Moisture content (%)	2.20 ± 0.08 [a]	2.97 ± 0.06 [a]
Water Activity (Aw)	0.32 ± 0.03 [a]	0.31 ± 0.03 [a]
pH	5.38 ± 0.03 [a]	5.43 ± 0.01 [a]
Ash (%)	1.35 ± 0.09 [a]	1.10 ± 0.01 [a]
Total Sugar (g / 100g)	49.70 ± 0.70 [a]	50.50 ± 0.30 [a]

Table 2. Microbiological analysis results of sugarcane juice powder with and without anticaking agent

	Sugarcane juice powder with anticaking (ASJ)	Sugarcane juice powder without anticaking (XSJ)
Total Plate Count (cfu/ml)	2.1 x 10^3 [a]	2.0 x 10^3 [a]
Yeast & Mould Count (cfu/ml)	<15 x 10^2 [a] est (1.0Y x 10^2)	<15 x 10^2 [a] est (1.0Y x 10^2)
Coliform (3M Petrifilm) (cfu/ml)	<25 x 10 [a] est (3.0 x 10)	<15 x 10 [a] est (1.5 x 10)
Escherichia coli (3M Petrifilm) (cfu/ml)	<1 x 10 [a]	<1 x 10 [a]
Staphylococcus aureus (cfu/ml)	<1 x 10^2 [a]	<1 x 10^2 [a]

CONCLUSIONS

In conclusion, the presence of anticaking agent in spray drying process did not show any significant differences in the physical and chemical properties of sugarcane juice powder except for hygroscopicity. Therefore, there is a need to continue the shelf-life study on the physical and chemical characteristics of the sugarcane juice powder during storage and proceed with the caking test using a powder rheometer.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
Borneo Convention Centre, Kuching, Sarawak

MARKET SOLUTION AND STRATEGY FOR AGRICULTURAL TRADE: CRYOGENIC FROZEN JACKFRUIT

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Abstract: The global market size for frozen tropical fruit is expected to reach US$4.9 billion and US$5.59 billion in 2026 and 2027, respectively, with an increasing CAGR rate of 6.7% annually. In line with the trends, MARDI has developed frozen jackfruit technology using the cryogenic freezing technique to extend the shelf-life, while maintaining the quality and nutritional values, thus providing solutions for exporters. Both primary and secondary data were utilized to evaluate the consumers' acceptance, economic viability and industry reviews on the frozen jackfruit variety J33, relative to the fresh segment. The findings discovered there was no significant difference between the frozen and fresh Jackfruit, implying the product is acceptable to local and foreign consumers, albeit the mean score showed a significant difference between the whole and minimal processed techniques among locals, excluding foreigners. The contingent valuation model confirmed that both categories of consumers are willing to pay higher market prices than the stated bidding prices. The economic measures indicated frozen jackfruit with both whole fruit and minimal process cryogenic freezing techniques is viable, but the latter is estimated more feasible, cost-effective and more practical for exports, thus providing not only a solution but also a strategy to explore global markets.
Keywords: frozen jackfruit, cryogenic, minimal process, variety J33, export

INTRODUCTION

Despite being a relatively marginal market, the frozen fruit market indicated rapid growth globally as the market size is forecasted to reach US$ 5.59 billion at a CAGR of 6.7% during 2020 – 2027. An analytical review of the global market for frozen berries, fruits, and vegetables noted that the fruit and vegetable freezing segment is one of the most promising segments of the entire horticultural business (FAO, 2023). Furthermore, frozen fruits offer immense promise in food industries as consumers are increasingly shifting towards healthier food products while the demand for frozen fruits witnessing substantial growth due to high nutritional values, progressively being used in industry applications, and thus gaining momentum in research and development activities along with the development of the food science sector (Transparency Market Research, 2023). Citrus and berries dominated the frozen fruit market for many years, yet the tropical frozen fruit market is standing higher in recent trends. Previously, Durian has been given attention in the frozen tropical fruit line due to its premium value as the commodity could guarantee the market while recording successful business stories of export penetration, particularly in China. Frozen jackfruit will be the next target as it provides a technical solution to the most critical challenge of maintaining the quality of fresh jackfruits due to their relatively short shelf-life, thus limiting market activities. The global jackfruit market size reached a value of USD 311.71 million in 2022 and is expected to further grow at a CAGR of 3.40% between 2023 and 2028, to reach a value of USD 380.96 million by 2028 (Expert Market Research, 2023). The fresh jackfruit can be stored no longer than 14 days to maintain its quality under ambient temperature and conditions. With the country gaining market access to export fresh jackfruit to several regions including Australia, Japan, the Middle East, Hong Kong and European countries, the current shelf life would not be possible for the country to explore the markets globally - for instance, exporting to European countries would take at least 30 days through sea shipment. Therefore, MARDI has developed a technology so-called cryogenic frozen jackfruit, focusing on variety J33, both in whole fruit and minimally processed product forms to provide market solutions to the agro-food industry players, mainly traders and exporters. In short, cryogenic is a freezing technique when applying liquid nitrogen as a freezing agent that can extend shelf-life as maximum as six (6) months while maintaining the quality of freshness, taste, colour and most importantly its nutritional values, while rapid freezing of fruits provides better quality and improves the internal microstructure

(Allan-Wojtas et al., 1998). Prior to the technology being adopted by industries, the final research output of frozen jackfruit must be evaluated from the economic and market perspectives to ensure the technology is applicable and acceptable to both user categories of industry and household consumers. Thus, this study evaluates the consumers' acceptance, economic viability and industry reviews on the frozen jackfruit, relative to the fresh segment. Both primary and secondary data are collected and analyzed using both quantitative and qualitative methodologies. In general, frozen jackfruit using a cryogenic technique is acceptable to the locals, and foreign consumers. In fact, the results showed no significant difference between the frozen and the controlled sample - fresh Jackfruit. The economic viability analysis suggested that frozen jackfruit with both blast freezing and cryogenic techniques is viable, but the latter required lower production costs, hence proving more economical and cost-effective with minimal processing product form that could be targeted for exportation.

MATERIALS AND METHODS

In with conjunction an international annual event in Malaysia in the year 2022, this study managed to collect primary data from 200 household consumer respondents, not only local but also foreign consumers who could represent the international markets using a purposive and random sampling approach. Participation in this study is voluntary basis. During the consumer survey, each person is given three (3) blind samples; 1) frozen jackfruit pulp (cryogenic whole fruit), 2) frozen jackfruit pulp (cryogenic minimally processed), and 3) fresh jackfruit pulp as a controlled sample. After tasting, they are required to evaluate the attributes, willingness to pay and acceptance using a structured questionnaire. From the industry reviews, primary data was collected through focus group discussions with the main players, mostly fruit exporters. Several quantitative methods were used for data analysis comprising descriptive analysis, ANOVA, contingent valuation model (CVM) and financial analysis to measure the economic viability, whereas thematic analysis was applied for the qualitative approach. The CVM is a survey-based method of estimating how much individuals would be willing to pay and often used to evaluate the economic values of the non-goods market, however is a highly flexible method for the estimation of values for goods, using a hypothetical market (Sajisee et al, 2021; Ekstrand and Draper, 2000; Hanemann, 1984).

RESULTS AND DISCUSSION

The composition of local and foreign respondents was 77.5% and 22.5%, respectively with various demographics. The major research findings consist of consumer acceptance and willingness to pay for frozen jackfruit (J33), the economic viability and industry reviews. Table 1 displays results of consumer preference including mean score and ANOVA. The mean score refers to the hedonic scale of seven (1= strongly dislike to 7= strongly like) across the three samples. The results for Malaysians showed that the mean score of frozen jackfruit using cryogenic minimal processing (5.303) was higher than the whole fruit method (4.645), meaning that the frozen minimal processing is more acceptable, albeit the score is lower than the fresh that could not beat the frozen form for local who accustomed to the freshness. However, the frozen indicated a slightly higher mean score than fresh for foreign consumers meaning that they are acceptable and have no significant difference between both samples, though the pulp color, juiciness and crunchy texture indicate a significant difference between samples. Heinrichs (2016) found the similarities between fresh and frozen nutritional values did not have a significant effect on preferences, albeit the taste, texture, or quality factors driving consumers to prefer fresh to frozen vegetables.

Table 1. Consumer acceptance of frozen jackfruit (variety J33) using ANOVA

Fruit Attributes	Type of samples	Malaysian (n=155)	Sig.	Foreign (n=45)	Sig.
Pulp Colour	Sample 1	5.323a	.051	5.644	.017**
	Sample 2	4.897b		5.222	
	Sample 3	5.103ab		5.733	
Aromatic	Sample 1	5.174a	.000**	5.378	.788
	Sample 2	4.645b		5.200	
	Sample 3	5.310a		5.267	
Juiciness	Sample 1	4.271b	.000**	4.889	.097*
	Sample 2	3.987b		5.044	
	Sample 3	5.206a		5.467	
Sweetness	Sample 1	5.426a	.000**	5.689	.44
	Sample 2	4.594b		5.467	
	Sample 3	5.529a		5.356	
Crunchy texture	Sample 1	4.510b	.000**	4.978	.008***

Fruit Attributes	Type of samples	Malaysian (n=155)	Sig.	Foreign (n=45)	Sig.
	Sample 2	4.374b		4.667	
	Sample 3	5.716a		5.667	
Overall Acceptance	Sample 1	5.303b	.000**	5.689	.834
	Sample 2	4.645c		5.533	
	Sample 3	5.723a		5.600	

Note: Sample 1=frozen jackfruit (cryogenic minimally processed); Sample 2=frozen jackfruit (cryogenic whole fruit; Sample 3=fresh jackfruit
Source: Primary data (2022)

The results of willingness to pay using the contingent valuation method suggested that consumers would pay higher than the bidding prices – both Malaysians and foreigners – for frozen jackfruits. The Local consumers tend to pay RM12.40/ 500 grams which is slightly higher than the bidding price, RM 12.00/ 500 grams, while the internationals are willing to pay RM16.40/ 500 grams, much higher than the bidding price at RM 14.00/ 500 grams. The attribute factor of preferred sweet taste indicated a significant influence on the prices for both consumer categories (Table 2).

Table 2. Contingent valuation model of willingness to pay by consumer category

Variables	**Malaysian**			
	B	**S.E.**	**Wald**	**Exp(B)**
Bidding price	-.164	.201	.665	1.178
Preferred attribute: Sweetness	1.360*	.754	3.257	3.898
Preferred attribute: Aromatic	.210	.440	.226	1.233
Repeat purchase: Good Taste	-.841	.714	1.390	.431
Product availability	1.917**	.805	5.666	6.798
Constant	-4.885*	2.964	2.716	.008
Variables	**International**			
	B	**S.E.**	**Wald**	**Exp(B)**
Bidding price	-.486	.500	.945	1.627
Preferred attribute: Crunchy texture	-1.072	1.722	.388	0.342
Preferred attribute: Aromatic	1.071	1.320	.659	2.919
Preferred attribute: Sweetness	-1.200**	1.420	.714	.301
Nutritional values	.046	1.840	.001	1.047
Gender x Household income	.167	.313	.284	1.181
Constant	-6.795	7.605	.798	0.001

Note: *, ** sig. at .1 and .05, respectively
Source: Data analysis (2022)

A thematic analysis was used to analyze qualitative data gathering from focus group discussions with agro-food industry players. The results showed that frozen jackfruit with a minimally processed method has a better taste relative to the whole fruit. In fact, the taste is highly acceptable with not much different from the fresh sample, thus the frozen jackfruit is strongly recommended for export markets, especially to the countries where Malaysia has gained market access and export protocols – Japan and Australia – and is accustomed to frozen fruits while growing demand for tropical fruits. The packaging and labelling should also provide useful information on how to prepare before consuming (i.e. thawing process).

The financial measures manifest that frozen jackfruit is economically viable using cryogenic freezing for both whole fruit and minimal process techniques. However, the minimal process technique revealed more positive returns with a larger net present value, exceeding 50% of the internal rate of return, and a shorter payback period, implying positive returns on investment (Table 3). Therefore, the results strongly suggest that minimal processing is more feasible and cost-effective relative to the whole fruit freezing technique. This economic estimation considers the assumption of the existing cryogenic processing facility for frozen Durian producers, a seasonal food crop, which can be utilized for non-seasonal, jackfruit.

Table 3. Economic viability analysis on cryogenic freezing techniques

	Cryogenic Freezing Techniques	
	Whole Fruit	**Minimal Process**
Net Present Value; RM Mill.	2.2	2.5
Internal Rate of Return; %	46	55
Benefit Cost Ratio; RM	1.78	1.61
Payback Period; year	3	2.2

Source: Data analysis (2022)

CONCLUSIONS

In line with the global uptrend frozen fruit market for tropicals, Malaysian Agriculture Research and Development (MARDI) has developed technology for frozen jackfruit using a cryogenic freezing technique that could extend its shelf life until six months when the fresh could only remain no longer 14 days, becoming a critical issue for marketing jackfruit. This innovation, thus, is expected to contribute a market solution for agro-food industry players, mainly those who are engaging with international trade markets. Prior to the application, it requires the evaluation of quality and preferences for frozen jackfruit from both household consumers and the agro-food industry perspectives and the economic viability assessment. This study suggests that frozen jackfruit (variety J33) using cryogenic freezing and with minimal processing technique is acceptable from multiple sides – household locals, international consumers and representative industries. In fact, the results denoted insignificant differences between frozen and fresh jackfruits among the international respondents. With the economic viability measures strongly suggesting the minimal process form would be more returnable than the whole fruit freezing approach, the frozen jackfruit in a minimal process is more practical, and cost-effective, and is expected could go further to target export markets globally. The frozen jackfruit technology not only provides a storage and marketing solution for the industries but also becomes a strategy to explore and penetrate the global market, in line with the current National agro-food policy agenda to boost the country's economy through export expansion and spillover effects.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
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MICROBIOLOGICAL QUALITY ASSESSMENT OF SMOKED MEAT AND FRESH SEAWEED DURING STORAGE STUDY UPON HIGH PRESSURE PROCESSING

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Abstract: Natural and healthier foods such as smoked meat and fresh seaweed are in greater demand on a global scale. However, both food products have naturally limited shelf life, thus it is crucial to investigate the effect of high pressure processing (HPP) on products' safety parameter including HPP potential to prolong shelf life. Specifically, HPP treatments of 6000 Bar for 4.5 min on Nylon/PE packaging for smoked meat and 6000 Bar for 3 min on PE/PET packaging for fresh seaweed were applied based on the optimization of HPP parameter previously done. The HPP-treated smoked meat showed a minimum storage of 56 days while the untreated smoked meat demonstrated Total Plate Count of 10^5 CFU/g at day 0 which increased to 10^6 CFU/g at day 14 of storage at chilled temperature. It was also found that throughout the 140 days of storage period, the microbial levels of HPP-treated fresh seaweed remained below the detection limit while the microbial levels of untreated fresh seaweed exceeded the microbial safety limit at day 14 of storage at chilled temperature. In summary, HPP effectively maintained the microbiological quality of smoked meat and fresh seaweed for a minimum of 56 days and 140 days, respectively.
Keywords: Microbiology, high pressure processing, smoked meat, fresh seaweed, storage study.

INTRODUCTION

Nowadays, there is a general demand to produce fresh and minimally-processed food in line with the increasing consumer awareness on the intake of healthier ready-to-eat (RTE) products with good nutritional qualities and guaranteed safety. This market trend has strived the food industry to develop innovation especially in traditional food such as smoked meat and fresh seaweed involving the application of novel processing technologies to preserve products, extend shelf life and improve microbiological safety. Smoked meat was the result of preparing red meat with smoking methods for flavour enhancement, appearance improvement and food preservation. Smoked meat also known as *daging salai* in Malay is one of the local favourite dishes that is widely consumed with rice, sandwich or spaghetti, however has limited shelf life when kept chilled. Meanwhile, fresh seaweed (*Gracilaria changi*) also known as sea vegetable is widely grown in Malaysia, can be consumed fresh or used as food condiments in the form of dried or wet seafood. However, *Gracilaria* has a relatively short shelf life once harvested due to its watery texture and high nutrient content that provide resources for microbial growth.

Therefore, the application of high pressure processing (HPP) is possible as an efficient alternative technique that involves non-thermal treatment to the packaged food in order to inhibit harmful pathogens and spoilage microorganism, and to inactivate undesirable enzymes, with minimal effects in sensorial and nutritional quality. The effect of HPP on the food characteristics' quality has been mainly derived from the the stability of covalent bonds under high pressure, without inducing any food structure molecules. HPP also encourages minimal usage of additives in product development as well as compliance with food safety regulatory requirements. Previous studies on the shelf life extension of local products such as jackfruit, durian, cow milk and goat milk have shown encouraging results (Tan *et al*., 2019; Chin *et al*., 2020). However, to the best of our knowledge, studies on the application of HPP to smoked meat and fresh seaweed in Malaysia are still fragmented. Thus, the aim for the present study was is to investigate the effect of optimized HPP parameters on the microbiological properties of smoked meat and fresh seaweed over the storage period under chilled temperature.

MATERIALS AND METHODS

Preparation of smoked meat and fresh seaweed including pressurization study

Smoked meat samples were provided by local entrepreneur at Rembau, Negeri Sembilan. Samples were prepared at entrepreneur's premise by smoking methods by using mixture of woods, subsequently packed into a 12 cm (width) and 17 cm (length) food grade nylon/polyethylene (Ny/PE) as primer packaging (approximately 100 g of sample per packet) and into biaxially oriented polypropylene/kraft paper/cast polypropylene (BOPP/Kraft/CPP) as secondary packaging. Then, packed smoked meat were transported to Food Science & Technology Research Centre, MARDI at Serdang, Selangor in chilled temperature (2 ± 7 ºC) within the same day of processing. Meanwhile, fresh seaweed (*Gracilaria changii*) samples were supplied from entrepreneur's farm at Muar, Johor and delivered to MARDI, Serdang within 3 to 5 hours at 2 ± 7 ºC. Once arrived, fresh seaweed samples were subjected to pre-treatment which consisted of washing with filtered water, soaking with chlorine for 30 secs, tossing and rinsing with filtered water, squeezing using spinner and finally packaging in polyethylene/polyethylene terephthalate (PE/PET) (approximately 50 g of sample per packet).

A HPP unit (Hiperbaric 55, Burgos, Spain) located at Faculty of Food Science and Technology, Universiti Putra Malaysia (UPM) was used to process the smoked meat and fresh seaweed, respectively. The high pressure was generated by a cylindrical pressure chamber, a pressure pump and a hydraulic unit with water as the pressure medium in the HPP chamber. HPP parameters of 6000 Bar for 4.5 mins for smoked meat and 6000 Bar for 3 mins for fresh seaweed with pressurization maintained at 20 ºC processing temperature were used in this study based from the optimization study using Response Surface Methodology (RSM) previously done. In this experiment, microbiological test was carried out 1 hr after HPP treatment with all samples analysed by triplicate. HPP-treated samples were kept at 2 ± 7 ºC for further analysis. The microbiological quality of the smoked meat and fresh seaweed was evaluated every 14 days throughout a 56-day and 140-day storage period, respectively.

Microbiological analysis

Microbial analysis of Total Plate Count (TPC), Yeast & Mould (Y&M), Coliform, *Escherichia coli*, *Staphylococcus aureus* and Psychrotrophic bacterial counts was performed on smoked meat and fresh seaweed according to United States-Food and Drug Administration (US-FDA) Bacteriological Analytical Manual (BAM) standard methods (Feng *et al*., 2002). All microbial data were expressed as number of colony forming units (CFU/g) with plates enumeration based on 25 to 250 CFU/g, respectively except 15 to 150 CFU/g for Y&M. Additional analysis of *Salmonella* was performed on smoked meat during the storage period by a method modified from the US-FDA BAM (Feng *et al*., 2002). Isolated colonies that showed typical reactions (Xylose Lysine Deoxycholate and Xylose Lysine Tergitol-4; dark red colonies with black centre, Rambach; bright red colonies) according to manufacturer's instructions were considered as presumptive *Salmonella*. Well isolated colonies were subjected to biochemical tests for confirmation (Merck, Germany).

RESULTS AND DISCUSSION

Microbiological properties during storage study

Specifically, the TPC in untreated smoked meat was 1.8 x 10^5 CFU/g, however reduced to <1 x 10 CFU/g after HPP treatment at week 0 (Table 1). Meanwhile, TPC growth in untreated fresh seaweed (control) was 2.7 x 10^4 CFU/g but decreased to <25 x 10 CFU/g after HPP treatment at week 0 (Table 1). These results proved that aforementioned HPP treatments successfully reduced the microbial load by 5 and 4 log reduction for smoked meat and fresh seaweed, respectively. Notably, the initial load of microbial counts in untreated smoked meat and fresh seaweed was possibly contributed from the environmental factors or poor handling at processing facilities. For HPP-treated smoked meat samples, TPC was observed incremental fortnightly until it reached 1.7 x 10^6 CFU/g at the end of the storage period at day 56 (Table 1). In contrast, TPC was found relatively higher for untreated smoked meat where it reached unsatisfactory level at week 2 of storage period. The microbial safety limit for smoked meat was referred to Malaysia's Food Act 1983 (Act 281) & Regulations in this study whereas 10^6 CFU/g was already considered unsatisfactory for meat and meat product category (Table 2).

Noteworthy, fresh seaweed showed a total reduction in microorganism after HPP at the pressure conditions applied, and remained so throughout the 140-day of storage period (Table 1). In comparison, TPC growth for untreated fresh seaweed (control) reached unsatisfactory level at day 14 during storage at chilled (Table 1). The

Compendium of Microbiological Criteria for Food (Food Standards Australia – New Zealand) was particularly referred in this study (Table 2) due to the absence of microbial safety limit in the Food Act 1983 for fresh seaweed category. These results are in accordance with the results of Queiros *et al*. (2014) and Tan *et al*. (2019) who reported the lethality of HPP on aerobic mesophilic microorganisms. This study also demonstrated Y&M and Psychrotrophic counts were significantly reduced to non-detectable levels for smoked meat and fresh seaweed as compared to untreated sample with the presence of these microorganisms at higher rate. Although not mentioned in both standards in Table 2, Y&M and Psychrotrophic could be used as food decay indicator including spoilage of refrigerated foods. In this experiment, HPP-treated smoked meat showed 10^4 CFU/g of Yeast and 10^2 CFU/g of Psychrotrophs at day 56 of storage period, meanwhile Y&M was only detected at day 98 only at a very low level whereas no Psychrotrophic count was detected in HPP-treated fresh seaweed, respectively (Table 1).

This study also demonstrated HPP at optimized parameter successfully inhibited Coliform with 4 log reduction for smoked meat and 5 log reduction for fresh seaweed (Table 1). *E. coli* and *Salmonella* were not detected in all samples which in line with the Compendium of Microbiological Criteria for Food (Food Standards Australia – New Zealand) (Table 2) except *Salmonella* that was found in control samples of smoked meat at week 14. In the present study, *Staph. aureus* was also successfully reduced to non-detectable level for smoked meat and fresh seaweed, respectively under the influence of HPP. Overall, based from the data obtained, a minimum level of pressure and holding time of 6000 Bar for 4.5 mins and 6000 Bar for 3 mins were sufficient for microbial spoilage prevention and pathogen deactivation in smoked meat and fresh seaweed, respectively. Since one of the consequences of foodborne illnesses is loss of revenue, HPP offers pasteurisation advantages to produce safe and high-quality food products. Therefore, it is crucial to understand the effects of HPP on microbial shelf life of both food matrices as the safety parameter provided by this study could serve as a foundation for product development strategy and food safety initiatives.

CONCLUSIONS

In summary, our results suggested HPP parameters of 6000 Bar for 4.5 mins and 6000 Bar for 3 mins were effective in retaining the microbiological quality of both products for a minimum of 56 days and 140 days, respectively.

ACKNOWLEDGEMENTS

This work was supported by a grant from MARDI, under the Development of Standard Operating Procedure (SOP) for Minimally-Processed Product using HPP Technology (Project No. K-RFS03-1001-KSR999). The authors are grateful to laboratory staff from MARDI and UPM for their overall technical assistance during the study.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
Borneo Convention Centre, Kuching, Sarawak

EFFECT OF DIFFERENT CARBONATE SUPPLEMENTATION ON ARTIFICIAL REEF MINERAL ACCRETION FOR FOOD SECURITY

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Abstract: Reefs play a pivotal role in fish abundance which contributed to food security. This study explored the usage of carbon dioxide supplementation on mineral accretion and its potential to enhance growth rates in reef corals. Mineral accretion technology is utilized to assess the effects of different levels of carbonate supplementation on the accretion properties. Calcium hydroxide ($Ca(OH)_2$) and carbonated water (H_2CO_3) were used in this study. Ca(OH)2 of 0%, 1.2% and 1.6% (w/v) were supplemented at 0, 5, and 10 mL/h with constant current 0.22 A. Water chemistry includes carbonic species, pH, and mineral accretion were measured. The use of $Ca(OH)_2$ significantly ($p<0.05$) increases the amount of H_2CO_3 available for supplementation compared to control. The mineral accretion showed no significant difference ($p<0.05$) between all treatments compared to control. The water chemistry measured shows significant difference ($p<0.05$) for day 0 compared to day 3 and 7 for all treatment. The water chemistry for day 0, 3 and 7 shows no significant difference ($p<0.05$) for every treatment. The result concludes that carbonate supplementation showed no significant difference in the mineral accretion but significant difference ($p<0.05$) for the water chemistry between day 0, 3 and 7.
Keywords: Mineral accretion technology, carbonate species, water chemistry, carbonate supplementation, electric current.

INTRODUCTION

This study was conducted to identify the effect of carbonate ions (CO_3^{2-}) supplementation to the mineral accretion system in idea to improve the condition of artificial reefs for coral growth and restoration. Coral reef plays a huge role as one of the key ecosystem services which is in supplying and sustaining food supplies supporting the livelihoods of over 500 million people worldwide due to its fish abundance (Mellin et al., 2022). Unfortunately, multiple studies have shown that coral reefs have been facing numerous threats from both natural and human caused stressor including climate change, ocean acidification, pollution, overfishing, and destructive fishing practices (A. El-Naggar, 2021; Benkwitt et al., 2020). The deterioration of coral reefs affects both marine biodiversity and human's dependent on these ecosystems (Eddy et al., 2021). Study shows that the recovery of coral reefs could be achieved through reduction of greenhouse emission and development of coral sanctuaries (Woesik et al., 2022). Climate change related mortality of coral reef might have been unavoidable, but it is proven in research over 15 years that local management actions may improve the regrowth of juvenile coral in optimal condition therefore increase the resilience and recovery of these ecosystems (Steneck et al., 2019). Carbon dioxide (CO_2) contributed the most towards global temperature rise compared to other gases (Myers & Subban, 2022). The ocean absorbs about 30% of carbon dioxide released from the atmosphere. The continuous increase of CO_2 released through daily human activities thereby increases the amount of CO_2 absorbed by the ocean resulting in ocean acidification (NOAA, 2020). The increase of hydrogen ion concentrations caused shift in carbonic species thus significantly implicates the ocean and marine organisms. Manipulation of the said carbonic species concentration is the general idea of many ocean CO_2 capture technologies (Myers & Subban, 2022). This study emphasizes the application of mineral accretion technology by improving the mineral deposits at cathode as done in a past study (Margheritini et al., 2020). This study emphasizes the usage of

mineral accretion technology by applying constant current of 0.22 A to the artificial reefs system with the supplementation of carbonate ions. The method of deacidification of carbonic acid shows a significant result as well as the condition of water throughout the seven-day period of experiment. Mineral deposition can also be observed at the cathode (artificial reef) by the end of experiment.

METHODS & MATERIALS

Mineral Accretion Technology Experiment

The laboratory scale experiment system consists of three 30×15×15 inches glass aquariums with a capacity of 100 L each. The experiment setup used are following a previous study (Margheritini et al., 2020) with slight modification on the tank setup. Calcium hydroxide treated carbonic acid solution was introduced to the system as carbonate supplementation. The anode used in the experiment is a titanium rod with the length of 10 cm and diameter of 10 mm. The cathode which acts as the artificial reefs are made of 304 stainless steel wire mesh that are shaped into rectangular structures. Carbonate ions with the concentration of 0%, 1.2% and 1.6% (w/v) are supplemented directly to the cathode through a plastic diffuser at 0, 5 and 10 mL/h using a dosing pump. Water samples of day 0, 3 and 7 were taken to measure the pH and carbonic species. The mineral accretion was also measured by calculating the difference between the initial mass of cathode and the final mass of cathode after every seven days experiment. Salinity of water was also measured at the start and end of experiments using a salinity refractometer. Figure 1 shows the experiment setup.

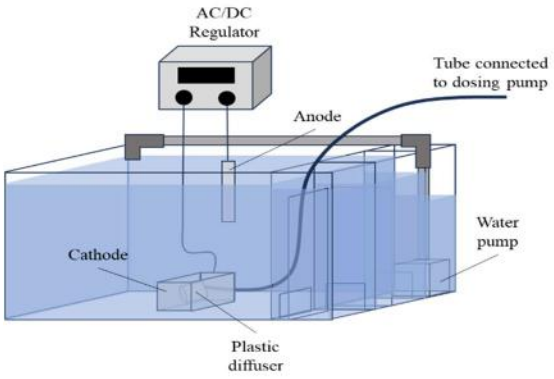

Figure 1. The experiment setup for mineral accretion system

Deacidification of Carbonic Acid

Calcium hydroxide ($Ca(OH)_2$) is used to deacidify carbonic acid (H_2CO_3) solution prepared to produce carbonate ions. The $Ca(OH)_2$ was weighed and introduced into 50 mL of H_2CO_3 solution before being filtered through a filter paper (Sartorius 1288). The deacidification procedure is done at a controlled temperature of 26 ºC using a refrigerated water bath. The deacidification process is followed by Gran titration method as conducted by Verma (2005) with slight modification to determine the carbonate alkalinity.

Determination of Carbonic Species

The carbonic species was determined using calculation of alkalinity relationship (Rodger B. Baird et al., 2017). The titration is conducted and recorded following a Gran titration method (Verma, 2005).

RESULTS AND DISCUSSION

Mineral Accretion Technology Experiment

Result shows that there is a significant difference ($p<0.05$) between the control and every other treatment conducted showing that there is a mineral accretion activity that occurred. The result unfortunately shows no significant difference ($p>0.05$) between each treatment. This shows that the supplementation of CO_3^{2-}at the concentration of 0%, 1.2% and 1.6% did not increase the mineral accretion activity. This is due to various factors such as the high concentration of calcium and carbonate ions exist in the artificial seawater making CO_3^{2-} to not being a limiting factor for this experiment and the rapid accretion that occurred at 0.22 A. Margheritini et al. (2020) also mentioned how the breakdown of water at anode and cathode would induce the production of carbonate and bicarbonate ions.

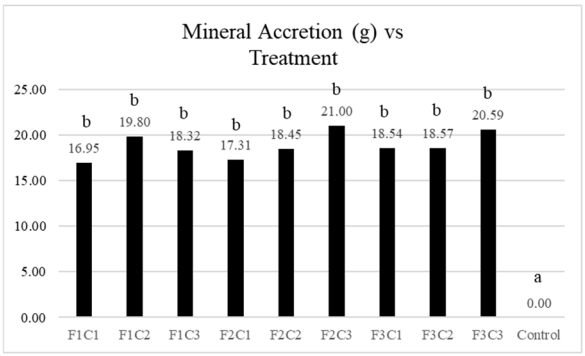

Figure 2. The graph of mineral accretion against treatment. Mean that do not share a letter is significantly different ($p<0.05$).

Deacidification Of Carbonic Acid

Table 1 shows that there is a significant difference ($p<0.05$) between the control (0%) and the sample treated with 1.2%, 1.4% and 1.6% ($Ca(OH)_2$) in hydroxide alkalinity as $CaCO_3$. Carbonate alkalinity as $CaCO_3$ shows a significant difference ($p<0.05$) between control and sample treated with 1.2% $Ca(OH)_2$. The result for bicarbonate concentration as $CaCO_3$ shows that there is a significant difference between control and sample treated with 0.8% $Ca(OH)_2$. This proves that the process of deacidification occurs during the treatment with ($Ca(OH)_2$). The concentration of 0%, 1.2% and 1.6% were selected to be introduced into the mineral accretion system as they show to be statistically different.

Table 1. The carbonate alkalinity shows the result of carbonic acid deacidification at different levels of calcium hydroxide introduced.

Calcium Hydroxide (w/v)	Hydroxide Alkalinity as $CaCO_3$	Carbonate Alkalinity as $CaCO_3$	Bicarbonate Concentration as $CaCO_3$
0%	0^{c}	0^{b}	1.48 ± 0.07^{b}
0.8%	0^{c}	0^{b}	54.55 ± 6.78^{a}
1.2%	150.94 ± 4.61^{b}	47.45 ± 16.10^{a}	0^{b}
1.4%	228.20 ± 18.00^{a}	19.69 ± 9.15^{b}	0^{b}
1.6%	251.49 ± 15.80^{a}	13.01 ± 5.40^{b}	0^{b}

$^{a-c}$ Means that did not share a letter is significantly different ($p<0.05$)

Determination of Carbonic Species

Table 2 shows that there is a significant difference ($p<0.05$) between day 0 and the other 2 days for the bicarbonate concentration as $CaCO_3$ of samples treated at different levels of $Ca(OH)_2$. The carbonic species was determined using calculation of alkalinity relationship (Rodger B. Baird et al., 2017). The titration of samples shows that the phenolphthalein alkalinity for every sample taken from the water tank during the experiment is 0. Thus, the only calculation that could be done following method by Roger B. Baird et al. (2017) is the determination of bicarbonate concentration as $CaCO_3$. This is due to the electrolysis that occurs between anode and cathode where the bicarbonate predominates at lower pH (Margheritini et al., 2020).

Table 2. The bicarbonate concentrations as $CaCO_3$ of samples conducted at different levels of Ca(OH) on day 0, 3 and 7.

Level of Factor	Bicarbonate Concentration as $CaCO_3$		
Day	**Ca(OH)2 (w/v)**		
	0%	**1.2%**	**1.6%**
0	25.33 ± 3.84^{a}	25.56 ± 4.59^{a}	25.67 ± 5.05^{a}
3	4.00 ± 5.66^{b}	1.22 ± 2.22^{b}	0.67 ± 1.41^{b}
7	2.44 ± 4.88^{b}	2.56 ± 6.58^{b}	0.67 ± 2.00^{b}

^{a} Means that did not share a letter is significantly different ($p<0.05$)

CONCLUSIONS

This conclude that the experiment of supplementation of carbonate to the mineral accretion technology does not produce the expected result due to the rapid mineral accretion that occurs and the high concentration of carbonate and bicarbonate ions existing in the artificial seawater. Further study should be conducted on different level of current to the supplementation of carbonate to the mineral accretion system and the addition of supplementation at higher concentrations while taking account the water condition which might affect the health of coral when used as artificial reefs.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
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COCONUT SPORTS POWDER: DEVELOPMENT, CHARACTERIZATION AND SENSORY EVALUATION

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Abstract: Medium-chain triglycerides (MCTs) have been reported to improve endurance exercise performance as MCTs can be readily absorbed and oxidized to produce energy. This study aims to develop coconut sports powder (CSP). Coconut provides an excellent source of MCTs and coconut water replenishes the electrolytes lost during exercise. A mixture was prepared with coconut meat: coconut water: virgin coconut oil at ratios of 10:3:0.6. Other ingredients include sugar and maltodextrin. The mixture was homogenized, pasteurized, and freeze-dried into powder. The CSP contained 422 calories/100g, 78.00% carbohydrate, 11.35% fat, 2.18% protein, 5.72% moisture, 2.76% ashes, 1008 mg potassium/L, and 726 mg sodium/L. The CSP exhibited DPPH free radical scavenging activity of 15.83% with a total phenolic content of 1.39 mg GAE/g. The mean overall acceptability score of the reconstituted coconut sports drink (CSP: water at 1:10) was 5.83 out of 7 with an acceptance index of 83%. The sugar content of the reconstituted coconut drink was 7.05% which meets the requirement for isotonic drink. In summary, a new MCT-containing CSP with relatively high minerals, antioxidants and acceptable sensory quality was developed. Further study on the effects of consuming CSP on physical endurance performance is required to explore its potential as an alternative ergogenic aid.

Keywords: Coconut sports powder, medium-chain triglycerides, electrolytes.

INTRODUCTION

Sports nutrition is a booming sector. Most of the ergogenic nutritional aids in the market are carbohydrate-based. Over-consumption of sugar from ergogenic nutrition aids may result in lipogenesis and insulin resistance leading to hyperglycemia, and possibly Type 2 diabetes (Geigl-Flueck et al., 2021; DiNicolantonio & O'Keefe, 2022). It was documented that MCTs could enhance exercise performance. Due to the shorter chain fatty acids, MCTs are readily absorbed and undergo beta-oxidation to provide an immediate source of energy with < 2% of MCTs turned into fat storage (Chapman-Lopez & Koh, 2022).

Profound electrolytes are lost through sweat during exercise. Electrolytes play critical roles in normal skeletal muscle contraction and bone health. Their deficiencies have been correlated with muscle cramps, and abnormal heart rhythm which leads to shortness of breath, and increased risk of fractures (Schafer & Shoback, 2016). During strenuous exercise, body metabolism and oxygen utilization increase leading to exercise-induced oxidative stress and the formation of free radicals. Excess free radicals may promote cellular oxidation and damage, mitochondria dysfunction, and impair skeletal muscle function, thereby affecting exercise performance (Sahlin et al., 2010; Yfanti et al., 2012). Antioxidants have been reported to reduce exercise-induced oxidative stress.

Coconut is a good source of MCTs and electrolytes. Hence, this study aims to develop coconut sports powder (CSP) using coconut and pandan extract as functional ingredients. The ingredients were homogenized, and freeze-dried into stable powders. Proximate composition, physicochemical properties, and sensory quality of the developed CSP were determined. The CSP was reconstituted to CSP drink which potentially provides fast fuel, electrolytes, and pandan antioxidants that reduce oxidative stress and enhance overall physical performance.

MATERIALS AND METHODS

Preparation of coconut sports powder (CSP). A mixture was prepared with coconut meat: coconut water: virgin coconut oil: pandan extract at ratios of 10:3:0.6:1. Other ingredients include sugar, maltodextrin, pectin and salt. The mixture was homogenized (10,000 rpm, 3 mins) using an Ultra-Turrax homogenizer and pasteurized (90°C, 5 mins). The emulsion was then freeze-dried (-98°C, 24 hrs) into powder.

Physicochemical analyses. References for physicochemical analyses are listed in Table 1. Carbohydrates were calculated by difference.

Table 1. Analyses and References

Analysis	Reference	Analysis	Reference
Fat	AOAC 945.16A (2007)	DPPH	AOAC 2012.04 (2012)

Crude protein	AOAC 920.152 (2005g)	Total phenolic content	AOAC SMPR 2015.009 (2005)
Moisture	AOAC 930.15 (2005h)	Emulsion stability	Abdel-Razek et al. (2022)
Ashes content	AOAC 920.153 (1920)	Encapsulation efficiency	Calvo et al. (2012)
Minerals (K, Na)	Mir-Marques et al. (2016)	Solubility & bulk density	Dirim & Caliskan (2012)
Total sugar	Dubois et al. (1956).	Dispersibility	Jinapong et al. (2008)

Sensory evaluation. Sensory attributes of the reconstituted coconut sports drink (CSP: water at 1:10) were evaluated by 40 untrained panelists using a 7-point hedonic scale (1: dislike strongly to 7: like strongly). The Acceptance Index (AI) of CSP drink was calculated using the formula: AI = (Mean overall acceptability score/7) x 100.
Data analysis. Data collected were analyzed using IBM SPSS Statistics 21.0.

RESULTS AND DISCUSSION

Composition of CSP and reconstituted CSP drink (Table 2). The coconut powder contains 11.35% fat which is attributed to the presence of MCT-rich virgin coconut oil (VCO) and coconut meat. VCO contains 62-70% MCTs, predominantly lauric acid (49%); while coconut meat contains lauric acid (48-60%), capric acid (10%), and caprylic acid (11%) (Boateng et al., 2016). MCTs are hydrolyzed in the intestine to yield medium-chain fatty acids which are directly absorbed into the bloodstream for rapid energy production. MCTs enhanced skeletal muscle performance by promoting mitochondrial biogenesis and metabolism (Wang et al., 2018). Energy generated from fat may help to reduce the lactate buildup in muscle and muscle soreness (Chapman-Lopez & Koh, 2022).

Table 2: Properties of Coconut Sports Powder (CSP) and Reconstituted CSP Drink

<table>
<tr><td colspan="6">Composition of CSP and reconstituted CSP drink</td></tr>
<tr><td colspan="2" rowspan="2">Coconut Sports Powder</td><td colspan="4">Reconstituted Coconut Sports Drink</td></tr>
<tr><td colspan="2">Sugar Content</td><td colspan="2">Mineral Content</td></tr>
<tr><td>Calories (kcal/100g)</td><td>422.81 ± 1.02</td><td rowspan="3">Total sugar content (%)</td><td rowspan="3">7.05 ± 0.32</td><td rowspan="3">Sodium (mg/100 mL)</td><td rowspan="3">72.64 ± 8.22</td></tr>
<tr><td>Carbohydrate (%)</td><td>78.00 ± 0.18</td></tr>
<tr><td>Fats (%)</td><td>11.35 ± 0.12</td></tr>
<tr><td>Crude protein (%)</td><td>2.18 ± 0.09</td><td rowspan="3">Brix value</td><td rowspan="3">7.47 ± 0.06</td><td rowspan="3">Potassium (mg/100 mL)</td><td rowspan="3">100.88 ± 6.37</td></tr>
<tr><td>Moisture (%)</td><td>5.72 ± 0.13</td></tr>
<tr><td>Ashes (%)</td><td>2.76 ± 0.01</td></tr>
<tr><td colspan="6"></td></tr>
<tr><td colspan="6">Antioxidant and physicochemical properties of CSP</td></tr>
<tr><td colspan="2">Antioxidant Properties</td><td colspan="4">Physicochemical Properties</td></tr>
<tr><td rowspan="3">TPC (mg GAE/g)</td><td rowspan="3">1.39 ± 0.05</td><td colspan="2">Emulsion stability (%)</td><td colspan="2">100.00 ± 0.00</td></tr>
<tr><td colspan="2">Encapsulation efficiency (%)</td><td colspan="2">49.92 ± 0.43</td></tr>
<tr><td colspan="2">Solubility (sec)</td><td colspan="2">55.85 ± 2.81</td></tr>
<tr><td rowspan="3">DPPH (% Radical scavenging activity)</td><td rowspan="3">15.83 ± 1.51</td><td colspan="2">Dispersibility (%)</td><td colspan="2">84.48 ± 9.55</td></tr>
<tr><td colspan="2">Bulk density (g/mL)</td><td colspan="2">0.29 ± 0.00</td></tr>
<tr><td colspan="2">Tapped bulk density (g/mL)</td><td colspan="2">0.36 ± 0.01</td></tr>
</table>

Coconut water contains (per 100 mL) 216.8-361.2 mg potassium and 7.3-58.6 mg sodium (Halim et al., 2018). According to Malaysia Food Regulations (Federal Gov. Gazette, 2019), isotonic beverages shall contain 23-92 mg sodium/100 mL, and the Na:K ratio must be less than 1. In this study, the reconstituted CSP drink contained (per 100 mL) 72.64 mg sodium and 100.88 mg potassium with a Na:K ratio of 0.7, which meets the requirements of the regulations. Potassium helps to maintain electrochemical equilibrium across cell membranes, which is essential for the transmission of nerve signals and muscle contraction (Lobo et al., 2002). Sodium stimulates the dephosphorylation of ATP and ADP, inducing muscle contraction (Fuller & Morgan, 1960). Pratama et al. (2022) reported that the provision of coconut water is as good as isotonic beverages in maintaining the hydration status of football athletes.

According to Malaysia Food Regulations 2019, isotonic beverages shall contain 3-10% total sugar. The reconstituted CSP drink contained 7.05% total sugar which falls within the range required by the regulations. The sugar content is attributed to the added sugar and maltodextrin. Isotonic beverages have an osmolality similar to that of blood, facilitating the absorption of water, carbohydrates, and electrolytes.

Antioxidant properties. The total phenolic content and DPPH radical scavenging activity of CSP were 1.39 mg GAE/g and 15.83%, respectively (Table 2). Coconut contains gallic acid, caffeic acid and catechin (Mahayotee et al., 2015), while pandan extract contains gallic acid, cinnamic acid and ferulic acid (Ghasemzadeh & Jaafar, 2013). These antioxidants enhance the antioxidant activity of CSP. Antioxidants reduce oxidative stress via free-radical scavenging; support faster recovery from sports injuries via anti-inflammation; reduce muscle fatigue via reduction of kinase and lactic acid levels; and increase the length of workout via enhancement of lipid metabolism (Wolf et al., 2009).

Physicochemical properties. The high emulsion stability could be attributed to the thickening effect of coconut meat, maltodextrin, and pectin. An emulsion can be made more stable by increasing the viscosity (thickening) of the continuous phase in the o/w emulsion as this makes it more difficult for the dispersed oil droplets to move and combine. The lower encapsulation efficiency (~50%) might be due to the partial coalescence of fat droplets during low-temperature freeze-drying (Nurhadi et al., 2022). In this study, plant biomass (coconut and pandan) was used as encapsulating matrices, capitalizing on the physical functionality of the proteins, carbohydrates in the disrupted plant materials to bind oil droplets in emulsion and providing structural integrity to the powder upon drying of the emulsion. Solubility is defined by the duration required for all particles to dissolve homogeneously in solvent through stirring. The coconut powder demonstrated satisfactory solubility (55.85 sec) and dispersibility (84.48%) which conformed to the characteristics of a porous freeze-dried product. The high porosity of freeze-dried samples eases solvent penetration, which shortens the amount of time needed to dissolve them. The low bulk density could be due to the morphological characteristics of freeze-dried powders with typical irregular shapes and surfaces.

Sensory evaluation results are displayed in Figures 1 and 2. Mean scores for the various attributes of CSP drink: appearance=4.50 $\pm$1.65 , taste=5.93 $\pm$1.35 , aroma=5.80 $\pm$1.27 , and texture=5.35 $\pm$1.56 . The mean overall acceptability score of the reconstituted coconut sports drink was 5.83 out of 7 with an acceptance index of 83%.

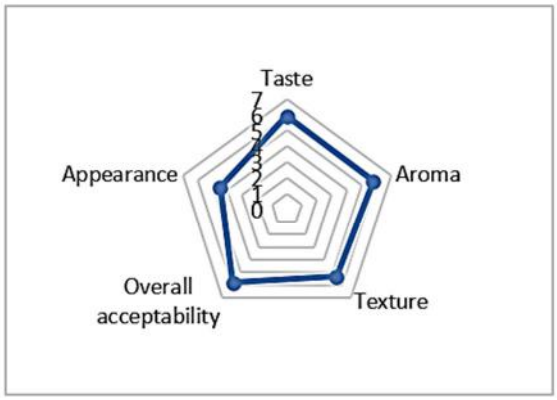

Figure 1: Mean Scores of Sensory Attributes

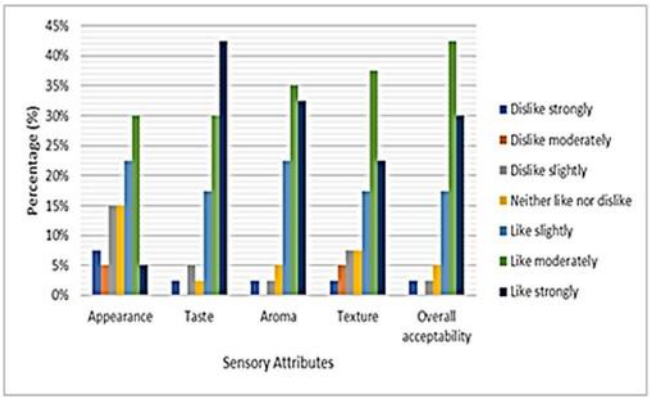

Figure 2. Percentage Distribution of Sensory Scores

CONCLUSION AND RECOMMENDATIONS

This project aligns with the Global Sustainable Development Goal SDG 3 - promote good health and well-being. A new MCT-containing coconut sports powder with acceptable sensory quality was developed. CSP powders are packed with MCTs (fast fuel), minerals (K, Na), and antioxidants. Additionally, the powder is one of the most versatile and cost-effective methods of preservation, for both storage and transportation of finished goods as it can increase the durability and shelf-life of the plant biomass materials – a key factor in an era of rising fuel costs and environmental concern. Further study on the effects of consuming CSP drink on physical endurance performance is required to explore its potential as an alternative ergogenic aid.

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The 17th ASEAN Food Conference 2023 (AFC2023)
24-27th October 2023
Borneo Convention Centre, Kuching, Sarawak

Potential Bioactive Peptides Derived from *Chlorella vulgaris* Microalgae Applying Natural Deep Eutectic Solvent (NaDES)-Biocatalytic System

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Abstract: Chlorella vulgaris are green microalgae widely known for their high nutritional value. Microalgae contain a significant amount of protein (more than 50%) with many potential applications in various industries. These microalgae proteins can also be transformed into bioactive peptides with antioxidant, antimicrobial, and angiotensin I-converting enzyme (ACE) inhibitory properties. However, the protein is less accessible due to the complexity of the microalgae cell wall. Natural deep eutectic solvent (NaDES) can break down microalgae cells and plausibly assist in situ proteolysis of the protein. Thus, the aim is to review the potential mechanism for simultaneous NaDES-Protease interaction in cell disruption of Chlorella vulgaris and in situ proteolysis of the protein released. The usage of NaDES which consists of choline chloride and glycerol was previously studied to extract the lipids from microalgae. Hence, NaDES could enhance the ability of this solvent in extracting the protein content in Chlorella vulgaris species as well. NaDES could assist in disrupting the rigid cell wall of the microalgae, and the presence of proteolytic enzyme will simultaneously mediate the protein extraction and bioactive peptides production. We hypothesize that the NaDES-Biocatalyse system would penetrate the cell wall by interacting with the cell wall components and enhance the protease activity by protecting their protein conformation. Thus, using NaDES would broaden the application of microalgae as a source of protein for future food.

Keywords: Microalgae; Natural Deep Eutectic Solvent (NaDES); Proteolysis; Chlorella vulgaris; Bioactive Peptides

INTRODUCTION

Microalgae is known as the unicellular with a complex organ as well as structure and it has the ability to accomplish the photosynthesis process using sunlight, carbon dioxide and water. This is due to it the presence of photosynthetic pigments such as chlorophyll in their cells (Tan et al., 2020). Many species may contain high levels of protein and it appears that *Chlorella vulgaris* and *Arthrospira* sp are the most commonly exploited industrial species due to their high protein content of 51-58% of dry matter and favourable essential amino acid profile (Wang et al., 2021). Previously, microalgae were also utilised to capture the carbon dioxide from the atmosphere/flue gas emissions and for nutrient removal from wastewaters (Zieliński et al., 2023). Besides, microalgae were also used in other industries such as bioenergy, pharmaceutical, human, and animal nutrition, as well as biodiesel production (Zhang et al., 2022). Hence, the good biological chemical composition that contained in microalgae makes it favourable as the new source for protein extraction (Vale et al., 2020).

However, studies on utilising protein derived from microalgae, especially *Chlorella vulgaris* for bioactive peptides are limited. Cunha et al. (2022) was the only relevant literature utilising *Chlorella vulgaris* for bioactive peptides. It may be due to its cell wall rigidity which hinders the accessibility of the protein (Cunha et al., 2022). Natural deep eutectic solvent (NaDES) can be an alternative to the conventional approach in extracting the protein within the microalgae. NaDES has low toxicity, is environmentally friendly, easy to prepare, has high biodegradability, and is low cost (Gómez et al., 2019). Ling & Hadinoto (2022) reported that the use of NaDES (choline chloride and glycerol) showed an excellent protein yield of 0.3462 g from soy protein compared to the conventional approach (0.3192 g) (Ling & Hadinoto, 2022). NaDES can interact with the cell wall component due to its high polarity allowing the penetration of the microalgae cell (Osamede Airouyuwa et al., 2022). The cell wall disruption would enable the exposure of the protein in the cell wall and intracellular components. Furthermore, Varriale et al. (2022) showed that NaDES could enhance enzyme activity (Varriale et al., 2022). Sun et al. (2020) speculated that deep eutectic solvent (DES) could form a hydrogen bond network to prevent ions from penetrating the

helical structure of the enzyme, thus exerting a role in stabilising enzyme structure (Sun et al., 2020). Thus, the proposed amalgamation of NaDES and protease is plausible to allow simultaneous cell disruption and proteolysis of the microalgae. Furthermore, using NaDES on *Chlorella vulgaris* and in situ proteolysis requires further research to discern its mechanism.

Chlorella vulgaris: CHARACTERISTICS AND BIOCHEMICAL COMPONENTS

Chlorella vulgaris belongs to the group of green algae, with the morphological properties such as 2 to 10 μm in diameter, with the shape of spherical, subspherical or ellipsoid without the presence of flagella. It appears as single cells, and it can form the colonies up to a maximum of 64 cells. *Chlorella vulgaris* consists of a single, cup-shaped chloroplast, with/without the presence of pyrenoids that act as storing starch granules. Besides, it is also made of cell wall, plasmic membrane, chloroplast, thylakoid, vacuole, nucleus, lipid droplet, Golgi body, mitochondrion and cytosol (Jubeau & Michaud, 2013) This unicellular living thing reproduce through asexual autospores, by the division of the mother cell into 2-32 autospores (daughter cells). The mother cell wall will burst upon the maturation of autospores. Mother cell debris becomes food for the daughter cells in the process known as auto sporulation (Ru et al., 2020). The composition of biomass produced *by Chlorella vulgaris* such as lipids, proteins and carbohydrate are affected by the environmental conditions such as the favours light intensity for green microalgae absorb light for photosynthesis via chlorophyll in the range of 450-475nm and 630-675 nm. While for temperature of growth is in the range of 25-35°C, as it shows better growth with higher biochemical content and better nutrient composition. The optimal pH of these cells is in the range of 6.5 to 8.5. Other than that, the carbon dioxide concentration, configuration of bioreactor, as well as nutrient composition such as nitrogen and phosphorus also play an important role (Metsoviti et al., 2020).

The cell wall of *Chlorella* consists of extracellular polysaccharides such as rhamnose, cellulose and hemicellulose. It also consists of algaenan, and protein can be found in the cellular membrane (Orusmurzaeva et al., 2022). The microalgae cell wall can be divided into two, known as the external cell wall and the internal cell wall. The matrix of polysaccharides such as pectin, agar, alginate and algaenan polymers are formed at the former layer, while for hemicellulose, cellulose, pectin, and soluble protein can be found at the latter layer. The composition of these compounds determined the rigidity of the cell wall, as it was previously reported, *Chlorella* sp has a harder cell wall that difficult to break compared to cyanobacteria *Arthrospira* sp and *Scenedesmus* sp (Pôjo et al., 2021). The function of microalgae's cell wall was to protect its vulnerable intracellular components. It was reported previously that the cell wall thickness of *Chlorella vulgaris* is different depending on the growth stages. The mean of cell wall thickness, for *Chlorella vulgaris, Coelastrella* sp, *H.pluvialis* sp and *Scenedesmus* sp increased per growth stage, respectively. A thickness of 0.05 μm for *C.vulgaris* cell wall thickness was observed at the exponential phase (Day 5) and increased to 0.11 μm at the stationary phase (Day 10). The quantity of polysaccharides, lipids, and protein content of *C.vulgaris* also differs as the growth phase shifts (Spain & Funk, 2022). In addition*, Chlorella vulgaris* is also rich in protein, lipids, vitamins, and minerals.

BIOACTIVE PEPTIDES

Bioactive peptides can be defined as the specific protein fragments that carries positive impact towards body function and condition as well as good impact for health (Zaky et al., 2022). The proteins will be broken down into smaller peptides chains, which carries specific peptides sequence and bioactivities, through a process known as hydrolysis (Akbarian et al., 2022). The respective bioactive peptides exhibit their potential bioactivities such as antimicrobial, antioxidative, angiotensin-converting enzyme (ACE) inhibitory activity and etc. (Pujiastuti et al., 2019). Bioactive peptides displayed drug-like or hormone activities and can be classified depending on its mechanism of action as anti-thrombotic, opioid, antimicrobial, immunomodulatory, antioxidative as well as mineral binding. Bioactive peptides can be obtained from various sources such as bovine milk, dairy-products, cheese, bovine blood, meat, eggs, and another various fish species, are the example of the excellent source of this bioactive peptide. Another source of bioactive peptides that can be obtained from vegetal such as mushrooms, maize, soy, as well as pumpkins (Sanchez & Vazquez, 2017). The molecular mass of bioactive peptide is the range of 0.4-2 kDa and build of between two, dipeptides and 20 amino acid residues. In rare case reported the presences of longer peptides, for example such as lunasin that made of 43 amino that were produced from soy and demonstrates the properties of hypocholesterolemia and anti-cancer (Zaky et al., 2022). Barkia et al. (2019) previously has hydrolyses six strains of marine diatom microalgae by using proteases and has resulting in the bioactivities such as angiotensin I converting enzyme (ACE) and oxidative stress marker (Acquah et al., 2020). Another microalgae species that has the unique bioactivities are such as *Chlorella ellipsoidea* consist of the antioxidant properties, as well as antihypertensive and ACE inhibition. Arthrospira maxima carries the bioactivity activities such as antimicrobial properties, anti-collagenase as well as

antioxidant and for *Nannochloropsis oculata* has the bioactivities for ACE -inhibition as well (Acquah et al., 2020). The limitation of microalgae-based bioactive peptides happened due to lack of studies in microalgae and the complexity structure of the microalgae cell wall itself. Thus, the combination of NaDES-biocatalytic process in obtaining the desired protein will help to increase the study in bioactive peptides that can be produced by microalgae.

NATURAL DEEP EUTECTIC SOLVENT (NaDES)

Deep eutectic solvents, known as DES, are the subclass of ionic liquid (IL) that are formed from a mixture of hydrogen bond donors (HBD) and hydrogen bond acceptors (HBA). DES refers to the mixture of substances with a lower melting point than any of the individual components (Figure 1). The hydrogen bond interaction between selected components creates the eutectic mixture (Moldes et al., 2022). The depression of the melting point of these selected components for NaDES is caused by the hydrogen bond. The magnitude of this depression depends on the stoichiometric ratio of the selected component. The stronger the interaction, the larger the depression of the DES melting point. The melting point of a 1:3 molar ratio of Choline chlorine: Glycerol is at -40°C which is much lower than those of Choline chloride at 302°C and Glycerol at 18°C (Hansen et al., 2020).

Natural Deep Eutectic Solvent (NaDES) is considered a natural approach as the elemental components of its eutectic mixtures consist of the primary metabolites that plants utilise for survivability. Those metabolites include organic acids and bases, sugars, and amino acids. NaDES groups can be divided into 5, such (1) the ionic liquid obtained for NaDES is made from acid and base, (2) the neutral NaDES that made from either sugars only or sugars and polyalcohol, (3) the neutral NaDES combined with acids that are made either from sugar or polyalcohol and organic acids, (4) the neutral NaDES combined with bases that made either sugar or polyalcohol and organic bases, (5) the amino acids-based NaDES that are made of amino acid and organic acids or sugar (Mehariya et al., 2021). NaDES is economical, easy to prepare, and environmentally friendly. NaDES can be employed to extract targeted bioactive compounds from the matrix of plants, used in the chemical or enzymatic process as a catalyst, as well as the carrier of insoluble compounds such as hydrophobic compounds in pharmaceutical industries (Yang, 2019).

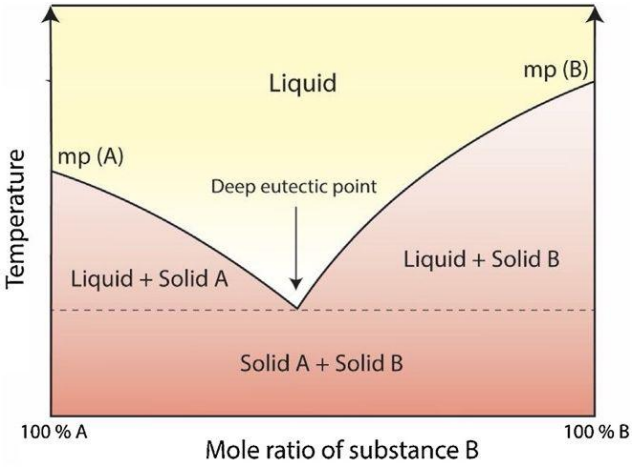

Figure 1 Deep eutectic formation of combined mixtures (Hansen et al., 2020)

NATURAL DEEP EUTECTIC SOLVENT (NaDES) - BIOCATALYST IN PROTEIN HYDROLYSIS

The use of NaDES in biocatalysis is still new; however, research in this area is increasing. NaDES viscosity, density, freezing point, polarity and pH directly influence the enzymatic reactions. These properties can be adjusted by changing the HBA, HBD, molar ratios, and water content (Pätzold et al., 2019). Recent work by Passos et al. (2023) showed that different combinations of NaDES containing sugar and acid as HBA and HBD, demonstrated different elastase activity. NaDES of Glucose: tartaric acid: water at a 1:1:5 ratio showed the highest activity of the elastase. However, the sugar: acid combination in this study showed lower activity than the ionic liquids, which according to the literature, is unexpected since NaDES is frequently described as a greener alternative to ionic liquids. A different combination of HBA and HBD, such as choline chloride (ChCl) and glycerol, can be a plausible option. The combination of ChCl and glycerol has excellent polarity and good ability for the donation and acceptance of hydrogen through the matrix of the natural product (Sakti et al., 2019). ChCl: glycerol has been demonstrated for its ability to extract protein. Chen et al. (2021) have reported that different ChCl: Glycerol molar ratios affect the extraction of protein obtained from soy due to the interaction of glycerol with the hydrophobic functional groups in protein, which

also acts as the amphiphilic interface between the hydrophobic surface as well as ChCl. Glycerol also acts as a stabiliser in the protein extraction process. Furthermore, Sun et al. (2020) reported that ChCl: glycerol enables cytochrome P450 enzyme catalytic function enhancement. The authors suggested that the hydrogen bond network formed by ChCl: Glycerol can provide a protective layer to prevent ions from penetrating the helical structure of the protein domain, thus exerting the role of stabilising the enzyme structure. These findings are crucial as they may be adapted to other enzymes, such as proteases, for the *Chlorella vulgaris* cell wall breakdown and in situ hydrolysis of the protein to bioactive peptides. Further depth on the structural changes through molecular interaction is crucial to improve the elucidation of the enhancement or inhibition of the enzyme by NaDES using computational study.

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GINGER PUREE IN A PACK AND ITS PHYSICAL CHARACTERISTIC

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Abstract: Due to consumer familiarity and the abundance of fresh spices in the local market, consumers always prefer fresh ginger over ginger byproducts. fresh spices are perishable, so the product needs to be upgraded to extend shelf life. This paper discusses the various processing methods for ginger puree and the effects of their physicochemical properties. The ground ginger puree was frozen and retorted. Samples were stored for up to three months for preliminary evaluation and analyzed at monthly intervals. The initial ginger puree contained a TPC and TAC value of approximately 10^4 cfu/g. Both the frozen and ginger purees exhibited stable moisture content, pH, and TSS values throughout the storage period. The overall colour difference, ΔE was greater for the retort puree than for the frozen puree. The colour of the ginger puree was primarily affected by the processing condition as it showed visually a brownish colour and quantitatively, a large deviation to the fresh sample, with a total browning index, *BI* of 45.74 for retort ginger puree and 35.57 for frozen ginger puree after 60 days storage. Therefore, it was suggested to optimize the processes, especially heat treatment to obtain a comparable fresh-like ginger puree in the future.
***Keywords*:** ginger processing, ginger byproducts, ginger puree, frozen ginger, retort ginger

INTRODUCTION

Ginger is one of the aromatic plants with a distinctive spicy flavour that is commonly used in Malaysian dishes as a spice, culinary ingredient or condiment. Ginger, a spice in the *Zingiber Officinale* family, is planted from its rhizomes and is best grown using fertigation technology (M. Yaseer Suhaimi et al., 2014). It is grown as an annual plant (Vasala, 2012), and its maturation period is long, about 7-9 months, to produce fibrous and strongly flavoured ginger. In Malaysia, young ginger is harvested at 4-6 months of age and matured ginger is harvested at 9 months (Mohd & Manas, 2016). Ginger in Malaysia is commonly sold as fresh and rarely processed into byproducts because of consumer familiarity and the abundance of fresh spices in the local market. However, fresh spices are perishable, so the product needs to be upgraded to extend shelf life.

There are a number of papers have studied on ginger paste, minced ginger, chopped ginger (Ahmed, 2004a; Choi et al., 2012; Devi et al., 2016; Unni et al., 2015) and some of them combined ginger with garlic (Topno et al., 2013). This study is an attempt to produce a microbially safe and fresh-like quality of ginger puree that treated under retort and frozen processes. In this paper, a quality evaluation towards their physicochemical characteristics and microbial load during storage were analyzed. However, this study do not specifically concentrate on a specific variety of ginger.

MATERIALS AND METHODS

Ginger puree processing

Fresh ginger was purchased from local Pasar Borong Selangor and kept in a 5°C chiller before processing. The cleaning process of the ginger was done mechanically using a vegetable washer (ATIR III, Nilma, Italy) for 15 minutes with a constant whirlpool of clean tap water. The ginger was then manually trimmed to remove the injured and damaged rhizomes. The peeling was done by mechanical means, using an abrasive bottom surface that spun with the aid of water to peel the ginger rhizomes. Peeling exposed the inner tissue of the ginger, therefore, a pretreatment is required to prevent further oxidation in the ginger rhizomes. The peeled ginger was then immersed in 0.2% sodium metabisulphate solution for a minute. It was then continued with blanching in hot water for a minute, then immersed in chilled water for a minute. The ginger was then ground using a tabletop vegetable chopper, commonly named a bowl chopper (EMS Saarbrucken, Germany) for 10 minutes to get fine particles.

The ground ginger was then divided equally into two different processing methods. For frozen puree processing, the ginger paste was pasteurized at 80 °C for 1 minute, manually hot filled (150g/pack) in a nylon laminated pouch, blast frozen until -18°C and stored frozen in -18°C cold room for three months. The other half portion of the ginger puree was then retorted. Retort processing of ginger paste was done after the ginger was ground. It was

then manually filled (150g/pack), and heat sterilized at 121 °C (Manaf & Yusof, 2021) for 1 hour and stored at room temperature.

Quality and safety analysis

The quality analysis of ginger puree was an important determinant of consumers' preferences. During these preliminary studies, the processed ginger puree was stored for 60 days as an initial study. The physicochemical analysis -moisture content, pH, brix and colour analysis was done throughout the storage of ginger puree. The pH of the samples was measured using a benchtop pH meter (Mettler Toledo, USA). The moisture content of the samples was measured using a moisture analyzer (Model HX 204, Mettler Toledo, USA). The total soluble solids (TSS) of the samples were measured using a pocket refractometer (Model PAL-1, ATAGO, Japan), while the colour analysis was carried out using a chromameter (Model CR-400, Konica Minolta, Japan). The total colour difference was calculated according to the equation below:

$$\Delta E* = \sqrt{(\Delta L*^2) + (\Delta a*^2) + (\Delta b*^2)} \quad (1)$$

The Browning Index, (BI) is used to characterise the overall changes in browning colour. It is defined as brown colour purity and is one of the most common indicators of browning in food products containing sugar (Kasim & Kasim, 2015)

$$BI = 100 \times \left(\frac{X-0.31}{0.17}\right), where\ X = \left(\frac{a+1.75L}{5.645+a-3.012b}\right) \quad (2)$$

Microbiological analyses were also carried out to verify the success of the technology using standard procedures. Total Plate count (TPC), Yeast & Mould, Coliform, *E. Coli*, Total Anaerobic Count (TAC) and *S. aureus* analysis were carried out monthly for both frozen and retort samples.

RESULTS AND DISCUSSION

Quality and safety analysis

Total soluble solids (TSS), pH, colour, moisture content (MC) and microbial counts, of both treated samples were analysed immediately after the process. The same analysis was also carried out in monthly intervals for preliminary analysis. The physicochemical analysis of the ginger - moisture content, pH and TSS values are shown in Table 1 below. The MC of fresh ginger was comparable to the study of Topno et al. (2013), who reported the MC of the ginger paste MC is 83.12 %. The pH of the samples was slightly lower than the fresh ginger probably because of the usage of sodium metabisulphate during the pretreatment process. However, the pH of the samples was higher than found by Devi et al. (2016) and Ahmed (2004b) mostly because of no addition of other preservatives in this processing. This pH value is almost similar to ginger puree by Choi et al. (2012) who obtained a pH ranging from 5.50 to 6.23.

Table 1. Physicochemical Analysis

Storage (days)	Samples	Moisture Content, MC (%)	pH	TSS (° Bx)
0	Fresh Ginger	89.5 ± 2.12	6.54 ± 0.01	2.85 ± 0.05
	Frozen Ginger	91.5 ± 0.70	5.90 ± 0.03	3.88 ± 0.12
	Retort Ginger	91.5 ± 0.08	5.66 ± 0.02	4.00 ± 0.32
30	Frozen Ginger	90.0 ± 1.06	5.93 ± 0.03	2.77 ± 0.14
	Retort Ginger	91.9 ± 1.06	5.58 ± 0.02	3.62 ± 0.15
60	Frozen Ginger	91.6 ± 1.23	5.55 ± 0.04	2.67 ± 0.10
	Retort Ginger	89.2 ± 2.18	5.85 ± 0.11	3.00 ± 0.10

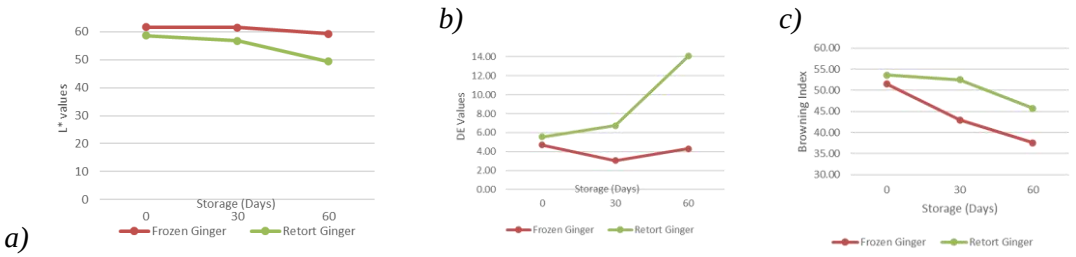
The TSS values of the ginger puree found in this study was lower than Ahmed (2004b) and Devi et al. (2016), probably because of the different variety of ginger used in their study.

The
most pressing parameter, colour analysis was significantly affected. The values showed that after the heat treatment, i.e. pasteurization in the frozen processing and sterilization in retort processing, the L* value decreased, showing the darker colour of the ginger puree. Degradation of colour due to heat treatment is because of the Maillard condensation, caramelization and diminishing of pigment (Unni et al., 2015). Our finding was in agreement with Ahmed (2004b) that the values of L* and b* decreased during storage, however, our a* values did not increase. The colour difference, ΔE, L* values and the browning index were also shown in the graph in Figure 2. All these parameters were obtained from the combination of the L*, a* and b* parameters and indicating the variation of the stored samples to the initial samples.

Ginger puree colour varies from light brown to yellowish and degrades after storage (Ahmed, 2004b). The largest colour difference, ΔE was found in the retort sample after 60 days of storage, with a value of 14.05. The ΔE of frozen samples was almost stagnant during the storage period. Choi et al. (2012) also found that the frozen ginger puree showed no change in colour values after 10 months of storage. These large ΔE, correlate with the greater colour difference from the 0-day samples (Kasim & Kasim, 2015). Devi et al. (2016) found higher values of ΔE in Suprabha varieties of ginger during storage after 120 days than our studies indicating that their ginger puree experienced a larger colour variation probably because of longer storage studies.

The colour of the ginger puree was primarily affected by the processing condition as it showed visually a brownish colour and quantitatively, a large deviation to the fresh sample, with a browning index, *BI* of 45.74 for retort ginger puree and 35.57 for frozen ginger puree after 60days storage. However, the *BI* of both samples showed a declining trend towards the 60 days of storage, probably as the degradation of colour due to oxidation of the pigment took place during storage as mentioned by Ahmed (2004b) and Unni et al. (2015). Min-Seek (2002) concluded that samples with the addition of selected additives would inhibit discolouration during storage. This finding was also in accordance with the study from (Devi et al., 2016) and (Choi et al., 2012) that the colour parameters were significantly affected during the storage period.

Microbiological analysis of the ginger puree is shown in Table 2. The fresh ginger puree before treatment contained a total plate count (TPC) and total anaerobic count (TAC) at approximately 10^4 respectively while total coliform and *S. aureus* were detected at an amount of approximately 10^2. Immediately after treatment, the amount of TPC and TAC of the frozen ginger puree was almost similar to the control sample with approximately 10^4 respectively. However, the amount of *S. aureus* was detected slightly higher, 10^3 compared to the control sample which was only 10^2, probably due to the manual handling during packaging. The coliform count showed an instant decrease after treatment of frozen
and retort as no growth was detected. Topno et al. (2013) reported that ginger-garlic paste after heat treatment at



85°C showed a reduction to nil growth of coliform. The same trend of microorganisms was noticed after 30 and 60 days of storage of the frozen ginger puree. No growth of Y&M and *E. Coli* in the samples of ginger puree throughout the study was detected. Devi et al. (2016) reported that Suprabha ginger showed the growth of mould and was not acceptable in HDPE and PET packaging after 105 days of storage.
The ginger puree that went into retort processing contained no microorganisms from the day of the processing until the 60 days of storage indicating that the ginger puree is microbially stable and the heat sterilization was able to kill all the microorganisms during the processing. As ginger puree is considered a fresh, non-processed food product, the amount of TPC, TAC and *S.aureus* count is considered satisfactory.

Figure 1. The a) L* values, b) ΔE values and c) *BI* values for the ginger puree during the 60 days of storage

CONCLUSIONS

In summary, our studies on frozen and retort ginger puree were microbially stable and the colour of the ginger puree was primarily influenced by processing. Since colour is the first parameter for consumers when making a purchase, it

is always important to give priority to this physicochemical characteristic during processing. While it is common to note that the fresh-looking colour of ginger puree is achieved by adding additives, it is also important to optimise the heat treatment process to preserve the other characteristics and the important health-promoting properties of ginger
puree. This work would be beneficial to small-scale farmers interested in processing ginger puree either frozen or in a shelf-stable format and to transfer the processing method to other ginger varieties.

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FORECASTING POTENTIAL DEMAND PLANTING AREA OF SHALLOT (*ALLIUM CEPA VAR. AGGREGATUM*) IN MALAYSIA

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Abstract: Malaysia has been reliant on the full importation of fresh onions, including large, small, and white varieties, since the 1990s. This practice has been a long-standing trade strategy aimed at meeting the local demand for onions, a crucial ingredient in Asian cuisine. Local onion production in Malaysia has been limited to green onions (spring onions), with no successful attempts to cultivate onion bulbs to date. Before the onset of the COVID-19 pandemic (2003-2019), the annual demand for shallots in Malaysia had been steadily increasing at a rate of 2.7%. However, the pandemic (2020-2022) caused a slight decline in demand, although it remained consistently above an average of 400 thousand metric tons per year. The situation was further complicated by disruptions in supply during the COVID-19 pandemic, as Malaysia's primary onion suppliers, India and China (which accounted for 85% of Malaysia's supply), halted their exports. In 2020, a crisis in the supply of imported onions, particularly from India, occurred due to export restrictions resulting from a major flood disaster in that country. In response to these challenges, the Malaysian Agricultural Research and Development Institute (MARDI) proactively implemented measures to cultivate shallots in temperate regions, which proved to be successful. Consequently, this study has been conducted to forecast the potential future demand for onions, with the aim of informing the development of new policies and strategies for shallot farming in Malaysia.
***Keywords*:** Forecasting, Malaysian Demand, Shallot estimate, Mardi's Farming Model, Import Supply

INTRODUCTION

Data extracted from the Malaysian Selected Agricultural Commodity Supply and Utilization Account (2017-2021) reveals that in 2021, the aggregate imports of onions, shallot and garlic amounted to 622.2 thousand metric tons, with a total worth of RM 1,477.6 million. Among these, large onions constituted the largest share, accounting for 71.5% of the total imports, equivalent to 445.1 thousand metric tons. Garlic followed at 22.1%, representing 137.6 thousand metric tons, while shallots comprised 6.3% of the imports, totalling 39.5 thousand metric tons (Department of Statistic Malaysia, 2022). When considering per capita consumption, it becomes evident that onions are the most commonly used variety, with an average annual consumption of 13.6 kg per person, followed by shallots at 1.2 kg per person and garlic at 0.2 kg per person.

Although onions are consumed in greater quantities overall, shallots stand out in terms of value and sales returns. The import price of shallot is RM 1,880 per metric ton, whereas onions are priced at RM 1,759 per metric ton (Noorazura , 2021). Shallots hold particular significance as a culinary garnish, particularly in the production of fried shallot products within Malaysia. Malaysia ranks third among the highest importing countries, following the United States and Germany, contributing 5.4% to the total import share. Apart from India, other major onion importers to Malaysia include Thailand, China, Pakistan, Myanmar, Netherlands, New Zealand, Australia, and Indonesia (Figure 4). India serves as the primary exporter of onions to Malaysia, accounting for 37.30% of the imports, with a substantial contribution of 57% in the case of shallot. Based on interviews conducted with suppliers and importers, imported red onions are labelled as either "onion" or "big onions," but are differentiated by size (big onions: M, L, and XL) and (shallot: S). Technically, the distinction between these onions is determined by their diameter size, with big onions having a diameter greater than 35 mm and small onions or shallot falling within the range of 25-35 mm.

The growing significance of onions in Malaysian cuisine has led to a continuous rise in demand each year. However, in December 2020, a crisis emerged as a result of an onion shortage in India due to supply constraints, impacting several major importing countries, including Malaysia. Consequently, onion prices in

the market doubled. To address this issue, initial research efforts were made to introduce onion cultivation in Malaysia, which proved to be successful. In order to obtain precise insights into demand and anticipated production, a demand forecasting study has been initiated. The primary objective of this study is to aid the government in formulating policies and allocations that can effectively balance domestic onion production with imports in the future.

METHODOLOGY

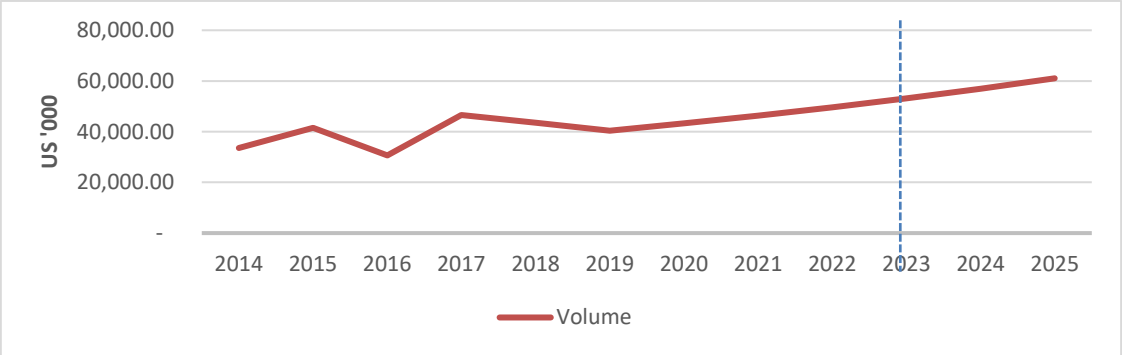
The secondary data used is time series data, namely the import including both quantity and value in the last 8 years, from 2014 to 2022. Data was obtained from International Trade Centre (TRADEMAP) data based. Forecasting data from 2024 until 2025 is forecasted using the Compound Annual Growth Rate (CAGR). The formula can be outlined as follows:

$FV=PVx(1+CAGR)^n$

Where:

FV = Forecasted Value (Value or quantity in the future year)

PV = Present Value (Value or quantity in the latest available year)



CAGR = Historical Compound Annual Growth Rate

n = Number of years into the future

Figure 1. Shallot import forecast for Malaysia for 2023 to 2025
Reference : TRADEMAP, 2023 ; Data processed by Microsoft Excel

The next step involves utilizing the simulation pilot shallot planting model, which has been developed by Mardi for a 1-hectare area. This information is employed to lower the country's import rate by applying a 0.1% expectation at the initial stage. Consequently, a reverse calculation method is employed to determine the planting requirements and the necessary land area for planning shallot cultivation within the country.

RESULTS AND DISCUSSION

Trend analysis forecasting

Figure 1 illustrates the anticipated information derived from time series data spanning from 2014 to 2023. By employing a trend analysis formula, forecasting analysis determined that there is about 7.13% increase in quantity during this period. Consequently, it is projected that shallot imports will continue to rise at the same rate, going from 46.3 thousand Mt in 2022 to 56.9 thousand Mt in 2025.

Mardi's pilot shallot planting model

The Mardi pilot shallot planting model has been employed to delineate the potential yields achievable through local agriculture. The implementation of this model is currently underway in the research plot established in 2022. Nevertheless, in the present year, efforts are being made to enhance crop cultivation to acquire more precise planting data.

Table 1. Technical parameter for shallot planting (1 ha)

Parameter	Item
Plant spacing	15 x 15 cm
Total plant / ha	250,000
Planting period	75 days
Planting cycle / year	2 times
Harvest weight / plant	50 gram
Production / cycle	10,225 kg (fresh weight) ; 8,180 kg (dry weight - after curing)

Based on calculations, it is recommended to conduct a planting trial equivalent to 1% of the total imports. In 2024, the import requirement is projected to reach 53.2 metric tons. If the 0.1% planting trial is successful in reducing the import volume, it would result in the production of 531 metric tons of shallots locally. Moving on to 2025, there is an estimated 7.13% increase in demand, reaching 56.9 metric tons for shallots. Taking into account an estimated production rate of 8.2 metric tons per cycle or 16.4 metric tons annually (accounting for two growing seasons), it would be necessary to allocate an area of 32 to 35 hectares in Malaysia.

Table 2. Shallot forecasting planting area

Year	Forecasting import volume (Mt)	1% from total import (Mt)	Projected land area (Ha) (1 ha = 16.4 Mt/year – 2 cycle)
2024	53,189	531.89	32.4
2025	56,984	569.84	34.7

CONCLUSION

The importance of shallots to Malaysians lies in their role as a crucial daily cooking ingredient. The majority of the nation's cuisines rely on onions, which have historically been entirely imported, lacking local production. However, in response to supply constraints in 2020 due to reduced imports from India, the MARDI research team has successfully introduced a shallot cultivation package. According to model forecasts, there is a projected annual demand increase of 7.13% in 2024 and 2025. To meet the initial goal of a 1% increase in cultivation, an area spanning 32 to 35 hectares is required for shallot cultivation. Ultimately, this initiative is expected to reduce imports by 500 to 570 metric tons, resulting in estimated savings of RM 1.05 million to RM 1.2 million per year. With the findings of this technical study conducted by MARDI, there is hope that onion cultivation in Malaysia can thrive, reducing the country's reliance on imports. This could mitigate the associated risks of import dependency and potentially lead to an increase in the value of domestic production.

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DEVELOPMENT AND MARKETABILITY OF A VACUUM-FRIED MIXED ORANGE AND PURPLE SWEETPOTATO [*IPOMOEA BATATAS* (L.) Lam], BANANA (*MUSA ACUMINATA* × *BALBISIANA* VAR. CARDABA), AND JACKFRUIT (*ARTOCARPUS HETEROPHYLUS*) CHIPS

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Abstract: *There is a growing concern in the consumption of high-fat food products among consumers which can lead to the development of chronic diseases. Studies have shown that vacuum frying technology can produce nutritionally quality fried food products. Binignit* is a traditional food usually made from rice flour, sweetpotato, banana, jackfruit, and tapioca pearls cooked in coconut milk prepared during Lenten Season. This study aimed to develop a healthy alternative *binignit*-inspired chips by using orange and purple sweetpotato, cardaba banana, and jackfruit fried in coconut oil as well as determine its market potential. The product was subjected to the Stage-Gate® System and iterative process in product development. On the third iteration, the mixed chips was able to retain its vibrant color and texture. It had less fat content compared to other fried chips in the market due to de-oiling mechanism of the vacuum fryer used. Based on the market testing, the product had the highest acceptability score on aroma, crispiness, and mouthfeel. Financial and market studies showed that the product is feasible for commercialization and young professionals including parents with an age between 26 and 35 years old as the market group. The results of this study showed promising investment in offering a convenient on-the-go and healthy alternative snack using vacuum-frying technology.
***Keywords*:** Philippine chips, food processing, vacuum frying technology

INTRODUCTION

Vacuum frying (VF) technology is one of the sought technologies in the food industry to cater consumers' preferences in low-fat food products. The demand is brought by the adverse effects of the excess consumption of fat which is a key contributor to the development of heart disease, diabetes, high blood pressure, and certain cancers. VF is a non-conventional deep fat frying that uses low pressure (<6.65 kPa) below the atmospheric level consequently lowering the boiling point of both water and oil. This condition enables to produce a high-quality fried food product (Troncoso and Pedreschi, 2009). Several studies have observed the use of VF technology preserves the natural color, flavor, nutritional components, (Da Silva and Moreira, 2008), reduces the oil content of the finished product (Ravli et al., 2013), decreases the formation of acrylamide (Ranasalva and Sudheer, 2017), and oil degradation is reduced (Ayustaningwarno et al., 2018).

Sweetpotato [*Ipomoea batatas* (L.) Lam] locally known as *camote* is a nutritious and staple crop. The fleshy roots are typically eaten boiled, baked, fried, or used as animal feed. Banana (*Musa acuminata* × *balbisiana* var. Cardaba) is a triploid hybrid (ABB) cultivar that originated in the Philippines. This cultivar is one of the most commonly used in processing banana chips for local and export markets. Jackfruit (*Artocarpus heterophyllus)* is an underutilized fruit in the Philippines. It is commonly known as *nangka/langka* and is usually eaten as fresh fruit. Several studies mentioned that it contains significant amounts of vitamins, minerals, and some carotenes. These commodities are widely cultivated throughout the country primarily for local consumption and export. Consequential to the high production of these commodities, significant amounts of it are rejected and dumped due to out-of-specification qualities, especially for bananas and sweetpotatoes (Calderon and Rola 2003; Lirag & Estrella 2017). Thus, to alleviate the losses of Filipino farmers, this study aimed to utilize the out-of-specification

bananas and sweetpotatoes and utilization of jackfruit into *binignit*-inspired mixed chips and to address the growing demand for nutritionally quality fried snack food.

Stage-Gate® System was employed in the development of vacuum-fried mixed chips (VFMC). It is a conceptual and operational method for moving product ideas to launch. The system consists of five (5) information-gathering gats wherein each gate is a Go/Kill decision point for the idea to progress. Multi-national companies, e.g. 3M, Abbot Nutrition, and Proctor and Gamble, recognized the system as part of their innovation process for creating both products and services (Cooper, 2023). Furthermore, the voice of the customer (VoC) is integrated into the development wherein a sample portion of the market gives feedback and an iterative process happens to address immediately the concerns from respondents to VFMC. Series of iteration were done to improve its color and texture. Three (3) iterations were done before the product became ready for commercialization. Market studies including the development of business model canvas, financial, and technical feasibilities through this method were generated for potential technology adopter of VFMC.

MATERIALS AND METHODS

Plant materials and sample preparation. Orange and purple sweetpotato, cardaba banana, and jackfruit were obtained from an out-of-specification retailer located in a public market at Davao City, Philippines. The samples were selected free from any chemical and microbiological deterioration and had no foul odor. All materials were stored in a well-ventilated area and were processed the following day. The experiment was carried out in a vacuum frying machine equipped with a de-oiling mechanism developed by the Department of Science and Technology (DOST) - Metals Industry Research and Development Center (MIRDC), Taguig City, Philippines. Samples were prepared using the registered Utility Model (UM) for producing the vacuum-fried mixed chips. The process has a Registration Number of 22018050158 from the Intellectual Property Office of the Philippines (IPOPHL).

Product development. The VFMC undergone the Stage-Gate® System in new product development as described by Cooper (2023). The VoC approach was employed during the development process. Product development was conducted at the Food Processing Innovation Center (FPIC) – Davao, Philippine Women's College (PWC) of Davao, Davao City, Philippines. The minimim marketable product (MMP) version of VPMC was subjected to microbiological analysis using the Food and Drug Administration (FDA) Bacteriological Analytical Manual (8th ed.) and Rapid Tests by 3M Petrifilm. The tests were in accordance with the Philippine FDA's Revised Guidelines for the Assesment of Microbiological Quality for Snack Foods.

Market research. Quantitative and qualitative market research were carried out in Davao City, Philippines. There were 200 respondents' information collected in this study using a 28-item pre-tested questionnaire pertaining their demographic profiles, awareness, and interests. Focus group discussions and sensory analysis were conducted for the sensory attributes like color, aroma, taste, flavor, crispiness, and over-all product acceptability.

RESULTS AND DISCUSSION

Product development and iterative process. Preliminary market, technical, and business assessments (Gate 1: Idea Screening) were conducted for VFMC. This gate identified FPIC-Davao's capability, product feasibility, opportunity, and market potential. Business model canvas and financial analysis (Gate 2: Second Screen) were developed for mixed chips as shown in Figure 1. The product is positioned as a locally-source and healthy alternative fried snack food for on-the-go young professionals and parents.

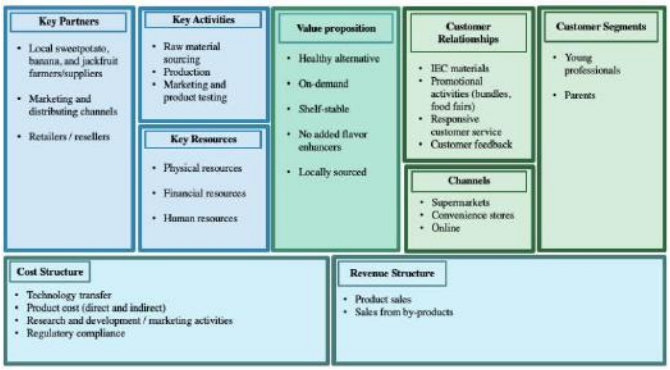

Figure 1. Generated business model canvas for vacuum-fried mixed chips.

Payback period of VFMC was estimated at 2.5 years with a return of investment (ROI) of 62%. Ruegg and Marshall (1990) suggests the acceptable payback period must be shorter than the useful life expectancy of invested capital outlays, e.g. building and equipment. In this study, vacuum-frying machine equipped with de-oiling mechanism and building's life expectancy are both ten (10) years. Hence, the computed payback period is a positive indicator for the development to further proceed in the next gates. Table 1 shows the 3-year financial projection of vacuum-fried mixed chips. Pre-market studies, financial and technical feasibility also showed positive results and figures for investment.

Table 1. A 3-year financial projection of vacuum-fried mixed chips.

3-year financial projection (2022-2024)[1]	
Return of investment (ROI)	62%
Return of Equity (ROE)	44%
Payback period	2.5 years
Net present value (NPV)	P 825,431.92 ($ 15,008)
Internal rate of return (IRR)	53%

Note: [1]FPIC-Davao's vacuum frying capacity: 3,168 pouches (50g) per month Production Cost. Year 1: P 34.70 ($ 0.63); Suggested retail price (SRP): P 50.00 ($ 0.90). Year 2: P38.17 ($ 0.69); SRP: P 55.00 ($ 1.00). Year 3 P 41.98 (($ 0.76); SRP: P 60.00 ($ 1.09).

Mixed chips undergone three (3) iterations (Gate 3: Go to Development) before launching it to the market. Iterations were based on maturity, time-temperature combinations during blanching and vacuum-frying, and use of nitrogen flushing technology. Feedback from sample market showed higher acceptability scores in terms of color, aroma, and mouthfeel compared to its identified direct and alternative competitors using paired comparison test. Results of this study suggest the application of pretreatment such as water blanching and adding of nitrogen gas to further improve the product quality. Existing studies state that blanching and nitrogen gas improves color and texture by deactivating quality-associated enzymes and slows down the rate of oxidation. It was also subjected to microbiological analysis resulting in acceptable levels of molds, yeast and yeast-like fungi, coliform, and aerobic plate counts. Hence, the developed process for producing mixed chips is sufficient to render the product safe for consumption. Thus, the third product iteration served as the minimum marketable product (MMP) version to be sold and marketed for initial users and possible technology adopters. Figure 2 shows the developed VFMC used in market testing (Gate 4: Go to Testing).

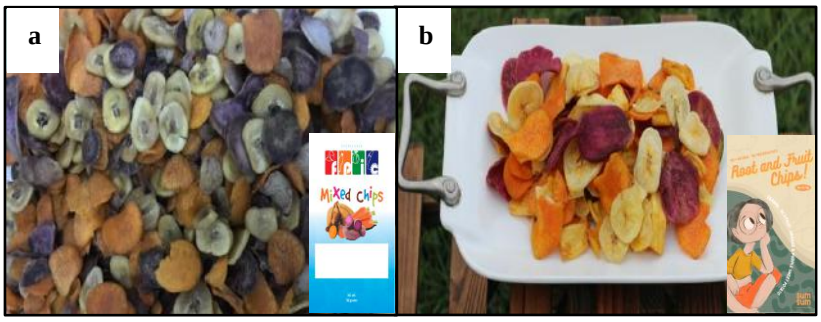

Figure 2. Developed vacuum-fried mixed chips prototype (a) first iteration and (b) minimum marketable product.

Market research. Test marketing was conducted in a high-foot traffic places such as schools, shopping malls, and plazas. Generally, the VFMC had a mean acceptability score of 8.26. This score can be described as 'like very much' on a 9-point Hedonic scale as shown in Table 2. Aroma, crispiness, and mouthfeel were most liked by the panelists. Conversely, taste and flavor had the lowest score of 8.00 and 7.40, respectively. Focus group discussion revealed that jackfruit contributes majorly to high acceptability in terms of aroma, taste, and flavor. Jackfruit contains several compounds that consequently contributes to its fruity aroma which volatilizes during frying. Additions of salt and flavorings to improve taste and flavor were also suggested. Panelists who participated in the market and consumer surveys were 43% male and 57% female with 66.5 % aged between 26 and 35 years old. Participants working in schools and offices were largely interested to buy the product at different channels such as supermarkets, convenience stores, and online shops. Results of this study coincide with the findings of Estrada *et al.* (2021). Product launching (Gate 5: Launch) was done at UP Town Center, Quezon City, Philippines

dated June 2022. The event was organized by DOST – Industrial Technology Development Institute (ITDI) during the culmination of the project titled "Development of FIC Competency in Moving New Products from Concept to Market Launch".

Table 2. Acceptability scores of vacuum-fried mixed chips.

Parameter	Acceptability score (9.00)	Acceptability description
Color	8.13±0.82	Like very much
Taste	8.00±0.56	Like very much
Aroma	8.50±0.67	Like very much
Flavor	7.40±0.83	Like moderately
Crispiness	8.46±0.62	Like very much
Mouthfeel	8.50±0.61	Like very much
Overall	8.36±0.30	Like very much

CONCLUSIONS

The demand for healthy fried food products was heightened with the increase of awareness and development of certain diseases brought by excess consumption of fat. Production of fried food products through vacuum frying technology showed favorable effects in lowering the risks of conventional deep fat frying. Results of this study revealed promising investment in the production of vacuum-fried orange and purple sweetpotato, banana, and jackfruit chips in relation to commercialization.

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DEVELOPMENT AND MARKETABILITY OF READY-TO-EAT TUNA CONGEE

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Abstract: The development of ready-to-eat (RTE), commercially safe, and consumer-focused food products is a practical solution to the increasing demand for convenient yet nutritious meals. This study aimed to develop an instant rice porridge and determine its market potential. The RTE Tuna Congee was subjected to the iterative process of design, development, and testing using the voice of customers. As an innovative rice porridge, the prototype contained tuna flakes and selected vegetables, packed in a retort pouch, and thermally processed at 116 degrees Celsius with a minimum process lethality (F_0) value of six minutes. Based on the commercial sterility test conducted, the established thermal process was sufficient to render the product safe for consumption by the general public. The acceptability test conducted also showed that the instant rice porridge was acceptable in terms of appearance, aroma, consistency, taste, and overall liking. Furthermore, the financial and market studies revealed that the product is feasible for commercial production with the highest potential market and age group were the time-strapped and on-the-go individuals, aged 26 to 35 years old, respectively. The results of this study showed its potential for commercial production, offering a convenient and safe RTE product through thermal processing technology.
Keywords: rice porridge, commercial sterility, thermal processing

INTRODUCTION

There is a growing demand for ready-to-eat (RTE) food due to its convenience, value, attractiveness, taste, and texture (Patel and Rathod, 2017). Belonging to this category are those foods that require minimal to no preparation before consumption such as canned foods, fast foods, instant products, dried foods, and preserved foods (Selvarajn, 2012). Consumer preference for this food type is influenced by a combination of factors including product quality such as taste, appearance, and texture; socio-economic such as availability, price, and culture; biological such as energy and nutrient requirements; and psychological such as behavior, moods, and attitude towards eating (Blades, 2001). Considering all these factors in new product development can be expensive and time-consuming. Hence, to maximize productivity, the Stage-Gate® methodology was introduced (Cooper, 2006).

This study is focused on the development and marketability of RTE Tuna Congee while adopting the Stage-Gate® system in product innovation. This approach involves a spiral process of design, development, and testing that is consumer-centered (Cooper, 2006). In this way, the feedback from the target market is highlighted throughout the development process, maximizing productivity. The results of this study showed the RTE Tuna Congee has potential for commercial production, offering a convenient and safe RTE product. Furthermore, the market studies suggested the highest potential market and age group for the RTE Tuna Congee were the time-strapped and on-the-go individuals, aged 26 to 35 years old, respectively.

MATERIALS AND METHODS

1. DEVELOPMENT OF RTE TUNA CONGEE

The Stage-Gate® system, popularized by Cooper (2006), was considered in this new product development. The RTE Tuna Congee was developed according to the Utility Model Registration No. PH22020050025, comprising the following steps: (1) rice soaked in water; (2) garlic, onion, ginger, and spring onion sauteed in vegetable oil to form a spice mixture; (3) spice mixture cooked with water, tuna meat, carrot,

sweet corn, moringa leaves, and at least one seasoning to form a pre-cooked tuna composition; (4) tuna congee composition pre-packed and (5) thermally-processed. Product development was conducted at the Food Processing Innovation Center (FPIC) – Davao, Davao City, Philippines.

1.1. PROTOTYPING

The RTE Tuna Congee formulation was subjected to the iterative process of design, development, and testing using the voice of customers (VoC). A total of six (6) iterations on the solid-to-broth ratio were conducted, from developing the minimum viable product (MVP) version to the minimum marketable product (MMP) version of the RTE Tuna Congee.

1.2. ESTABLISHMENT OF THERMAL PROCESS

Thermal processing of the product was conducted using a vertical water immersion retort. The final product prototype was subjected to a Heat Penetration Test (HPT) using the Ellab Thermal Validation System. Process calculation was done using the Ellab Valsuite Pro ver. 6.2.3.0 software, employing both the general and formula methods. The established thermal process schedule was then validated using a commercial sterility test (CST) following the AOAC Official Method for Canned Low-acid Foods.

2. MARKETABILITY OF RTE TUNA CONGEE

The acceptability of the RTE Tuna Congee by its intended market was determined by both quantitative and qualitative market research via survey and key informant interview, respectively. The Segmenting-Targeting-Positioning (STP) approach was followed during the strategic marketing. Prior to the conduct of the research, a letter of consent was given to the target participants, who were identified as time-strapped and on-the-go individuals based on the STP conducted.

2.1. QUANTITATIVE MARKET RESEARCH

The quantitative market research used a survey questionnaire to gather data from 200 respondents within Davao City. The 28-item questionnaire was pre-tested to check its clarity and reliability in collecting information on the respondents' demographic profiles, as well as their awareness, opinion, and interest in the developed product. A sensory evaluation was also conducted by quality scoring and acceptability test.

2.2. QUALITATIVE MARKET RESEARCH

The qualitative market research, on the other hand, used purposeful or snowball sampling since the target market was not easy to reach. Specifically, the researchers targeted Call Center Agents as key informants since they qualified as time-strapped and on-the-go individuals. A semi-structured interview was conducted among eight (8) respondents from selected Business Process Outsourcing (BPO) companies within Davao City.

RESULTS AND DISCUSSION

The Stage-Gate® approach was adopted in the development of the RTE Tuna Congee. This new product development system serves as a roadmap to guide innovation processes from idea to launch stages. Furthermore, to ensure that the product development is properly executed, preceding each stage is an entry gate that serves as a quality-control checkpoint (Cooper, 2010).

Stage 1. Scoping. This stage involves a quick assessment of the technical feasibility, market potential, and business soundness of the product concept. The RTE Tuna Congee was technically feasible since the raw materials and technology were readily available and accessible in the locality. In addition, the concept was deemed valuable to the overall Filipino palate since rice porridge is considered a comfort food, and making it into an instant and shelf-stable form is a sound innovation.

Stage 2. Build Business Case. This stage involves primary research with a more detailed investigation on the technical and market aspects of the product concept. At this particular stage, the business model canvas (BMC) for the RTE Tuna Congee was developed, as shown in Figure 1. The presented BMC illustrates the flow of the expense-and-profit structure for the development of the RTE Tuna Congee. To balance such structure, the value propositions of the developed products were highlighted at the center of the BMC. Particularly, the product had been propositioned as "ready-to-eat or convenient", "healthy and nutritious", "safe and shelf-stable", "lightweight and easy-to-open", "affordable", and "available and accessible". Based on its value propositions, the identified targeted markets or customer segments of the product were those time-strapped, on-the-go, and health-conscious individuals.

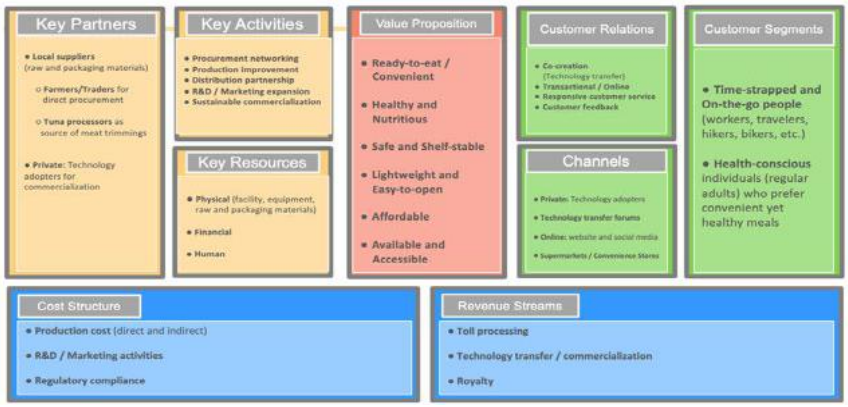

Figure 1. The business model canvas for the RTE tuna congee.

Stage 3. Development. This stage involves the actual product development with product prototyping. The RTE Tuna Congee formulation was subjected to the iterative process of design, development, and testing using the VoC. Iterations on the solid-to-broth ratio were conducted according to the feedback from the target participants. The solid-to-broth ratio was critical in the development of RTE Tuna Congee since the product is mainly made from rice which has varying degrees of water uptake during cooking. According to related studies, water uptake of milled rice heightened initially at an increasing rate before decreasing at a given cooking schedule. Furthermore, water uptake by rice during cooking was primarily dependent on its surface area as a function of varietal differences as manifested by physicochemical and quality characteristics such as amylose content, gel consistency, alkali spreading value, and protein content (Yadav and Jindal, 2007; Keawpeng and Venkatachalam, 2015; Zhu et al., 2020). To control the interfering effects of these factors, the rice variety, serving size, and thermal processing conditions were held uniformly throughout the study. The most acceptable formulation according to the target participants was considered the MMP version of the RTE Tuna Congee, as shown in Figure 2, which was subjected to further testing.

Figure 2. The minimum marketable product version of the RTE tuna congee.

The main technology employed in the development of the RTE Tuna Congee was thermal processing, which primarily involves in-container sterilization. As a basis for the establishment of a safe thermal process, the time-temperature profile of the developed product, packed in 12x20 cm retort pouch at 200 g/pouch, was determined by HPT. The resulting minimum F_0 value of six minutes was established at 116 degrees Celsius. This data is in agreement with Tucker (2001) and Drotz (2012), denoting that commercial processing operations are commonly done with substantially increased safety margins which would equate to log reductions upwards of 25 to have a minimum F_0 value of six minutes.

Moreover, the results of the CST showed that the product was commercially sterile, implying that the established thermal process schedule was sufficient to kill all microorganisms capable of surviving in the food at non-refrigerated conditions evident during manufacture, distribution, and storage Tucker (2001).

Stage 4. Testing and Validation. This stage involves testing in the marketplace to validate the proposed new product. Sensory evaluation of the RTE Tuna Congee revealed the quality and acceptability scores of the product, as summarized in Tables 1a and 1b, implying that the product is acceptable in terms of appearance, aroma, consistency, taste, and overall liking.

The market studies further revealed that the product is feasible for commercial production with the highest potential market and age group were the time-strapped and on-the-go individuals, aged 26 to 35 years old, respectively. The results are in agreement with the study of Loverio and Guevarra (2016) on the food practices and preferences of selected call center agents in Metro Manila. Accordingly, as a result of the stressful nature of their jobs, they prefer convenient and comfort foods such as rice meals and congee variants (Loverio and Guevarra (2016).

Table 1a. Mean quality scores of the product.

Attribute	Mean Score	Description
Appearance	3.90±0.79	Good solid-to-broth ratio
Aroma	4.15±0.75	Moderately perceptible aroma
Consistency	4.05±0.69	Moderately viscous
Taste	4.35±0.75	Good balance
Aftertaste	4.00±0.97	Slightly perceptible aftertaste

Table 1b. Mean acceptability scores of the product.

Attribute	Mean Score	Description
Appearance	7.78±0.81	Like very much
Aroma	8.00±0.69	Like very much
Consistency	7.89±0.68	Like very much
Taste	8.00±0.77	Like very much
Aftertaste	7.89±0.76	Like very much
Overall	7.78±0.81	Like very much

Stage 5. Launching. Finally, the launching of the developed product was successfully conducted during the "InnovEats: Food Ideas and Creations", which was organized by the Department of Science and Technology-Industrial Technology Development Institute (DOST-ITDI) dated 25 June 2022.

CONCLUSION AND RECOMMENDATION

The Stage-Gate® system adopted in this product innovation was deemed efficient. The spiral development approach that was market-driven offered a competitive advantage since meeting customer needs is vital for the success of new products.

The results of this study revealed that the developed RTE Tuna Congee showed potential for commercial production, offering a convenient and safe RTE product through thermal processing technology. As a complementary study, an accelerated shelf-life test is highly recommended in order to determine the keeping quality of the developed product

ACKNOWLEDGEMENT

The authors wish to extend their deepest gratitude to the DOST-ITDI, DOST Regional Office XI, PWC of Davao, and DOST Office of the Undersecretary for Research and Development for the capacity building and funding.

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MICROPROPAGATION OF BANANA (*MUSA* SP.) CULTIVAR 'BERANGAN' AND 'SABA' USING MALE FLOWERS

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Abstract: The present study was undertaken with a view to formulate growth medium for clonal micropropagation of two banana cultivar 'Berangan' and 'Saba' using male flower (inflorescence) for the production of planting material. This experiment consisted of the basal growth medium of Murashige and Skoog (1962) with combination of different concentration of plant growth regulator cytokinin (6-benzylaminopurine BAP and Kinetin) and auxin (1-Naphthaleneacetic Acid NAA). The trial design is Completely Randomize Design with five treatments namely T1 (devoid of plant growth regulator) as a Control, T2 (MS + 7.0 mg/l BAP + 0.5 mg/l NAA), T3 (MS + 15% Coconut Water + 5.0 mg/l BAP + 0.5 mg/l NAA), T4 (5.0 mg/l Kin + 0.5 mg/l NAA) and T5 (MS + 2.5 mg/l BAP + 2.5 mg/l Kinetin + 0.5 mg/l NAA). Each treatment consisted of three replications with 10 culture per replication. The results showed significant shoots response on 'Berangan' and 'Saba' cultivar after 13 weeks in treatment T2 (MS + 7.0 mg/l BAP + 0.5 mg/l NAA) and treatment T3 (MS + 15% CW + 5.0 mg/l BAP + 0.5 mg/l NAA) with an average of 38 and 32 shoots produced respectively. A total of 25 'Berangan' and 32 'Saba' banana plantlets were successfully transplanted out to nursery. MS medium supplemented with 5.0 - 7.0 mg/l BAP is recommended as a growth medium for clonal micropropagation of banana (*Musa* sp.) for the mass production of planting material.
Keywords: Micropropagation, *Musa* sp., banana male flower, 6-Benzylaminopurine, Kinetin

INTRODUCTION

Banana is globally ranked fourth, next to rice, wheat and maize in terms of gross value of production. Among the major producers, India accounts for 26.2 milion tonnes followed by Philippines, producing 9.01 milion tonnes and China, Brazil and Ecuador, with production ranging from 7.19 to 8.21 milion tonnes (Singh, *et al.*, 2011), while Malaysia produced more than 50,000 metric tonnes annually. New market areas has emerged for banana in Peninsular Malaysia where the Federal Agriculture Marketing Authority (FAMA) is seeking a constant supply of about 1,728 metric tonnes annually from Sabah (Sabahkini). The national food policy, outlined in the 'Dasar Agromakanan Negara' indicates that our nation will produce 440,566 metric tonnes of banana per annum by the year 2020 for the state of Sabah, banana is listed under food crops with the production of 47,424 tonnes in 2011 (Anon, 2011).

According to Singh, *et al.* (2011), with conventional field planted banana, the rate of multiplication in banana is restricted to 5-20 suckers per plant during its growth period, which makes it difficult to obtain sufficient amount of planting material. Advanced propagation method such as *in vitro* micropropagation facilitates production of large number of plantlets per unit time, thus helping in rapid introduction and dissemination of new varieties/cultivars. By using tissue culture technique, it is possible to develop planting material which is free from sucker borne disease and pests. Using of healthy planting material complemented with integrated pest management program is the key to a good crop stand in field.

In vitro propagation using shoot tips has been reported for many commercial banana cultivar, however, male flower (inflorescence) reported has potential regenerable explant (Cronauer and Krikorian, 1985a; b; Mahadev, *et al.*, 2011; Linta, 2018) for rapid micropropagation. This method reduces contamination rate as compared to soil grown suckers (Darvari, *et al.,* 2010). Therefore, the objective of this project is to formulate growth medium for clonal micropropagation of two banana cultivar 'Berangan' and 'Saba' using male flower (inflorescence) for the production of planting material.

MATERIALS AND METHOD

This study was conducted in Plant Tissue Culture Laboratory at Agriculture Research Centre Ulu Dusun (ARCUD), Sandakan, Department of Agriculture Sabah in the year 2019-2020.

Plant material

A total of 30 floral apices explant of 'Berangan' and 'Saba' banana were sampled for each designated treatment

medium from banana germplasm plot located at ARCUD, Sandakan.

Preparation of explants
Healthy explants from reduced male floral buds were first washed with running tap water, few of the spathe were removed, surface-sterilized using 75% (w/v) alcohol for 30s, then the explants spathe was removed again inside the laminar flow and subsequently isolated using surface-sterilized forceps. Innermost male flower buds were used in culture after their bracts have been carefully removed. The sizes of these floral apices were reduced to 4.0-5.0 cm long and were cut longitudinal section into three to four before cultured onto medium designated medium culture (Figure 1).

Culture medium
The explants were cultured onto the Murashige and Skoog (MS) medium (Murashige and Skoog, 1962), which was supplemented with two different types of cytokinins (6-benzylaminopurine (BAP) and kinetin (Kin) singly or in combination) and an auxin 1-Naphthaleneacetic Acid (NAA) as follows in Table 1. All media was set at pH 5.7 prior to autoclaving for 20 min 121ºC. The explants were cultured onto the media in a flask, placed on racks and incubated at 25 – 27ºC under 14/10 h (light/dark) photoperiod with 3000 Lux of light intensity provider by fluorescent lamps. Explant subculture was done every four weeks interval. Growing shoots with expanded leaves were then excised and cultured separately on 10 ml fresh medium in test tube to encourage shoot elongation and formation of basal roots.

Table 1: Plant Growth Regulators (mg/l) Treatment for Shoots Bud Multiplication.

No.	Banana cultivar	Treatments	Growth Media	Plant Growth Regulator mgL^{-1}		
				BAP	Kin	NAA
1.	Berangan	T1 (Control)	MS	-	-	-
		T2	MS	7.0	-	0.5
		T3	MS + 15% CW	5.0	-	0.5
		T4	MS	-	5.0	0.5
		T5	MS	2.5	2.5	0.5
2.	Saba	T1 (Control)	MS	-	-	-
		T2	MS	7.0	-	0.5
		T3	MS + 15% CW	5.0	-	0.5
		T4	MS	-	5.0	0.5
		T5	MS	2.5	2.5	0.5

CW = Coconut Water

Experimental Design
The trial design was arranged in a Completely Randomized Design (CRD) consists of four treatments and a control with three replications for each banana cultivar ‘Berangan’ and ‘Saba’.

Statistical Analysis
Regeneration frequency was calculated based on the number of shoots produced at every four weeks interval. All variables were presented in Mean, Standard Error (S.E.) and Coefficient of Variation (C.V %). Analysis of variance (ANOVA) was performed using statistical software, MSTAT Version 2.0 (Freed, 1985).

RESULTS AND DISCUSSION

All ‘Berangan’ and ‘Saba’ explants were found to expand and they became green as early seven days of inoculation. The explant responded to all treatments (T2, T3, T4 and T5) through induction of cauliflower-like bodies clusters (CLBs), which became visible after five weeks in culture (Figure 2) except for treatment T1 (MS medium devoid of PGR). These results shown that male flowers of both banana cultivar ‘Berangan’ and ‘Saba’ have shown their potentials as suitable explants for direct regeneration through induction of CLBs clusters as reported by Krikorian, *et al.* (1993).

After fifth cycles of subculture, vigorously growing shoots with expanded leaves were observed. All shoots then excised and cultured separately on fresh 10ml medium in test tube to encourage shoot elongation and formation of basal roots. The rooting basal tufts emerges in all of the transferred shoots after four weeks (Figure 3). The results (Table 2) shown that, significant shoots response was obtained after 13 weeks in treatment T2 for ‘Berangan’ and treatment T3 for ‘Saba’ with an average of 38 and 32 shoots production respectively, compared to treatment T1. This shows that BAP ranged from 5.0 - 7.0 mg/l were best to regenerate banana shoots. This study agrees with Cronauer, S. S., & Krikorian, A. D. (1984), Jarret, *et al.* (1985), Resmi and Niar (2007) that MS medium supplemented with 5.0 mg/l of BAP able to regenerate *Musa acuminate* cv. ‘Dwarf Cavendish’ (AAA)

with multiple shoot cluster. Studies by Harirah and Khalid (2006), also reveal that MS medium supplemented with 7.0 mg/l of BAP gave a large number of shoot formation from the male inflorescence of *M. acuminate* cv. 'Berangan'. This study agree that each banana cultivar has an optimum concentration for maximum response to proliferation as reported by Vuylsteke (1989).

Table 2: Effects of PGR (BAP and Kin) on Shoots Production From Male Flower Explants of 'Berangan' and 'Saba' Using MS Medium.

Treatments	Average Banana Shoots Production		Phenolics Rate (%)	
	Berangan	Saba	Berangan	Saba
T1 (Control)	0	0	100	100
T2	38[a]	12[c]	60.00	80.00
T3	9[c]	32[a]	56.67	66.67
T4	5[c]	4[d]	80.00	83.33
T5	21[b]	21[b]	66.67	66.67
Mean	**14.60**	**13.80**	**72.67**	**79.33**
S. E.	**0.968**	**1.472**		
Significance	*	*		
C.V (%)	**10.61**	**17.07**		

Means of 3 replicates; any two means within a column having a common letter are not significantly different from each other at the 5% level by LSD. *Significant at 5%; n.s not significantly different.

From this study, it was also observed that both banana cultivar explants in MS medium augmented with combination of 2.5 mg/l BAP and 2.5 mg/l Kin able to produce better shoots formation with an average of 21 shoots respectively. It indicates that the multiplication rate of the male flowers was found to be significantly dependent on the types, concentration and combination of cytokinins used. Thus, cytokinins are necessary as a pre-requisite of male flower regeneration as reported by Darvari, *et al.* (2010).

This study also shows high phenolics rate was observed in 'Saba' compare to 'Berangan' with 79.33% and 72.67% respectively (Table 2). It was observed that 'Saba' banana produces high phenolic compound which contribute to high contamination rate (Siddiqui, *et al.*, 1995; Titov, *et al.*, 2006; Ko, *et al.*, 2009; Ahmad, *et al.*, 2013). Shorter subculture interval has been conducted to reduce the phenolic effects. However, it did not reduce the mortality rate of the banana explants. Therefore, further study can be conducted to reduce the effect of phenolic compound on explants culture. A total of 25 'Berangan' and 32 'Saba' banana plantlets were successfully transplanted out to nursery for *ex-vitro* observation. Plantlets with expanded leaves and develop root system were planted to *Hyplug*® tray containing of coco peat (Figure 4).

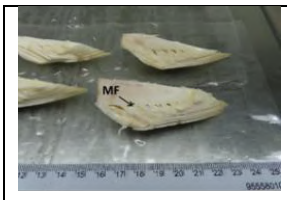

Figure 1: Male flowers (MF) ready for inoculation.

Figure 2: CLBs clusters became visible after 5 weeks in culture.

Figure 3: Shoot regeneration from CLBs.

Figure 4: Acclimatized and hardened plantlets in polybags containing top soil.

CONCLUSION

Findings of the present study elucidate that plant regeneration could be possible from the CLBs developed from banana male flowers explants. All medium culture supplemented with cytokinins were able to produce CLBs and shoots formation excluding treatment T1 (medium devoid of PGR). Significant shoots response was obtained in 'Berangan' and 'Saba' cultivar after 13 weeks in treatment T2 and treatment T3 with an average of 38 and 32 shoots production respectively. This shows that cytokinin concentration range from 5.0 to 7.0 mg/l BAP were best to regenerate banana shoots for clonal micropropagation of both banana cultivar for mass production of planting material. A total of 25 'Berangan' and 32 'Saba' banana plantlets were successfully transplanted out to nursery. It is recommended to conduct further experiments to evaluate the growth performance of both banana cultivar in the field before being mass-produced.

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MICROBIOLOGICAL QUALITY ASSESSMENT OF SMOKED MEAT AND FRESH SEAWEED DURING STORAGE STUDY UPON HIGH PRESSURE PROCESSING

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Abstract: Natural and healthier foods such as smoked meat and fresh seaweed are in greater demand on a global scale. However, both food products have naturally limited shelf life, thus it is crucial to investigate the effect of high pressure processing (HPP) on products' safety parameter including HPP potential to prolong shelf life. Specifically, HPP treatments of 6000 Bar for 4.5 min on Nylon/PE packaging for smoked meat and 6000 Bar for 3 min on PE/PET packaging for fresh seaweed were applied based on the optimization of HPP parameter previously done. The HPP-treated smoked meat showed a minimum storage of 56 days while the untreated smoked meat demonstrated Total Plate Count of 10^5 CFU/g at day 0 which increased to 10^6 CFU/g at day 14 of storage at chilled temperature. It was also found that throughout the 140 days of storage period, the microbial levels of HPP-treated fresh seaweed remained below the detection limit while the microbial levels of untreated fresh seaweed exceeded the microbial safety limit at day 14 of storage at chilled temperature. In summary, HPP effectively maintained the microbiological quality of smoked meat and fresh seaweed for a minimum of 56 days and 140 days, respectively.
Keywords: Microbiology, high pressure processing, smoked meat, fresh seaweed, storage study.

INTRODUCTION

Nowadays, there is a general demand to produce fresh and minimally-processed food in line with the increasing consumer awareness on the intake of healthier ready-to-eat (RTE) products with good nutritional qualities and guaranteed safety. This market trend has strived the food industry to develop innovation especially in traditional food such as smoked meat and fresh seaweed involving the application of novel processing technologies to preserve products, extend shelf life and improve microbiological safety. Smoked meat was the result of preparing red meat with smoking methods for flavour enhancement, appearance improvement and food preservation. Smoked meat also known as *daging salai* in Malay is one of the local favourite dishes that is widely consumed with rice, sandwich or spaghetti, however has limited shelf life when kept chilled. Meanwhile, fresh seaweed (*Gracilaria changi*) also known as sea vegetable is widely grown in Malaysia, can be consumed fresh or used as food condiments in the form of dried or wet seafood. However, *Gracilaria* has a relatively short shelf life once harvested due to its watery texture and high nutrient content that provide resources for microbial growth.

Therefore, the application of high pressure processing (HPP) is possible as an efficient alternative technique that involves non-thermal treatment to the packaged food in order to inhibit harmful pathogens and spoilage microorganism, and to inactivate undesirable enzymes, with minimal effects in sensorial and nutritional quality. The effect of HPP on the food characteristics' quality has been mainly derived from the the stability of covalent bonds under high pressure, without inducing any food structure molecules. HPP also encourages minimal usage of additives in product development as well as compliance with food safety regulatory requirements. Previous studies on the shelf life extension of local products such as jackfruit, durian, cow milk and goat milk have shown encouraging results (Tan *et al*., 2019; Chin *et al*., 2020). However, to the best of our knowledge, studies on the application of HPP to smoked meat and fresh seaweed in Malaysia are still fragmented. Thus, the aim for the present study was is to investigate the effect of optimized HPP parameters on the microbiological properties of smoked meat and fresh seaweed over the storage period under chilled temperature.

MATERIALS AND METHODS

Preparation of smoked meat and fresh seaweed including pressurization study

Smoked meat samples were provided by local entrepreneur at Rembau, Negeri Sembilan. Samples were prepared at entrepreneur's premise by smoking methods by using mixture of woods, subsequently packed into a 12 cm (width) and 17 cm (length) food grade nylon/polyethylene (Ny/PE) as primer packaging (approximately 100 g of sample per packet) and into biaxially oriented polypropylene/kraft paper/cast polypropylene (BOPP/Kraft/CPP) as secondary packaging. Then, packed smoked meat were transported to Food Science & Technology Research Centre, MARDI at Serdang, Selangor in chilled temperature (2 ± 7 ºC) within the same day of processing. Meanwhile, fresh seaweed (*Gracilaria changii*) samples were supplied from entrepreneur's farm at Muar, Johor and delivered to MARDI, Serdang within 3 to 5 hours at 2 ± 7 ºC. Once arrived, fresh seaweed samples were subjected to pre-treatment which consisted of washing with filtered water, soaking with chlorine for 30 secs, tossing and rinsing with filtered water, squeezing using spinner and finally packaging in polyethylene/polyethylene terephthalate (PE/PET) (approximately 50 g of sample per packet).

A HPP unit (Hiperbaric 55, Burgos, Spain) located at Faculty of Food Science and Technology, Universiti Putra Malaysia (UPM) was used to process the smoked meat and fresh seaweed, respectively. The high pressure was generated by a cylindrical pressure chamber, a pressure pump and a hydraulic unit with water as the pressure medium in the HPP chamber. HPP parameters of 6000 Bar for 4.5 mins for smoked meat and 6000 Bar for 3 mins for fresh seaweed with pressurization maintained at 20 ºC processing temperature were used in this study based from the optimization study using Response Surface Methodology (RSM) previously done. In this experiment, microbiological test was carried out 1 hr after HPP treatment with all samples analysed by triplicate. HPP-treated samples were kept at 2 ± 7 ºC for further analysis. The microbiological quality of the smoked meat and fresh seaweed was evaluated every 14 days throughout a 56-day and 140-day storage period, respectively.

Microbiological analysis

Microbial analysis of Total Plate Count (TPC), Yeast & Mould (Y&M), Coliform, *Escherichia coli*, *Staphylococcus aureus* and Psychrotrophic bacterial counts was performed on smoked meat and fresh seaweed according to United States-Food and Drug Administration (US-FDA) Bacteriological Analytical Manual (BAM) standard methods (Feng *et al*., 2002). All microbial data were expressed as number of colony forming units (CFU/g) with plates enumeration based on 25 to 250 CFU/g, respectively except 15 to 150 CFU/g for Y&M. Additional analysis of *Salmonella* was performed on smoked meat during the storage period by a method modified from the US-FDA BAM (Feng *et al*., 2002). Isolated colonies that showed typical reactions (Xylose Lysine Deoxycholate and Xylose Lysine Tergitol-4; dark red colonies with black centre, Rambach; bright red colonies) according to manufacturer's instructions were considered as presumptive *Salmonella*. Well isolated colonies were subjected to biochemical tests for confirmation (Merck, Germany).

RESULTS AND DISCUSSION

Microbiological properties during storage study

Specifically, the TPC in untreated smoked meat was 1.8 x 10^5 CFU/g, however reduced to <1 x 10 CFU/g after HPP treatment at week 0 (Table 1). Meanwhile, TPC growth in untreated fresh seaweed (control) was 2.7 x 10^4 CFU/g but decreased to <25 x 10 CFU/g after HPP treatment at week 0 (Table 1). These results proved that aforementioned HPP treatments successfully reduced the microbial load by 5 and 4 log reduction for smoked meat and fresh seaweed, respectively. Notably, the initial load of microbial counts in untreated smoked meat and fresh seaweed was possibly contributed from the environmental factors or poor handling at processing facilities. For HPP-treated smoked meat samples, TPC was observed incremental fortnightly until it reached 1.7 x 10^6 CFU/g at the end of the storage period at day 56 (Table 1). In contrast, TPC was found relatively higher for untreated smoked meat where it reached unsatisfactory level at week 2 of storage period. The microbial safety limit for smoked meat was referred to Malaysia's Food Act 1983 (Act 281) & Regulations in this study whereas 10^6 CFU/g was already considered unsatisfactory for meat and meat product category (Table 2).

Noteworthy, fresh seaweed showed a total reduction in microorganism after HPP at the pressure conditions applied, and remained so throughout the 140-day of storage period (Table 1). In comparison, TPC growth for untreated fresh seaweed (control) reached unsatisfactory level at day 14 during storage at chilled (Table 1). The Compendium of Microbiological Criteria for Food (Food Standards Australia – New Zealand) was particularly referred in this study (Table 2) due to the absence of microbial safety limit in the Food Act 1983 for fresh seaweed category. These results are in accordance with the results of Queiros *et al*. (2014) and Tan *et al*. (2019) who reported the lethality of HPP on aerobic mesophilic microorganisms. This study also demonstrated Y&M and

Psychrotrophic counts were significantly reduced to non-detectable levels for smoked meat and fresh seaweed as compared to untreated sample with the presence of these microorganisms at higher rate. Although not mentioned in both standards in Table 2, Y&M and Psychrotrophic could be used as food decay indicator including spoilage of refrigerated foods. In this experiment, HPP-treated smoked meat showed 10^4 CFU/g of Yeast and 10^2 CFU/g of Psychrotrophs at day 56 of storage period, meanwhile Y&M was only detected at day 98 only at a very low level whereas no Psychrotrophic count was detected in HPP-treated fresh seaweed, respectively (Table 1).

This study also demonstrated HPP at optimized parameter successfully inhibited Coliform with 4 log reduction for smoked meat and 5 log reduction for fresh seaweed (Table 1). *E. coli* and *Salmonella* were not detected in all samples which in line with the Compendium of Microbiological Criteria for Food (Food Standards Australia – New Zealand) (Table 2) except *Salmonella* that was found in control samples of smoked meat at week 14. In the present study, *Staph. aureus* was also successfully reduced to non-detectable level for smoked meat and fresh seaweed, respectively under the influence of HPP. Overall, based from the data obtained, a minimum level of pressure and holding time of 6000 Bar for 4.5 mins and 6000 Bar for 3 mins were sufficient for microbial spoilage prevention and pathogen deactivation in smoked meat and fresh seaweed, respectively. Since one of the consequences of foodborne illnesses is loss of revenue, HPP offers pasteurisation advantages to produce safe and high-quality food products. Therefore, it is crucial to understand the effects of HPP on microbial shelf life of both food matrices as the safety parameter provided by this study could serve as a foundation for product development strategy and food safety initiatives.

CONCLUSIONS

In summary, our results suggested HPP parameters of 6000 Bar for 4.5 mins and 6000 Bar for 3 mins were effective in retaining the microbiological quality of both products for a minimum of 56 days and 140 days, respectively.

ACKNOWLEDGEMENTS

This work was supported by a grant from MARDI, under the Development of Standard Operating Procedure (SOP) for Minimally-Processed Product using HPP Technology (Project No. K-RFS03-1001-KSR999). The authors are grateful to laboratory staff from MARDI and UPM for their overall technical assistance during the study.

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Table 1. Microbiological Count of Smoked Meat and Fresh Seaweed throughout Storage Period at Chilled Temperature (2 ºC ± 7 ºC)

Analysis / Day of Storage	Total Plate Count (CFU/g)		Yeast & Mould (CFU/g)		Coliform (CFU/g)		*Escherichia coli* (CFU/g)		*Staph. aureus* (CFU/g)		Psychrotrophic (CFU/g)		Presumptive *Salmonella* in 25 g
HPP	SM	FS	SM	FS	SM	FS	SM	FS	SM	FS	SM	FS	SM
0	<1 x 10	<25 x 10	<1 x 10^2	<1 x 10^2	<1 x 10	<1 x 10	<1 x 10	<1 x 10	<1 x 10^2	<1 x 10^2	<1 x 10	<1 x 10^2	Absent
14	9.5 x 10^2	<25 x 10	<15 x 10^2	<1 x 10^2	<1 x 10	<1 x 10	<1 x 10	<1 x 10	<1 x 10^2	<1 x 10^2	<1 x 10	<1 x 10^2	Absent
28	1.5 x 10^3	<1 x 10	<15 x 10^2	<1 x 10^2	<1 x 10	<1 x 10	<1 x 10	<1 x 10	<1 x 10^2	<1 x 10^2	<1 x 10	<1 x 10^2	Absent
42	2.1 x 10^4	<1 x 10	4.8Y x 10^4	<1 x 10^2	<1 x 10	<1 x 10	<1 x 10	<1 x 10	<25 x 10^2	<1 x 10^2	3.3 x 10	<1 x 10^2	Absent
56	1.7 x 10^6	<25 x 10	4.2Y x 10^4	<1 x 10^2	<1 x 10	<1 x 10	<1 x 10	<1 x 10	<25 x 10^2	<1 x 10^2	2.5 x 10^2	<1 x 10^2	Absent
70	Nil	<25 x 10	Nil	<1 x 10^2	Nil	<1 x 10	Nil	<1 x 10	Nil	<1 x 10^2	Nil	<1 x 10^2	Nil
84	Nil	<25 x 10	Nil	<1 x 10^2	Nil	<1 x 10	Nil	<1 x 10	Nil	<1 x 10^2	Nil	<1 x 10^2	Nil
98	Nil	<25 x 10	Nil	<15 x 10^2	Nil	<1 x 10	Nil	<1 x 10	Nil	<1 x 10^2	Nil	<1 x 10^2	Nil
112	Nil	<25 x 10	Nil	<15 x 10^2	Nil	<1 x 10	Nil	<1 x 10	Nil	<1 x 10^2	Nil	<1 x 10^2	Nil
126	Nil	<25 x 10	Nil	<1 x 10^2	Nil	<1 x 10	Nil	<1 x 10	Nil	<1 x 10^2	Nil	<1 x 10^2	Nil
140	Nil	<25 x 10	Nil	<1 x 10^2	Nil	<1 x 10	Nil	<1 x 10	Nil	<1 x 10^2	Nil	<1 x 10^2	Nil
Control	SM	FS	SM	FS	SM	FS	SM	FS	SM	FS	SM	FS	SM
0	1.8 x 10^5	2.7 x 10^4	1.4Y x 10^4 6.2M x 10^3	<15 x 10^2	1.7 x 10^4	2.5 x 10^5	<1 x 10	<25 x 10	1.5 x 10^2	<25 x 10^2	2.1 x 10^2	2.3 x 10^2	Absent
14	2.3 x 10^6	4.5 x 10^7	8.3Y x 10^5 1.1M x 10^5	7.7Y x 10^4	9.3 x 10^4	7.3 x 10^7	<1 x 10	3.3 x 10^3	1.5 x 10^7	4.1 x 10^4	2.4 x 10^2	3.4 x 10^4	Detected

Note: <1 x 10 and <1 x 10^2 indicate the microorganisms tested were not detected; <15 x 10^2 ,<25 x 10 and <25 x 10^2 indicate microorganisms tested were very low counts
Y = Yeast Count, M = Mould Count, SM = Smoked meat, FS = Fresh seaweed, HPP SM = HPP-treated at 6000 Bar for 4.5 mins (Optimised HPP for smoked meat), HPP FS = HPP-treated at 6000 Bar for 3 mins (Optimised HPP for fresh seaweed), Control = Fresh samples of smoked meat / fresh seaweed without HPP treatment

Table 2. Microbiological Safety Limit (Unsatisfactory Level) Based on National and International Standards

Analysis / Reference	Total Plate Count (CFU/g)	Yeast & Mould (CFU/g)	Coliform (CFU/g)	*Escherichia coli* (CFU/g)	*Staph. aureus* (CFU/g)	Psychrotrophic (CFU/g)	Presumptive *Salmonella* in 25 g
MAS	10^6 per g	Not stated	5 x 10 per g	Absent in 1 g	Not stated	Not stated	Not stated
ANZ	$\geq 10^6$	Not stated	$> 10^4$	$>10^2$	10^3 - $\leq 10^4$	Not stated	Detected in 25g

Note: MAS = Malaysia's Food Act 1983 (Act 281) & Regulations (Table 15) for meat and meat product in hermetically sealed containers (As at 5th May 2021)
ANZ = Compendium of Microbiological Criteria for Food (Food Standards Australia – New Zealand) for Ready-To-Eat (RTE) foods in which all components of the foods have been cooked but there is some handling before sale or consumption (As at October 2016)