JFHB
Journal of Food Health and Bioenvironmental Science

Journal of Food Health and Bioenvironmental Science

Vol. 15 No. 3 September – December 2022

Journal of Food Health and Bioenvironmental Science

http://jfhb.dusit.ac.th/
http://research.dusit.ac.th/new/e-journal/

ISSN 2630-0311 (Online)

Journal of Food Health and Bioenvironmental Science

Vol. 15 No. 3

September – December 2022

ISSN 2630-0311 (Online)

Copyright Research and Development Institute, Suan Dusit University
295 Nakhon Ratchasima Road, Dusit, Bangkok, Thailand 10300
Phone: +662 244 5801-2 Fax: +662 668 7460

Printing Graphicsite
295 Nakhon Ratchasima Road, Dusit, Bangkok, Thailand 10300
Phone: +662 244 5080-2 Fax: +662 243 9113

Journal of Food Health and Bioenvironmental Science

Journal of Food Health and Bioenvironmental Science is an international scientific journal which is the official publication of Research and Development Institution, Suan Dusit University, Thailand. The Journal publishes papers in the categories of peer reviewed original articles, review article and book review from all areas related to food, health, biological and environmental disciplines in order to exchange knowledge and concepts with the scientific community. The journal is published thrice annually, with the 1st issue covering the months of January to April, the 2nd issue May to August and the 3rd issue September to December. The journal is distributed in online (http://www.researchold.dusit.ac.th/new/e-Journal/jfhb.php) format. Those who are interest can submit the manuscripts via scholar one system (https://mc03.manuscriptcentral.com/jfhb).

All the manuscripts submitted to the journal must be plagiarism free and has never been published or submitted elsewhere unless the manuscript was published in another language and has permission from the journal. Before being published, the manuscripts must be peer reviewed by at least 3 specialized reviewers via double blinded review system and the manuscript must be approved by at least 2 reviewers.

The following areas are covered in the journal:

- Food science including food chemistry, food biotechnology, food processing, food technology, food microbiology, food safety, food nutrition, product development, sensory analysis and related fields
- Health sciences including epidemiology, nursing, pharmaceutical science, cosmetic, public health and related fields
- Biological science including microbiology, molecular biology & genetics, cell biology, biochemistry, biotechnology, biodiversity, biomonitoring & biomarkers, ecology, plant & animal biology, toxicology, applied biology, agricultural science and related fields
- Environmental science including aquatic environment, atmospheric environment, environmental pollution, environmental chemistry, alternatively fuels, green technology and related fields

Office of Management Research and Development Institute, Suan Dusit University
295 Nakhon Ratchasima Road, Dusit, Bangkok, Thailand 10300
Phone: +662 244 5801-2 Fax: +662 668 7460
e-mail: jfhb@dusit.ac.th

Journal of Food Health and Bioenvironmental Science

Vol. 15 No. 3 September - December 2022 ISSN 2630-0311 (Online)

Peer Reviewers

Prof. Dr.Sirithon Siriamornpun	Mahasarakham University, Thailand
Prof. Dr.Christian R. Encina-Zelada	Universidad Nacional Agraria La Molina, Peru
Prof. Dr.Vasudevan Subramanyan	Central Electrochemical Research Institute CSIR - Library, India
Assoc. Prof. Dr.Tawfik A. Saleh	King Fahd University of Petroleum and Minerals, Saudi Arabia
Assoc. Prof. Dr.Prapaisri Sirichakwal	Mahidol University, Thailand
Assoc. Prof. Dr.Nantawan Therdthai	Kasetsart University, Thailand
Assoc. Prof. Dr.Mayuree Kanlayavattanakul	Mae Fah Luang University, Thailand
Assoc. Prof. Dr.Sriwiang Rittisak	King Mongkut's University of Technology North Bangkok, Thailand
Assoc. Prof. Dr.Wanna Tungjaroenchai	King Mongkut's Institute of Technology Ladkrabang, Thailand
Assoc. Prof. Dr.Vasina Chandrasiri	Sukhothai Thammathirat Open University, Thailand
Assoc. Prof. Dr.Narisa Kamkaen	Eastern Asia University, Thailand
Assoc. Prof. Dr.Khongsak Srikaeo	Pibulsongkram Rajabhat University, Thailand
Assist. Prof. Dr.Sukhuntha Osiriphun	Chiang Mai University, Thailand
Assist. Prof. Dr.Orawan Chunhachart	Kasetsart University, Kamphaeng Sean Campus, Thailand
Assist. Prof. Dr.Pavalee Chompoorat	Maejo University, Thailand
Assist. Prof. Dr.Piyaporn Chueamchaitrakun	Mae Fah Luang University, Thailand
Assist. Prof. Dr.Tita Foophow	Suan Dusit University, Thailand
Dr.Masaaki Kurasaki	Hokkaido University, Japan

Content

Journal of Food Health and Bioenvironmental Science
Journal homepage : http://jfhb.dusit.ac.th/

Characteristics of Flattened Rice Flour Used with Daifuku

Yotsinee Huadong & Tita Foophow*

Department of Nutrition and Culinary Arts for Health Capability and Anti-aging Wellness, School of Culinary Arts, Suan Dusit University, Dusit, Bangkok, 10300 Thailand

Article info

Article history:
Received : 10 October 2022
Revised : 7 December 2022
Accepted : 9 December 2022

Keywords:
Flattened rice, Flour, Properties, Viscosity, Daifuku

Abstract

Flattened rice, an immature sticky rice product, contains high nutritional values and bioactive compounds. Based on its health values, this rice has been used as a functional ingredient in food. The objective of this study was to characterize the properties of sticky rice flour with substitution of flattened rice flour at 0-100% on Daifuku. Flattened rice flour was green-yellow in color and had very low amylose content (3.34%). The starch granule of flattened rice flour revealed an apparent pigment of phenolic compound and/or chlorophyll. According to pasting properties, peak viscosity, trough viscosity and breakdown of flattened rice flour were less than that of sticky rice flour. The FTIR spectra patterns of flattened rice flour showed it consisted of sticky rice polysaccharide structure as the main proportion. Daifuku with flattened rice flour at 75% had the highest sensory scores in all attributes especially in overall acceptance and texture. For nutritional analysis based on the calculated data, Daifuku with flattened rice flour had higher contents of calcium, iron and vitamin B1 than the control Daifuku. Therefore, the favorable properties of flattened rice flour and Daifuku are useful for further nutritional food product development.

Introduction

Rice (*Oryza sativa* L.) is the staple food for Asian populations and it is cultivated mainly in China, India, Indonesia, Bangladesh, Vietnam and Thailand. Based on amylose content, rice is classified as non-glutinous and glutinous rice (Itthivadhanapong & Sangnark, 2016). Glutinous rice, also called sticky rice, waxy rice, or sweet rice, is commonly grown in the Northeastern region of Thailand. One of sticky rice products is flattened rice (known as Khao Mao in Thailand), which is made from young sticky rice at the dough stage (13 to 19 days after anthesis) (Ekasit & Jiraporn, 2013). The flattened rice is consumed in many Asian countries such as Thailand, Lao, Cambodia, Myanmar, India, Bhutan and Tibet. Many varieties of sticky rice cultivars can be used to produce flattened rice such as Saifon, Lueang Boonma, Sawang, RD6, RD10 and Hang Yee 71 (Salitlertthanasin, 2017). The flattened rice production starts with immature sticky rice grains appearing light green in color (after the milk stage for 1-2 weeks) that are soaked in water for 3-4 hr, roasted and pounded to separate the rice husk. Rice grains are pounded several times and winnowed to obtain soft flattened rice (Nachaisin et al., 2016). Thai

* Corresponding Author
e-mail: tita_foo@dusit.ac.th

traditional desserts made from flattened rice (Khao Mao) such as sweet Khao Mao with coconut flesh (Khao Mao Khlook), fried Khao Mao and grated coconut banana rolls (Khao Mao Thaawt) and roasted Khao Mao served with shrimp relish (Khao Mao Mee). For nutrition information, 100 g of cooked flattened rice contains 364 kcal, 79.9 g carbohydrate, 6.8 g protein and 1.9 g fat which also contains varieties of vitamins and minerals including calcium, phosphorus, iron, vitamin B1 and B2 (Institute of Nutrition, 2013). Furthermore, it has been reported that flattened rice contains bioactive compounds including γ-oryzanols and GABA (Ekasit & Jiraporn, 2013). Considering its nutritional value, the application of flattened rice in food products is an alternative way to obtain a functional food product. The flattened rice can be used from whole grains or milled flour. Previous studies have reported that flattened rice grains can be used as the main ingredient in Thai rice cereal (Khao Mao) bars (Salitlertthanasin, 2017) and breakfast cereal products (Voraputhaporn, 2009). However, no prior report has used flattened rice flour in food products. Normally, sticky rice flour is widely used as an ingredient in snacks and desserts for example mochi, sweet soup balls, dumplings and gluten-free products because of its properties in softness and stickiness (Qin et al., 2016; Ji et al., 2007). Therefore, the application of flattened rice flour to replace sticky rice flour could provide a new appearance and good nutritional value in food products. In this study, the properties of flattened rice flour when substituted for sticky flour at 0-100% are characterized and tested with Daifuku (Japanese rice cake with red bean paste filling) with partial substitution of sticky rice flour with flattened rice flour. Characteristics of Daifuku with flattened rice flour are reported based on physical properties, sensory evaluation and nutritional values.

Materials and methods

1. Raw materials

The young sticky rice was obtained from local sticky rice varieties in Nang Rong District, Buri Ram Province, Thailand. Sticky rice flour (Golden Coins brand, Thai Flour Industry Co., Ltd.), red bean paste (Been brand, Zensprout Co., Ltd.) and sugar (Mitr Phol Sugar Co., Ltd.) were purchased from a local market.

2. Preparation of flattened rice flour

Flattened rice was made from young sticky rice (immature grains). The paddy was harvested on the 18th day after anthesis and was green in color. The young grains were roasted on medium heat. After cooling down, the grains were pounded with a mortar, sieved and winnowed using a threshing basket in order to separate the flattened grains from the rice husk. The grains were dried to be flattened rice. To obtain flattened rice flour, flattened rice was milled using the dry milling method, sieved at 100 mesh, packed in sealed plastic bags and kept at 4ºC until used.

3. Properties of flattened rice flour as a substitute for sticky rice flour

Flattened rice flour was used as a substitute for sticky rice flour at 0%, 25%, 50%, 75% and 100% (w/w), respectively. Each flour sample was blended and sieved at 60-mesh. Color values of the flours were determined by a Handy colorimeter (NR-3000, Japan) in CIE color scale as L (lightness-darkness), a (redness-greenness) and b (yellowness-blueness). Amylose content of the flours was determined accordingly to the method of ISO6647-2 (2007). Furthermore, morphological analysis of starch granules in flours was determined by using light field microscopy.

The pasting properties of the flours were determined accordingly to the method of AACC (2000) using a Rapid Visco Analyzer (RVA-4, Newport Scientific, Australia) controlled by Thermocline v.2.3 software. The sample mixture, containing 3 g of flour (12% moisture basis) and 25 mL distilled water, was transferred into an aluminum cup. The temperature profile was composed of heating the sample at 50ºC for 1 min and then raised to 95ºC within 3 min 48sec. After that, it was kept at 95ºC for 2 min 30sec., cooled to 50ºC within 3 min 48sec and kept at 50ºC for 2 min.

Fourier transform infrared spectroscopy (FTIR) of the flours was analyzed by attenuated total reflection-Fourier transform infrared spectrometer (ATR-FTIR) (IRTracer-100, Shimadzu, Japan). ATR-FTIR data were carried out between wavenumbers of 4000–500 cm^{-1} with 40 replicate collections.

4. Preparations of Daifuku

A control formulation for Daifuku (Boonchai, 2020), calculated as % weight of sticky rice flour, consisted of 100% sticky rice flour, 100% red bean paste, 22.5% sugar and 125% water. Four formulations of Daifuku were prepared by flour mixes of flattened rice flour substituted for sticky rice flour at 0, 25, 50 and 75% (w/w), respectively. Other ingredients for all formulations were fixed in weight. For Daifuku preparations, flour, sugar and water were mixed and stirred in a bowl until obtained batter. The bowl with the batter was covered

with plastic wrap on the top and heated by the microwave (R-250, Shape, Japan) at a moderate power level (800 watts) for 3 min. Next, the bowl was removed from the microwave and the dough was mixed with a wet spatula and then returned to microwave for another 3 min. If the dough was not smooth, it was returned to microwave for one more min. The cooked dough was sticky and translucent. The temperature of dough after microwave was 90°C. After that, the mochi dough was placed on a cutting board floured with tapioca flour, cut the dough (20 g) and flattened into a rectangle. Then, the red bean paste (20 g) was placed at the center of the mochi dough and wrapped with the dough. The end of the mochi dough was pinched together and made into a ball shape. Daifuku was sprinkled with tapioca flour and kept in a plastic box with a lid.

5. Physical properties of Daifuku without red bean paste

Color values of Daifuku surfaces (without red bean paste) were determined using CIE color scale. For texture analysis, five replications of each Daifuku were measured by a texture analyzer (TA-XT2i, Stable Micro System, UK). The sample (20 g with 1.5 diameters) was compressed using a double-cycle program with a 100 mm aluminum probe. The probe was declined at a speed of 1 mm/sec to 75% sample height. After 10 sec, the compression was repeated to complete the measurement (Modified from Wongbasg & Jangchud, 2011). Hardness and springiness were obtained from the texture profile analysis.

6. Sensory evaluation of Daifuku

The untrained panelists of 40 people were assigned for sensory evaluation of the Daifuku prepared from flour mixes with red bean paste. The 9-point hedonic scale of preference test (1 = dislike extremely and 9 = like extremely) was used for six attributes regarding appearance, color, flavor, taste, texture and overall acceptance. All samples were placed on white dishes and presented with random 3-digit code numbers. Drinking water was served to participants for mouth rinsing between samples.

7. Nutritional values of Daifuku

Nutritional values including energy, carbohydrate, protein and fat of Daifuku were acquired using the nutritional analysis program (Thai NutriSurvey version 2.0). The obtained data sets were reported as per 40 g of Daifuku.

8. Statistical analysis

All experiments were conducted in triplicates and data from three samples were analyzed statistically using ANOVA and significant differences were done using Duncan's multiple range test ($p \leq 0.05$). A completely randomized design was applied for preparations and property determinations of the flours and Daifuku, while a randomized complete block design was applied for the sensory evaluation. All analysis was conducted using SPSS software Version 22 (SPSS Inc.; Chicago, IL, USA).

Results and discussion

1. Properties of Flattened rice flour substituted for sticky rice flour

Due to the application of flattened rice flour in place of commercial sticky rice flour, therefore the properties of flattened rice flour were determined and compared with commercial flour at 0-100% substitution. The appearance and color of the flour mixes are shown in Fig. 1 and Table 1. The flour mixes containing high percentages of flattened rice flour displayed lower L and a values but higher b values, indicated by darker and more green and yellow than others. Flattened rice flour harvested at the dough stage (15-20 days after anthesis), is considered as immature grains. The paddy of this stage is green or green-yellow in color because of the chlorophyll and carotenoid pigment. After that, the grains were roasted in medium heat, the roasting time and temperature had an effect on the dark color of the grains due to Maillard reactions (Ekasit & Jiraporn, 2013). In regards to the flour preparation, flattened rice flour was milled using the dry milling method, in which the flour contains the pigments from the pericarp and seed coat. Therefore, the color of flour with an increase in flattened rice flour levels became green-yellow.

Table 1 Physicochemical properties of flattened rice (FR) flour substituted for sticky rice flour at different levels

FR flour substitution (%)	Amylose content (%)	Color		
		L	*a*	*b*
0	7.89	99.78 ± 0.31^{a}	-1.44 ± 0.27^{a}	5.99 ± 0.23^{e}
25	6.83	97.39 ± 1.57^{a}	-1.18 ± 0.25^{b}	7.73 ± 0.31^{d}
50	5.64	92.50 ± 2.53^{b}	-2.26 ± 0.08^{c}	10.66 ± 0.23^{c}
75	4.96	86.58 ± 1.83^{c}	-2.44 ± 0.09^{c}	13.89 ± 0.41^{b}
100	3.34	73.78 ± 4.46^{d}	-3.17 ± 0.33^{d}	20.39 ± 0.72^{a}

Remark: Means ± S.D. with different superscripts in the same column represent significantly different ($p \leq 0.05$)

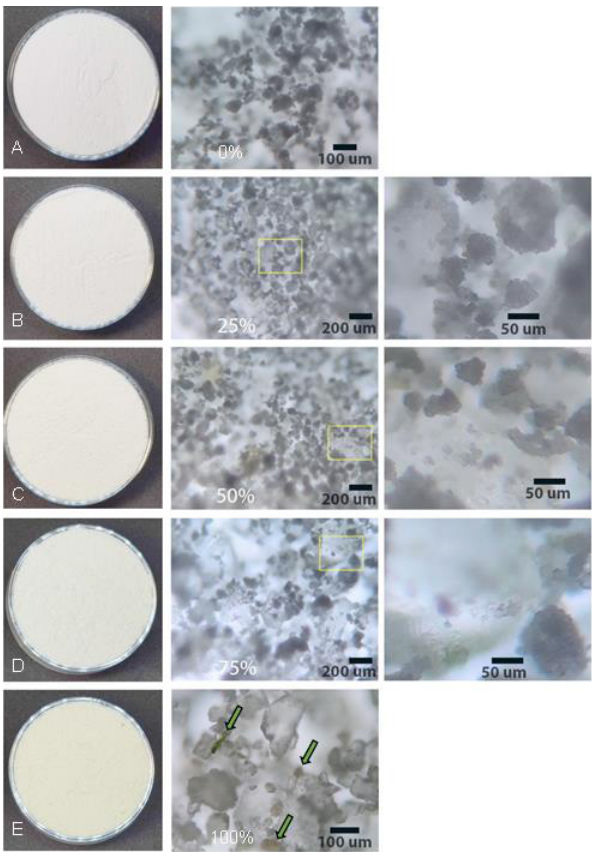

Fig. 1 The flour mix appearance and photomicrographs of starch granules of flattened rice flour substituted for sticky rice flour at 0 (A), 25 (B), 50 (C), 75 (D) and 100% (E) (w/w)

The amylose content of flour mixes are shown in Table 1. Flattened rice flour at 100% had the lowest amylose content (3.34%), whereas sticky rice flour had the highest (7.89%). According to previous studies, Thai sticky rice varieties, RD6 and short grain rice, had amylose content of approximately 2.04-2.61% and 2.03-3.14%, respectively (Keeratipibul et al., 2008). The amylose content of flattened rice flour was lower than that of sticky rice flour probably because of the milling method. The commercial sticky rice flour was milled using the wet milling method, while the flattened rice flour was milled using the dry milling method. The dry-milled flour had lower purity of starch than the wet-milled flour (Foophow et al., 2020), resulting in the lowest amylose content in flattened rice flour. Sticky rice containing high amylose content had a hard and less sticky texture, compared with rice containing low amylose content (Keeratipibul et al., 2008). However, flattened rice flour and sticky rice flour are classified as very low amylose rice (2-9%) (Sompong et al., 2011).

For morphological analysis, the starch granules in flours were determined using light-field microscopy as shown in Fig. 1. The starch granule of flattened rice flour at 100% had a smooth irregular and low sphericity sub-rounded surface structure (Fig. 1E) due to the thermal and mechanical pressure treatment in roasting and flaking at the moisture process of immature rice grain and endosperm morphology (Kumar et al., 2018; Kumar & Prasad, 2017). The starch granule morphology of flattened rice flour was similar to that of flattened rice (data not shown), which had a granular size ranging around 20-100 µm. The arrows in Fig. 1E represent the pigment of phenolic compound (Miraji et al., 2020, 2021; Ogawa et al., 2003; Ratseewo et al., 2019; Tamura et al., 2014) and/or chlorophyll from cell wall matrix as the characteristic color of native flattened rice. The starch granule morphology of sticky rice flour as shown in Fig. 1A represents a well-uniform micro-scale structure with particle size around 1–100 µm, corresponding to characteristics of rice starch granules in prior reports (Govindaraju et al., 2020; Rani & Bhattacharya, 1995). The starch granules of flattened rice flour at 25, 50 and 75% (Fig. 1B-1D) showed that the well-uniform particles dispersed in all mixture samples, which the enlargement photo of the rectangles inset represents at the flattened rice starch and sticky rice starch particles interface.

The pasting properties of flattened rice flour substituted for sticky rice flour at different levels are shown in Table 2. The peak viscosity, trough viscosity and breakdown decreased with the increase of flattened rice flour content, which was similar to black glutinous rice flour substitution (Itthivadhanapong & Sangnark, 2016). Flattened rice flour at 100% had the lowest peak viscosity and breakdown, resulting from the increase in the degree of starch damage during the flattened rice process. Therefore, flattened rice flour absorbed water and swelled higher than sticky rice flour, which had an effect on the increase of viscosity at low temperatures. In contrast, peak time and set back tended to increase with increasing flattened rice substitution. The pasting temperature slightly decreased and showed a significant difference only at 100% flattened rice flour. In addition, no statistically significant difference between flattened rice flour at 0% and 100% ($p > 0.05$) was found in the final viscosity. The pasting properties of flattened rice flour at 100% corresponded to the result of Ekasit & Jiraporn (2013) for peak viscosity (1440.66-1638.33 cp), trough viscosity (1316.00-1382.66 cp) and breakdown

Table 2 Pasting properties of flattened rice (FR) flour substituted for sticky rice flour at different levels

FR flour substitution (%)	Peak viscosity (cP)	Trough viscosity (cP)	Breakdown (cP)	Final viscosity (cP)	Set back (cP)	Peak time (min)	Pasting temp (°C)
0	2811.00 ± 10.15^{a}	1462.67 ± 10.97^{a}	1348.33 ± 17.62^{a}	1824.67 ± 5.86^{a}	362.00 ± 9.86^{b}	3.74 ± 0.03^{c}	70.20 ± 0.44a
25	2686.67 ± 28.88^{b}	1404.33 ± 33.20^{b}	1282.33 ± 29.94^{b}	1794.33 ± 4.93^{bc}	390.00 ± 31.43^{b}	3.65 ± 0.07^{c}	70.50 ± 0.78a
50	2410.00 ± 23.07^{c}	1365.00 ± 3.61^{c}	1045.00 ± 24.88^{c}	1795.33 ± 21.03^{bc}	430.33 ± 24.58^{a}	3.67 ± 0.04^{c}	69.70 ± 0.00a
75	1905.33 ± 46.05^{d}	1410.33 ± 26.16^{b}	495.00 ± 23.64^{d}	1784.00 ± 24.25^{c}	373.67 ± 10.50^{b}	4.03 ± 0.08^{b}	70.95 ± 0.44a
100	1537.33 ± 24.11^{e}	1352.00 ± 10.82^{c}	185.33 ± 34.50^{e}	1814.33 ± 2.52^{ab}	462.33 ± 9.50^{a}	6.43 ± 0.27^{a}	67.30 ± 2.16b

Remark: Means ± S.D. with different superscripts in the same column represent significantly different ($p \leq 0.05$)

(124.66-255.66 cp). However, these results differed from a previous report (Ekasit & Jiraporn, 2013), which showed less final viscosity (1509.33-1671.66 cp), set back (193.33-289.00 cp), peak time (5.13-5.73 min) and pasting temperature (51.61-55.13 °C).

FTIR spectra of flour mixes are shown in Fig. 2A. Main spectral patterns corresponding to characteristics of sticky rice starch (Basnet et al., 2016; Soe et al., 2020; Wang et al., 2019) indicated that all flour mixes consisted of sticky rice polysaccharide as the main proportion. The green rectangle highlights the vibration mode region of C-H stretching and the polysaccharide fingerprint is presented in Fig. 2A inset, the enlargement is in Fig. 2B and 2C, respectively. Broad band at wavenumbers around 3200-3500 cm^{-1} was presented in all samples due to O-H stretching in carbohydrates as the main composition and may be contributed from the NH amide stretching mode of rice proteins (Rahmani & Mani-Varnosfaderani, 2022). The O-H band may also be contributed from the O-H group of small phenolic and chlorophyll components in immature rice by nature, agreeing with the small pigment present in the optical micrograph result. The stretching vibration mode of the C-H group region was identified as some of the main characteristics of rice polysaccharides. Moreover, the C-H stretching region in Fig. 2B revealed predominantly two peaks at 2923.1 and 2853.6 cm^{-1} of the 100 samples, while the sticky rice (flattened rice flour at 0%) indicated weak three peaks of C-H stretching mode at 2929.1, 2885.8 and 2849.7 cm^{-1}, respectively. The gradual gradient decrease of intensity in the C-H stretching region from 100 to 0 sample confirmed that the higher intensity of $C\text{-}H_2$ asymmetric and symmetric stretching contributes to higher chlorophyll content of the flattened rice characteristic than the conventional raw sticky rice. The polysaccharide fingerprint region in Fig. 2C represented similarly the main peak shape of glycosidic C-O-C linkage of pyranose with C-OH bending mode at wavenumber around 994-1077 cm^{-1} in all samples. In addition, the 100-sample showed broader and gradually sharper in sticky rice flour (flattened rice flour at 0%), probably due to the contribution of phenolic C-O-C stretching and chlorophyll C-O vibration mode (Chang et al., 2013; Dadwal et al., 2021). However, the crystallinity degree of amylose is also involved in this region because of the milling and heat treatment process (Man et al., 2014, 2012; Soe et al., 2020). Amide I contributed with in-plane NH bending peak due to rice protein associated with polysaccharide COO stretching located at wavenumber around 1744 cm^{-1}. The peak at 1643 cm^{-1} was also attributed to the COO stretch group of polysaccharides and OH group bending mode. Moreover, the couple peaks at around 1744 with 1643 cm^{-1} in flattened rice flour at 100% suggesting the strong intensity of high phenolic C=O stretching mode proportion in flattened rice flour. This result suggested strong peaks at 2923, 2853 and 1730 cm^{-1} were due to characteristics of flatted rice flour and proportions of flattened rice flour to sticky rice flour ratio. In addition, no evidence of new absorption band energy of the flour mixes in FIIR analysis was observed, which could be assumed that no bonding between flattened rice flour and sticky rice flour occurred by a simple physical mixing process.

The flatted rice flour was applied to use as an ingredient in Daifuku based on the properties in soft and sticky texture in products and easily increased viscosity at low temperatures. Daifuku is a Japanese rice cake with red bean paste filling, which is a popular dessert. It is usually shaped into a ball and composed of a chewy and soft outer layer. Therefore, the application of flattened rice flour could provide the new appearance and good nutritional value in food products.

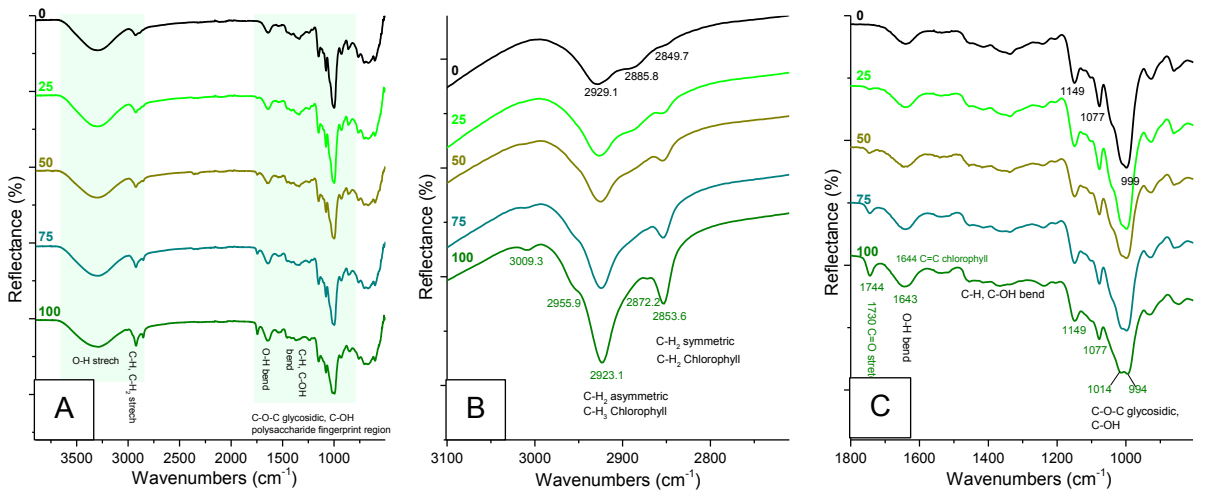

Fig. 2 ATR-FTIR spectra of flattened rice flour substituted for sticky rice flour at 0, 25, 50, 75 and 100% (w/w) with full spectra (A), the region of C-H asymmetric/ symmetric stretching between wavenumbers of 2700-3100 cm-1 (B) and polysaccharide fingerprint region between wavenumbers of 800- 1800 cm^{-1} (C)

2. Physical properties of Daifuku

Daifuku dough prepared with 100% flattened rice flour proved unable to make a ball shape. The dough at 100% was so wet and sticky, probably due to the pasting properties in the lowest peak viscosity, breakdown and pasting temperature. Therefore, the dough at 100% flattened rice flour had higher water absorption and swelling than others, which had an effect on the increase of viscosity at low temperatures. Thus, four formulations of Daifuku with the substitution of flattened rice flour at 0, 25, 50 and 75% (w/w) were used for physical property determinations. The physical properties of Daifuku are shown in Table 3. The color values of Daifuku containing high percentages of flattened rice flour displayed lower a values but higher b values. The result corresponded to the result of flour mixes in Table 1. Therefore, Daifuku had a darker green color with an increase in flattened rice flour levels.

According to the texture analysis, the increase of flattened rice flour substitution did not significantly influence the hardness of Daifuku ($p > 0.05$). Normally, the high amylose content in flour affects to the harder texture of the product (Lu et al., 2013). Therefore, the product with high amylose content was firm and fluffy, while product with low amylose was soft and sticky. However, flattened rice flour substituted for sticky rice flour at 0-75% had an amylose content of 7.89-4.96% (Table 1), which all flour mixes were classified in the same group as very low amylose rice (2-9%). Therefore, the amylose content of flour mixes had no significant effect on the hardness of Daifuku. These results corresponded with a previous study about the mixed flours between non-glutinous and glutinous rice with amylose content at 1.6-6.4%, in which the hardness of rice crackers (arare) from the mixed flour showed non-significant differences (Keeratipibul et al., 2008). Regarding springiness, the substitution of flattened rice flour at 0% and 25% showed no significant difference ($p > 0.05$), but significantly increased the springiness of Daifuku at 50% and 75% ($p \leq 0.05$). Springiness is related to the elasticity of food. The texture of food with high springiness requires more energy for chewing in the mouth. Therefore, the substitution of flattened rice flour for sticky rice flour significantly increased elastic texture, probably due to the low amylose content in flattened rice flour. The previous report also found that the springiness of cooked noodles increased with a low amylose content of mixed flours (Heo et al., 2012).

3. Sensory evaluation of Daifuku

Sensory evaluation of Daifuku in terms of appearance, color, flavor, taste, texture and overall acceptance are shown in Table 3. Daifuku with the substitution of flattened rice flour at 75% had the highest sensory scores in all attributes, especially in texture and overall acceptance, probably because of the consumer acceptability of the high springiness of Daifuku texture. Daifuku with flattened rice flour had a higher sensory score in appearance, color, flavor and taste compared to Daifuku at 100% sticky rice (control). The results showed that flattened rice flour significantly affected the

Table 3 Physical properties and sensory evaluation scores of Daifuku with different contents of flattened rice (FR) flour

FR flour substitution (%)	Color		Texture		Sensory evaluation					
	a	*b*	Hardness^{ns} (N)	Springiness	Appearance	Color	Flavor	Taste	Texture	Overall acceptance
0	-0.86±0.85^{a}	4.42±0.30^{c}	26.88±1.56	0.0097±0.001^{b}	7.45±1.28^{b}	7.12±1.22b	6.57±1.37^{b}	6.97±1.70^{b}	6.75±1.69^{c}	6.20±2.00^{c}
25	-2,19±0.21^{b}	11.61±1.40^{b}	29.62±2.17	0.0103±0.001^{ab}	7.70±0.95^{ab}	7.72±1.28^{a}	7.40±1.63^{a}	7.62±1.28^{a}	7.35±1.40^{bc}	7.20±1.26^{b}
50	-3.14±0.28^{c}	14.85±0.96^{a}	29.93±2.78	0.0117±0.002^{a}	7.90±0.86^{ab}	7.82±1.22^{a}	7.47±1.39^{a}	7.95±1.04^{a}	7.67±1.09^{ab}	7.67±0.91^{ab}
75	-3.30±0.16^{c}	16.17±0.38^{a}	30.02±3.02	0.0120±0.000^{a}	8.15±1.13^{a}	8.07±1.12^{a}	7.90±1.51^{a}	8.12±1.23^{a}	8.20±1.40^{a}	8.30±1.34^{a}

Remark: Means ± S.D. with different superscripts in the same column represent significantly different ($p \leq 0.05$)
Means ± S.D. with ns in the same column represent not significantly different ($p > 0.05$)

consumer acceptability of the appearance, flavor and taste of Daifuku. The control Daifuku had the lowest sensory scores in all attributes, probably due to the heating condition by the microwave. In this study, the process for Daifuku preparation was fixed controlled variables, which affected the Daifuku characteristics. From the result of sensory evaluation, flattened rice flour can be used as raw material for a partial substitute of 75% of sticky rice flour in Daifuku.

4. Nutritional values of Daifuku

Nutritional values of the control Daifuku and that with the substitution of flattened rice flour at 75% were calculated using the nutritional analysis program (Thai NutriSurvey version 2.0) and the results were shown in Table 4. Daifuku with flattened rice flour (40 g) had 35.6 g carbohydrate, 3.5 g protein, 0.4 g fat, 15.85 mg calcium, 1.26 mg iron 0.093 mg vitamin B1, 0.187 mg vitamin B2 and 158 kcal of energy. From the results, Daifuku with flattened rice flour had a little bit higher content of fat than the control (0.3 g fat). For vitamins and minerals, Daifuku with flattened rice flour had higher contents of calcium, iron and vitamin B1 than Daifuku with sticky rice flour. However, the carbohydrate, protein, energy and vitamin B2 contents of Daifuku with flattened rice flour were similar to the control. A previous report also showed that the protein content of flattened rice was similar to that of sticky rice (Ekasit & Jiraporn, 2013). From prior results of nutritional dough stage rice, it contained higher contents of fat, dietary fiber, calcium, iron, phosphorus, potassium, vitamin B1, vitamin B2 and niacin than sticky rice (Institute of Nutrition, 2013). Furthermore, flattened rice is a rich source of bioactive compounds such as γ-oryzanols and GABA (Ekasit & Jiraporn, 2013). In the future, the nutritional values and bioactive compound of Daifuku with flattened rice flour should be studied with the experimental data to confirm the chemical composition and nutrition quality in product.

Table 4 Nutritional values of Daifuku

Nutritional value	Sticky rice flour Daifuku (control) (40 g)	75% Flattened rice flour Daifuku (40 g)
Carbohydrate (g)	35.8	35.6
Protein (g)	3.4	3.5
Fat (g)	0.3	0.4
Calcium (mg)	5.62	15.85
Iron (mg)	0.98	1.26
Vitamin B1 (mg)	0.084	0.093
Vitamin B2 (mg)	0.186	0.187
Energy (kcal)	158	158

Remark: Nutritional values were calculated using the nutritional analysis program (Thai NutriSurvey version 2.0)

Conclusion

The dry-milled flattened rice flour presented as green-yellow in color and had very low amylose content. The substitution of flattened rice flour for sticky rice flour increased phenolic compound and/or chlorophyll and easily increased viscosity at low temperatures. In Daifuku, flattened rice flour can be substituted with up to 75% sticky rice flour, with the highest scores in all sensory attributes and high nutritional values especially vitamins and minerals. This study shows that flattened rice flour could be applied as a nutritional ingredient for developing healthy and functional food products. However, further studies are suggested to determine the functional properties of flours and chemical properties and bioactive compounds of products in order to apply the flattened rice flour to various food products.

References

American Association of Cereal Chemists (AACC). (2000). Approved methods (10th ed.). Minnesota: Author.

Basnet, P., Amarasiriwardena, D., Wu, F., Fu, Z., & Zhang, T. (2016). Investigation of tissue level distribution of functional groups and associated trace metals in rice seeds (*Oryza sativa* L.) using FTIR and LA-ICP-MS. *Microchemical Journal, 127,* 152-159.

Boonchai, C. (2020). *Culinary of Asia I*. Bangkok: Graphic Site.

Chang, H., Kao, M.J., Chen, T.L., Chen, C.H., Cho, K.C., & Lai, X.R. (2013). Characterization of natural dye extracted from wormwood and purple cabbage for dye-sensitized solar cells. *International Journal of Photoenergy,* 159502.

Dadwal, V., Joshi, R., & Gupta, M. (2021). Formulation, characterization and in vitro digestion of polysaccharide reinforced Ca-alginate microbeads encapsulating *Citrus medica* L. phenolics. *LWT-Food Science and Technology, 152,* 112290.

Ekasit, O., & Jiraporn, B. (2013). Some physical characteristics and bioactive compounds of young flattened rice (Khao-Mao). *International Food Research Journal, 20*(3), 1327-1332.

Foophow, T., Phoohinkong, W., Lertkowit, P., & Ketkoom, N. (2020). Fortification of iron in brownies with Sinlek brown rice flour. *Songklanakarin Journal of Science and Technology, 42*(5), 1027-1033.

Govindaraju, I., Pallen, S., Umashankar, S., Mal, S.S., Kaniyala Melanthota, S., … Mazumder, N. (2020). Microscopic and spectroscopic characterization of rice and corn starch. *Microscopy Research and Technique, 83*(5), 490-498.

Heo, H., Baik, B.K., Kang, C.H., Choo, B.K., & Park, C.S. (2012). Influence of amylose content on cooking time and textural properties of white salted noodles. *Food Science and Biotechnology, 21*(2), 345-353.

Institute of Nutrition. (2013). *Nutrient calculation computer software INMUCAL-Nutrients V3 database NB1*. Nakornpathom, Thailand: Mahidol University.

International Organization for Standardization (ISO). (2007). International Standard ISO/IEC 6647-2: 2015-Rice-Determination of amylose content, Part 2: Routine Method, 2nd Ed., Geneva.

Itthivadhanapong, P., & Sangnark, A. (2016). Effects of substitution of black glutinous rice flour for wheat flour on batter and cake properties. *International Food Research Journal, 23*(3), 1190-1198.

Ji, Y., Zhu, K., Qian, H., & Zhou, H. (2007). Staling of cake prepared from rice flour and sticky rice flour. *Food Chemistry, 104,* 53-58.

Keeratipibul, S., Luangsakul, N., & Lertsatchayarn, T. (2008). The effect of Thai glutinous rice cultivars, grain length and cultivating locations on the quality of rice cracker (arare). *LWT - Food Science and Technology, 41,* 1934-1943.

Kumar, S., Haq, R.U., & Prasad, K. (2018). Studies on physico-chemical, functional, pasting and morphological characteristics of developed extra thin flaked rice. *Journal of the Saudi Society of Agricultural Sciences, 17*(3), 259-267.

Kumar. S., & Prasad, K. (2017). Optimization of flaked rice dry roasting in common salt and studies on associated changes in chemical, nutritional, optical, physical, rheological and textural attributes. *Asian Journal of Chemistry, 29*(6), 1380-1392.

Lu, S., Lu, S., Cik, T.T., Lii, C.Y., Lai, P., & Chen, H.H. (2013). Effect of amylose content on structure, texture and α-amylase reactivity of cooked rice. *LWT-Food Science and Technology, 54*(1), 224-228.

Man, J., Lin, L., Wang, Z., Wang, Y., Liu, Q., & Wei, C. (2014). Different structures of heterogeneous starch granules from high-amylose rice. *Journal of Agricultural and Food Chemistry, 62*(46), 11254-11263.

Man, J., Yang, Y., Zhang, C., Zhou, X., Dong, Y., Zhang, F., . . . Wei, C. (2012). Structural changes of high-amylose rice starch residues following in vitro and in vivo digestion. *Journal of Agricultural and Food Chemistry, 60*(36), 9332-9341.

Miraji, K.F., Linnemann, A.R., Fogliano, V., Laswai, H.S., & Capuano, E. (2020). Nutritional quality and in vitro digestion of immature rice-based processed products. *Food & Function, 11*(9), 7611-7625.

Miraji, K.F., Linnemann, A.R., Fogliano, V., Laswai, H.S., & Capuano, E. (2021). Dry-heat processing at different conditions impact the nutritional composition and in vitro starch and protein digestibility of immature rice-based products. *Food & Function, 12*(16), 7527-7545.

Nachaisin, M., Nillseangrat, P., & Sutthapintu, A. (2016). Improvement qualities for shredded Thai-style instant rice. *Journal of Research for Development Social and Community, Rajabhat Maha Sarakham University, 3*(2), 88-95.

Ogawa, Y., Glenn, G.M., Orts, W.J., & Wood, D.F. (2003). Histological structures of cooked rice grain. *Journal of Agricultural and Food Chemistry, 51*(24), 7019-7023.

Qin, Y., Liu, C., Jiang, S., Cao, J., Xiong, L., & Sun, Q. (2016). Functional properties of glutinous rice flour by dry-heat treatment. *PLoS One, 11*(8), e0160371.

Rahmani, N., & Mani-Varnosfaderani, A. (2022). Quality control, classification, and authentication of Iranian rice varieties using FT-IR spectroscopy and sparse chemometric methods. *Journal of Food Composition and Analysis, 112,* 104650.

Rani, M.R.S., & Bhattacharya, K.R. (1995). Microscopy of rice starch granules during cooking. *Starch, 47*(9), 334-337.

Ratseewo, J., Warren, F.J., & Siriamornpun, S. (2019). The influence of starch structure and anthocyanin content on the digestibility of Thai pigmented rice. *Food Chemistry, 298,* 124949.

Salitlertthanasin, P. (2017). Product development of Thai rice cereal (Khao-Mao) bar with garlic flavor and its shelf-life using accelerated method. Bangkok, Thailand: Assumption University, BSc special project.

Soe, M.T., Chitropas, P., Pongjanyakul, T., Limpongsa, E., & Jaipakdee, N. (2020). Thai glutinous rice starch modified by ball milling and its application as a mucoadhesive polymer. *Carbohydrate Polymers, 232,* 115812.

Sompong, R., Siebenhandl-Ehn, S., Linsberger-Martin, G., & Berghofer, E. (2011). Physicochemical and antioxidative properties of red and black rice varieties from Thailand, China and Sri Lanka. *Food Chemistry, 124*(1), 132-140.

Tamura, M., Nagai, T., Hidaka, Y., Noda, T., Yokoe, M., & Ogawa, Y. (2014). Changes in histological tissue structure and textural characteristics of rice grain during cooking process. *Food Structure, 1*(2), 164-170.

Voraputhaporn, W. (2009). Production and development of breakfast cereal products from young puffed rice. In *Proceedings of the Khon Kaen University University annual Conference: Sustainable Rural Development* (pp.30-37). Khon Kaen, Thailand: Khon Kaen University.

Wang, H., Wu, Y., Wang, N., Yang, L., & Zhou, Y. (2019). Effect of water content of high-amylose corn starch and glutinous rice starch combined with lipids on formation of starch–lipid complexes during deep-fat frying. *Food Chemistry, 278,* 515-522.

Wongbasg, C., & Jangchud, K. (2011). Effect of ingredients on the quality of Daifuku from germinated glutinous brown rice flour. In *Proceedings of 49th Kasetsart University Annual Conference* (pp.173-181). Bangkok, Thailand: Kasetsart University.

Journal of Food Health and Bioenvironmental Science
Journal homepage : http://jfhb.dusit.ac.th/

Preparation and Adsorption Properties of a Biosorbent from Banana Peel for Use as Natural Vitamin Beads in Cosmetic Products

Pornpassanan Dechprasitichok[a],* Tasanee Panichakul[b], Khwunjit Itsarasook[b], Napaporn Boonthoem[c], Christoph Sontag[d] & Sanchai Luachan[e]

[a] *Department of Chemical Technology, Faculty of Science, Suan Dusit University, Bangkok, 10300 Thailand*
[b] *Department of Cosmetic Science, Faculty of Science, Suan Dusit University, Bangkok, 10300 Thailand*
[c] *The Research and Development Institute, Suan Dusit University, Bangkok, 10300 Thailand*
[d] *Department of Chemistry, Faculty of Science, Phayao University, Phayao, 56000 Thailand*
[e] *Division of Mineral Resources Analysis and Identification, Department of Mineral Resources, Bangkok, 10400 Thailand*

Article info

Article history:
Received : 26 July 2022
Revised : 16 December 2022
Accepted : 19 December 2022

Keywords:
Biosorbent, Cellulose, Banana peel, Natural beads

Abstract

The purpose of this research was to produce natural vitamin beads using a biosorbent from banana peel as an alternative to plastic vitamin beads for use in cosmetic products. The new biosorbents could be prepared by an extraction process in combination with a hydrothermal technique and physical processing. The biosorbent material has high fiber content, up to 45.25% by weight, particle sizes in the range of 10-160 µm, with a specific surface area of 21.5 m^2/g and a point of zero charge at pH 6.83. It has a high cellulose crystallinity index (I_{cr}) equal to 59.2%. It could be manufactured with a yield of 8.85%. The study on the adsorption equilibrium of this biosorbent material showed that the Langmuir isotherm fits better for the adsorption process (R^2 = 0.9912) than the Freundlich isotherm (R^2= 0.9532) which presented a monolayer surface adsorption mechanism confirmed by XRD of vitamin C from released solution. The biosorbent from banana peel has an effective adsorption capacity for vitamin C (5% solution) of 545 mg/g and the release efficiency of vitamin C was 80% in water. In addition, an increase of adsorption capacity from 27 to 50 °C showed that the adsorption reaction between the biosorbent and vitamin C was endothermic. We have concluded that biosorbent from banana peel can be prepared by a hydrothermal method that is energy-efficient and environmentally friendly. This biosorbent material can be used as a natural alternative to polyethylene beads for vitamin C release in cosmetic products for antioxidant effect. The product from this research is a new category that combines natural materials with active ingredients to be used in cosmetic applications to ensure health safety and environmental protection.

* Corresponding Author
e-mail: paunpassanan_dec@dusit.ac.th

Introduction

In the past few decades, people are increasingly paying attention to environmental impacts. Especially the problem of littering plastics into rivers, which affects the environment. Microplastics, which are extremely small plastic particles, may not be visible to the naked eye (smaller than 5 mm or 5,000 microns). They are used in households and industries such as vitamin beads and scrub beads in cosmetic products (NYS AOG, 2015). Microplastics contaminating water sources can affect the environment and the health of aquatic animals, for example, causing intestinal disorders of fish. In addition, the chemicals in microplastics can be toxic and threaten human health. Environmentalists therefore focus on solving the environmental problems caused by plastics, both relatively large (>5 mm) plastics called mesoplastics (Andrady, 2011) and microplastics (<5 mm) (Duis & Coors, 2016). There is an urgent need to develop biodegradable alternatives to plastics as sustained-release preservatives in cosmetics. In addition to solving problems by using various technologies and materials to treat wastewater, such as nanomaterials, polymers and green materials (Saleh, 2021), processes and products of environmentally friendly materials have been developed that are degradable such as natural materials to replace synthetic products (Jimenez et al., 2012). Plant cellulose is a very attractive natural material due to its outstanding adsorption and biodegradability properties (Bhasney et al., 2020).

Most natural fibers come from plants, with cellulose being the most abundant active ingredient. It is found in the cell walls of all plants. It plays a role in helping plants to be strong, solid and most importantly, insoluble in water. In nature, cellulose is often found in combination with lignin, hemicellulose, tannins, fats and pigments, etc. Cellulose is a natural fiber in plants that can be used extensively. It is a porous material that can be used as a filter material and a good biosorbent. Cellulose from fruits and vegetables is also used to make products that are safe to use and consume, such as ingredients in food to increase fiber content, as additives in cosmetics, etc. There are many fruits and vegetables that are commonly used to extract natural cellulose fibers for utilization, such as cellulose from rice husk (Yunus et al., 2019), corncobs (Garcia et al., 2022), banana pseudo-stem (Li et al., 2015; Nguyen et al., 2021), etc.

The selection of plant species and organelles with high fiber content is important for the preparation of the biosorbent. In addition, a fiber preparation method is necessary since other components such as proteins, lipids, carbohydrates, plant pigments and other soluble substances must be removed first through various fiber preparation processes. Fiber preparation methods are chemical extraction, mechanical processing and hydrothermal methods for efficient sorbent preparation (Shi et al., 2018; Phanthong et al., 2018). The hydrothermal method is one of the most efficient, energy-saving and environmentally friendly method (Tang et al. 2021) and is also convenient for scale-up in production from laboratory to semi-industrial and industrial scale.

Banana is a tropical fruit that is widely consumed around the world. It is found in tropical regions (Gowthaman et al., 2018), especially in Southeast Asia such as Thailand, Laos, Myanmar, Indonesia, Malaysia and Vietnam. It represents one of the most important fruit crops, with a global annual production of more than 50 million tons (Sharma et al., 2016). Banana is a fruit that can be consumed in both fresh form and used as an ingredient in many foods such as banana snacks, dried bananas and fried bananas (Mohapatra et al., 2010). Banana is also an economically important fruit for both domestic consumption and export, generating an annual income of more than 300 million baht (Singanusong & Sodchit, 2011). As a result, Thailand has a large amount of banana peels up to 200 tons per day that tends to increase continuously (Tibolla et al., 2018). Banana peels are generally littered. It was found that banana peels contain more than 53% of dietary fiber (Singanusong & Sodchit, 2011) and 15-17% of cellulose (Tibolla et al., 2018; Menon et al., 2017; Singanusong & Sodchit, 2011). Banana peels can be extracted as fiber for use in consumer products that are safe for health.

This project researched the preparation of cellulose fiber biosorbent from banana peels by a hydrothermal method, which is energy-efficient and environmentally friendly. The study focused on the adsorption efficiency of vitamin C by this biosorbent, which can be used as natural vitamin C beads instead of polyethylene beads in cosmetic products. The result of this research obtained a new type of product that combines natural materials with active ingredients to be used in cosmetic applications to ensure health safety and reduce plastic waste in water resources to protect the environment.

Materials and methods

1. Materials

"Hom Thong" banana peels were collected from King Fruit Company at Lamlukka, Pathum Thani Province, Thailand. The applied chemicals are sodium hydroxide (AR grade, from Ajax Finechem, Australia), potassium metabisulfite (AR grade, from Fluka, Switzerland), sodiumchlorite (AR grade, from Ajax Finechem, Australia), hydrogen peroxide (AR grade, from Qrec, New Zealand), ethanol (AR grade, from RCI Labscan, Thailand) and sulfuric acid (AR grade, from Qrec, New Zealand).

2. Preparation of biosorbent materials

The process of preparing biosorbent from banana peels is divided into three main processes starting from the banana peel pretreatment process. The next process is to isolate cellulose fibers by chemical processing. Finally, there is a process of reducing the size to microcellulose by a physical process. A summary diagram of the preparation process of biosorbent from banana peels is shown in Fig. 1 and consists of the following steps: Banana peels were extracted to isolate the active ingredient as the banana peel residues were prepared as dry coarse powder by a modified method of Chairgulprasert et al. (2013) for the preparation of biosorbent. The procedure was as follows: 1) Banana peels were prepared by separating the pulp and washing the peels with distilled water to remove dust and dirt. The banana peels were dried in the oven at 60°C for 12 hr, grinded with a high-speed blender to a coarse powder and placed in a refrigerator at 4°C before its chemical composition analysis by AOAC methods (2000). 2) The banana peel powder was extracted by soaking in 95% ethanol (in the ratio of 1:5 in w/v) for 24 hr to remove organic byproducts and the powder was dried in the oven at 60°C for 12 hr. 3) The banana peel powder was immersed in a solution of potassium metabisulfite (1% w/v) (in the ratio of 1:5 in w/v) for 12 hr. After that, the powder was filtered out, washed with distilled water and dried in the oven at 60°C for 12 hr. 4) The dry peel powder was weighed, ground, passed through a 40 mesh sieve and kept in a desiccator for further experiments.

The preparation of cellulose fibers from banana peel powder was chemically processed by a hydrothermal method in combination with the cellulose preparation method by Khawas & Deka (2016). The procedure was as follows: (1) The banana peel powder was mixed with 20% sodium hydroxide solution (in the ratio of 1:20 in w/v) and then filled in a hydrothermal reactor and heated at 100°C for 1 hr. The substance was filtered and washed with distilled water. (2) The resulting substance was dispersed in a 1% sodium chlorite solution (in the ratio of 1:20 in w/v) and the pH of the solution was adjusted to a value of 5. The mixture was placed in a hydrothermal reactor and heated to 80°C for 1 hr. The substance was filtered and washed with distilled water. (3) The resulting substance was added to a 5% potassium hydroxide solution and stirred at room temperature for 12 hr. The material was filtered and washed with distilled water. (4) The resulting substance was added to a 1% sulfuric acid solution (in the ratio of 1:20 in w/v) and then packed in a hydrothermal reactor to be heated at a temperature of 80°C for 2 hr. The substance was filtered and washed with deionized water to finally obtain cellulose as an adsorbent material from the banana peels.

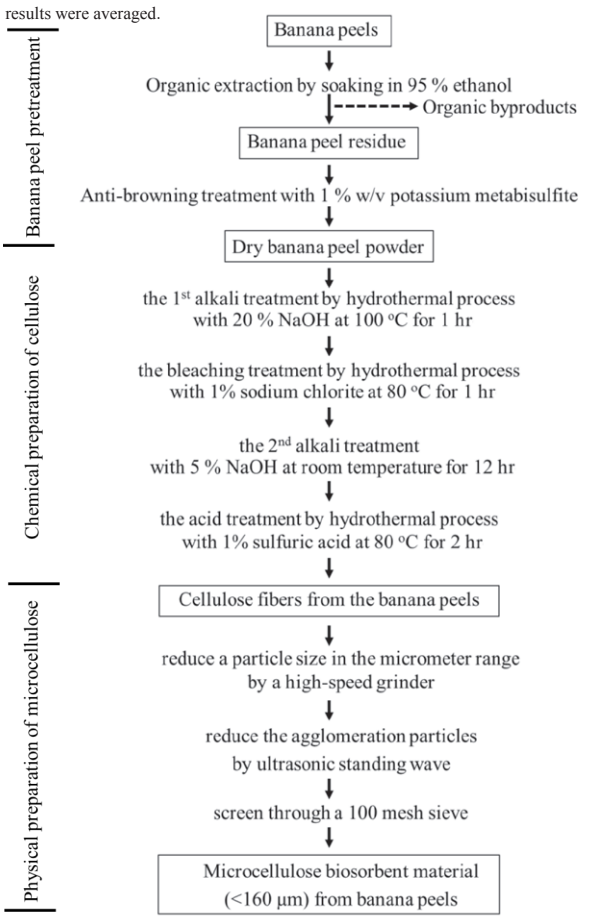

Fig. 1 Schematic diagram of the preparation of biosorbent from banana peels

Preparation of microcellulose from banana peels was done by a modified physical and mechanical process of Khawas & Deka (2016). The process was as follows: (1) The cellulose fibers from the chemically treated banana peels were reduced to a particle size in the micrometer range by a high-speed grinder. (2) The resulting substance was dispersed by ultrasonic standing wave in water for 30 min to reduce the agglomeration of the particles before drying. (3) Finally, the powder was screened through a 100- mesh sieve. The result was a microcellulose biosorbent material (<160 μm) made from banana peels. The biosorbent preparation experiment was repeated three times and the results were averaged.

3. Characterization and physical properties

3.1 Study the zero point charge of the biosorbent

The biosorbent prepared from banana peel was characterized by the point of zero charge (PZC; pH at the point of zero charge). Exact weights of 0.25 g of the prepared biosorbent material were added to beakers containing 50 mL buffer solution of pH 1-9, respectively. The mixtures were shaken at 250 rpm for 1 hr and then soaked for 24 hr. The mixture in each beaker was filtered and the pH was measured. The experiment was repeated three times, the measured pH values were averaged and the graph was plotted to determine the point of zero charge on the surface of the biosorbent material.

3.2 Surface analysis and characterization

The specific surface area of the biosorbent prepared from banana peel was analyzed by the BET method using a surface analyzer (BET), Model CIEX/X500RAnton.

The surface morphology of the biosorbent was characterized by scanning electron microscope (SEM: JSM-7610F, Oxford). The sample was fixed on 10 mm diameter aluminum stubs with double-sided tape and coated with a fine layer of gold using a sputter gold coater to improve conductivity. The surface characteristic of the coated samples were scanned under 1000x magnification.

3.3 X-ray diffraction (XRD)

X-ray powder diffraction profiles of the biosorbent were collected by a Bruker diffractometer, Model D8 Advance at ambient temperature, using Cu Kα radiation (λ = 0.15418 nm) with operating voltage and current of 40 kV and 30 mA, respectively. The diffraction intensities were recorded between the 2θ angle range of 5 and 50° with a scanning speed of 0.5°/min. The crystallinity index (I_{cr},%) of the biosorbent was calculated using equation (1), following the method of Segal et al. (1959).

$$I_{cr} = \frac{I_{002} - I_{am}}{I_{002}} \; x \; 100 \qquad \text{.....(1)}$$

With I_{cr} The crystallinity index

I_{002} The maximum intensity of diffraction corresponding to the 002 plane of cellulose crystals (diffraction intensity close to 2θ at 22°)

I_{am} The intensity of diffraction referring to amorphous cellulose (diffraction intensity close to 2θ at 18°)

The crystallinity index was used to describe the relative amount of crystalline cellulose in the biosorbent; the crystallinity percentage was calculated as the ratio of heights between the maximum intensity of the crystalline diffraction and the intensity of the non-crystalline (amorphous) diffraction materials.

3.4 Fourier-transform infrared spectroscopy (FTIR)

The functional groups of the biosorbent were analyzed by adsorption spectroscopy using a Fourier Transform Infrared Spectrophotometer (IR Tracer- 100, SHIMADZU, Japan). The samples were prepared by using the KBr disk (ultra thin pellets) technique with a pellet preparation as a carrier of the dry sample in the ratio of 1:100. IR spectra of samples were measured within the infrared region between 4000 and 400 cm^{-1}, with a resolution of 4 cm^{-1} and 20 scans.

4. Batch adsorption studies

To study the effect of concentration and contact time for the adsorption of Vitamin C on the surface of the biosorbent material, exact amounts of 0.25 g of the biosorbent were added into different Erlenmeyer flasks. Vitamin C solutions at concentrations of 0.50, 1.00, 2.00, 3.00, 4.00 and 5.00% w/v were added to 50 mL of the flasks. The solutions were shaken by a shaker (n-biotek, NB-205) at a speed of 120 rpm at 27°C with different periods of 0, 1, 3, 5, 10, 15, 30, 60, 120 and 180 min, respectively. The flasks were then removed from the shaker and the solutions filtered through filter papers to separate the biosorbent from the solutions. The final concentration of Vitamin C in the solutions was analyzed by a double beam UV-Visible Spectrophotometer (Shimadzu, UV-2401PC). The experiment was repeated three times and the results were averaged.

To study the effect of concentration and temperature for the adsorption of Vitamin C on the surface of the

biosorbent, exact amounts of 0.25 g of the biosorbent were added into different Erlenmeyer flasks. 50 mL of different concentrated solutions of vitamin C (0.50, 1.00, 2.00, 3.00, 4.00 and 5.00%w/v) were added to each of the flasks. Each sample was kept in a shaker at temperatures of 27 and 50°C for equilibrium time (120 min). The final concentration of Vitamin C in the solutions was analyzed by a double beam UV-Visible Spectrophotometer (Shimadzu, UV-2401PC). The experiment was repeated three times and the results were averaged. The adsorption capacity of vitamin C at equilibrium, q_e was calculated by equation (2).

$$q_e = \frac{(C_0 - C_e)V}{W} \qquad \text{.....(2)}$$

With q_e as adsorption capacity of vitamin C at equilibrium (mg/g)

C_0 is the initial concentration of the vitamin C solution (mg/L)

C_e is the equilibrium concentration of the vitamin C solution (mg/L)

V is the volume of the vitamin C solution (L)

W is the weight of the biosorbent (g)

5. Study the amount of desorption of vitamin C from the biosorbent

To study the efficacy of the release of vitamin C from the surface of biosorbents into water, exact amounts of 0.25 g of the biosorbent, which had adsorbed vitamin C in a solution with a concentration of 1.0%w/v, were added into Erlenmeyer flasks. 50 mL distilled water was added to each flask and shaken at 27°C for 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80 and 90 min, respectively. The amount of vitamin C which was released from the biosorbent was determined by a UV-Visible Spectrophotometer (Shimadzu, UV-2401PC). The experiment was repeated three times and the results were averaged.

Results and discussion

1. The preparation of biosorbent from banana peels

The ethanol extracted, crushed banana peel powder samples were treated to inhibit enzymatic browning by soaking in 1% potassium metabisulfite (anti-browning agent) solution at room temperature for 12 hr and drying at 80°C for 12 hr (Arora et al., 2018). The dry peel powder was weighed, ground and passed through a 40 mesh sieve. The resulting substance was banana peel coarse powder used for the preparation of biosorbent materials.

Chemical preparation of cellulose from banana peel powder by the method of Khawas & Deka (2016) combined with a hydrothermal method was performed. The chemical process is described as follows: Step 1 is the 1st alkali treatment. Sodium hydroxide was used to hydrolyze and solubilize pectins, starch, hemicelluloses and proteins. Step 2 is the bleaching treatment. Sodium chlorite solution was used to remove phenolic compounds and molecules with chromophoric groups (coloring groups). Most of the lignin was bleached by the rapid oxidation by chlorine and chlorite. As a result of this reaction, hydroxyl, carboxyl and carbonyl groups were generated, which contributed to greater solubility of lignin in alkali (Dufresne et al., 1997). Step 3 is the 2nd alkali treatment. Potassium hydroxide was used to remove other contaminants dissolved in alkali. Step 4 is the acid treatment. Sulfuric acid was used to remove residual pectin and to break down large fibers (digestion of bigger fibers). The resulting cellulose product consisted of white fine, small fiber material.

Physical preparation of microcellulose from banana peel cellulose included milling in a high-speed grinder to reduce the particle size of the fibers. The cellulose product was dispersed with an ultrasonic machine to fine-tune and shrink the fibers. It was found that the use of ultrasonic waves produces strong mechanical vibrations in which liquid molecules adsorb the energy of sound waves, expanding them into bubbles and forming cavitations. The resulting shock wave can separate the fibers or agglomerating particles. The resulting microcellulose was a white fine powder with a particle size < 160 μm.

The yield analysis of the microcellulose product as biosorbent showed that the percentage yield was 8.85.

2. Chemical Composition

The chemical composition of the biosorbents compared to the chemical composition analysis of dried banana peels (raw materials) are shown in Table 1. It was found that the highest part of dried banana peels were carbohydrates with 62.05% of total weight, followed by fiber content 13.54%, moisture 8.32%, ash 8.12%, protein 4.01% and fat 3.96%, respectively. In comparison, the biosorbent contained a lower carbohydrate composition (44.85%) and had a high fiber content, up to 45.25% by weight. This was less than the 72.36% fiber content of

commercial cellulose (Singanusong & Sodchit, 2011). However, the result of the research is that the preparation of cellulose from natural materials yields relatively high fiber content. This is the reason why banana peel fibers have the potential to be used as a natural adsorbent.

Table 1 Chemical Composition

Composition	Content (%)	
	Dried banana peel	Biosorbent from banana peel
Moisture	8.32 ± 0.05	4.05 ± 0.10
Fat	3.96 ± 0.48	1.56 ± 0.25
Protein	4.01 ± 0.45	2.28 ± 0.13
Carbohydrate	62.05 ± 0.12	44.85 ± 0.10
Ash	8.12 ± 0.54	2.01 ± 0.68
Fiber	13.54 ± 0.15	45.25 ± 0.47

Remark: Results are mean ±SD of triplicate analysis

3. Characterization and physical properties of the biosorbent

3.1 Point of zero charge of biosorbent

The analysis of the charge on the surface of the biosorbent is shown in Fig. 2. The two curves intersect at a pH of 6.83 indicating that the biosorbent has a zero point charge equal to 6.83. A pH value less than 6.83 will result in a positive charge due to the amount of hydronium ions (H_3O^+) on the surface of the biosorbent. Hydroxide ions (OH^-) ions at a pH greater than 6.83 will cause negative charges (Bharathi & Ramash, 2013).

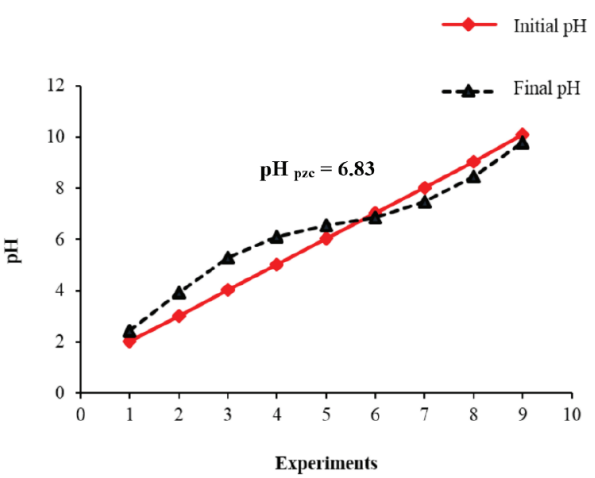

Fig. 2 Point of zero charge of biosorbent from banana peel: (◆) solid red line is initial pH and (▲) black dashed line is final pH

3.2 Specific surface area

The results of the analysis of the specific surface area of banana peels compared with the biosorbent material produced from banana peel revealed that the specific surface area of the biosorbent was 21.5 m^2/g which is substantially greater than the specific surface area of banana peels (15.7 m^2/g). The results were similar to the surface area of the natural cellulose (5.3-28.1 m^2/g) (Constant et al., 2016). However, the specific surface area of the biosorbent from banana peel was less than that of activated carbon produced from banana peel (311.18-625.82 m^2/g) (Wanprakhona et al., 2021).

3.3 Scanning electron microscopy

The morphological results of banana peel surfaces and biosorbent material produced from banana peels by scanning electron microscope (SEM) are shown in Fig. 3. It revealed that the microstructure of the banana peel surface was significantly different from the surface of the biosorbent. The surface of the banana peel (Fig. 3a) was irregular and had layers of deposits due to the presence of several components: carbohydrates, proteins, lipids, lignins, pectins, cellulose and hemicellulose (Li et al., 2015). In contrast, the surface

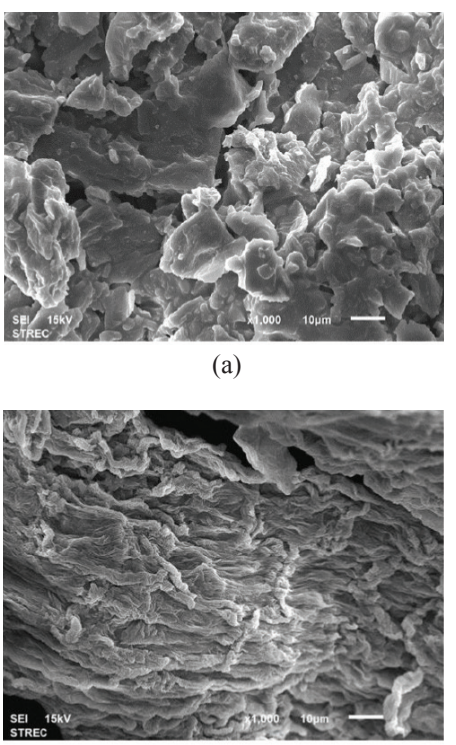

Fig. 3 SEM images of (a) banana peel and (b) biosorbent prepared from banana peel at magnification of 1000x

of the biosorbent (Fig. 3b) was uniform, smooth and clean, due to the non-fibrous constituents removed by the chemical pretreatment. It was also found that the chemical treatment helped in the removal of some amorphous components such as lignin, pectin and hemicelluloses (Pelissari et al., 2014).

3.4 Fourier transform infrared spectroscopy (FT-IR)

The results of the functional group determination of the material using Fourier transform infrared-spectroscopy (FTIR) technique to analyze the lignin, cellulose and hemicellulose composition of untreated banana peels were compared with the cellulose fiber composition of the biosorbent. The study was conducted in the wave number range between 400 and 4000 cm^{-1}.

The FTIR spectrum of untreated banana peels (Fig. 4) showed a broad vibrational band at wave number 3650–3000 cm^{-1}, corresponding to the stretching and bending modes of the hydroxyl (-OH) groups on the surface. Peaks at wave number 2950-2850 cm^{-1} showed asymmetric C-H stretched vibrations involving lignin and hemicellulose. Signals at wave numbers 1040-1020 cm^{-1} corresponded to stretching vibrations of C-O-C in the pyranose ring consistent with the cellulose composition. The peak at wave number 1730 cm^{-1} showed stretching vibrations of the C-O bonds of acetyl and ester in lignin, hemicellulose pectin. A peak at wave number 907-897 cm^{-1} was related to the β- bond glycosidic in cellulose and a peak at wave number 897 cm^{-1} indicated cellulose I.

The FTIR spectrum of the biosorbent prepared from banana peel (Fig. 5) showed decreased intensity of vibrational bands at wave number 2950-2850 cm^{-1}. The bands at wave number 1040-1020 cm^{-1} associated with the stretching bond of C-O-C in the pyranose ring clearly represent cellulose. The intensity of the stretching vibrational peak of C-O at wave number 1730 cm^{-1} was reduced because the hemicellulose and lignin components were eliminated in the chemical process. The vibrational bands at wave number 907-897 cm^{-1} showed a decrease in intensity after chemical treatment, indicating that lignin was removed.

3.5 X-ray diffraction (XRD)

A X-ray powder diffraction analysis of banana peel and biosorbent was performed, with XRD patterns shown in Fig. 5. It was found that the diffractogram of banana peel (Fig. 6a) displayed an amorphous characteristic. It shows a peak at 2θ = 17°, indicating a typical B-type pattern of starch (Tibolla et al., 2018). In comparison, the diffractogram of biosorbent from banana peels (Fig. 6b) showed X-ray diffraction peaks of 2θ angles at 16° and 22°, which are characteristic of cellulose I crystals with parallel structures (Yiying et al., 2015).

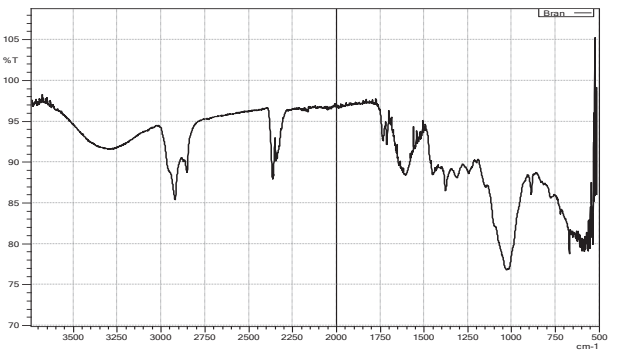

Fig. 4 FTIR spectrum of untreated banana peels

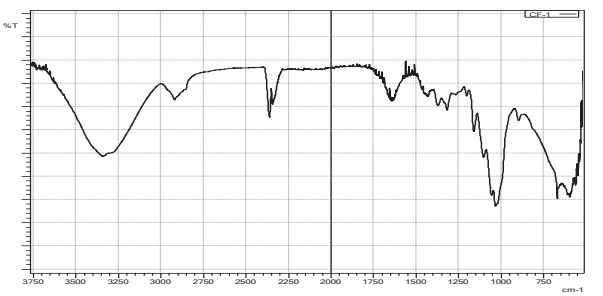

Fig. 5 FTIR spectrum of the biosorbent prepared from banana peel

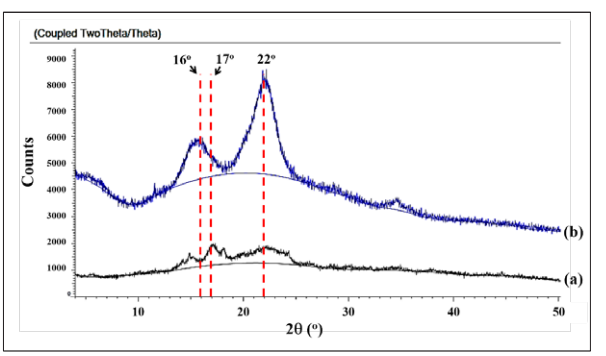

Fig. 6 XRD patterns showing amorphous characteristic of (a) banana peel and (b) cellulose I of biosorbent prepared from banana peels

X-ray diffraction peaks around 2θ =16° and 22° refer to 101 and 002 plane positions that are characteristic for cellulose. The crystallinity index (I_{cr},%) of banana peel and biosorbent was determined by equation (1) of Segal et al. (1959). The result showed that banana peel (raw material) had a low crystallinity index (I_{cr} = 11.1%) as amorphous material, while the

biosorbent showed a very high crystallinity index (I_{cr} = 59.2%). This value was higher than the prepared cellulose by alkaline treatment (I_{cr} = 36.6%) (Madhushani et al., 2020), mechanical extraction (I_{cr} = 50.8%) (Madhushani et al., 2020) and the natural cellulosic fibers derived from Senna auriculata for making light weight industrial biocomposites (I_{cr} = 49.6%) (Nagarajaganesh et al., 2019). In contrast, our result was lower than prepared nanocellulose by different methods such as enzymatic treatment (I_{cr} = 66.2%) (Tibolla et al., 2018) and NaOH/HNO_3-$NaNO_2$ oxidation (I_{cr} = 80.1%) (Kumar et al., 2019)

4. Adsorption of biosorbent from banana peel

4.1 Effect of contact time

The effect of contact time for the adsorption of vitamin C by the biosorbent is shown in Fig. 7. In the beginning the graph is very steep and became parallel to the x-axis later at equilibrium time. That means a large amount of vitamin C was adsorbed rapidly during the initial 15 min of contact and then the adsorption speed slowed down until equilibrium was achieved. The rapid initial adsorption could be attributed to the large amounts of adsorbent active sites available for adsorbate molecules (Ahmad & Kumar, 2010).

In the early start time, the biosorbent can adsorb vitamin C quickly because of a high surface area available for adsorption until the equilibrium time at 30 min when the active sites are saturated with vitamin C molecules.

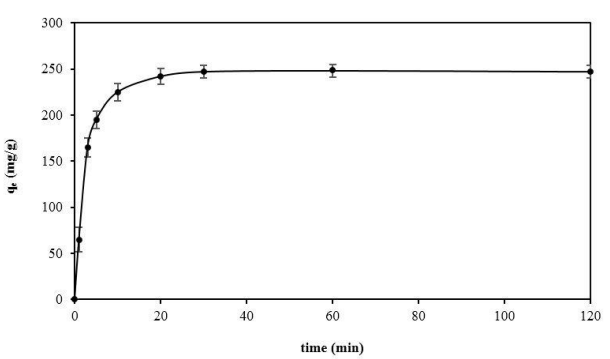

Fig. 7 Effect of contact time for the adsorption of vitamin C on biosorbent from banana peels (solution pH 7, temperature 27 °C). Data points are the mean of the results of three replicates and error bars show standard deviation

4.2 Effect of concentration

In the present study, the concentration of vitamin C solutions varied from 0.50, 1.00, 2.00, 3.00, 4.00 and 5.00% w/v. The effect of the concentration on the amount adsorbed is shown in Fig. 8; the adsorption capacity of the biosorbent increased steadily when the vitamin C concentration increased up to 5.0% w/v indicating higher amounts of vitamin C molecules occupy the surface of the biosorbent at higher concentrations (Ahmad & Kumar, 2008).

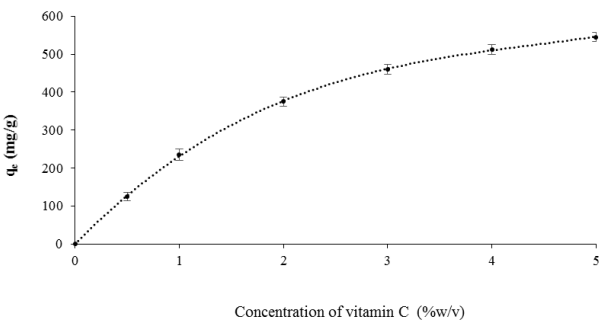

Fig. 8 Effect of concentration for the adsorption of vitamin C on biosorbent from banana peels. Data points are the mean of the results of three replicates and error bars show standard deviation

4.3 Effect of temperature

Table 2 and Fig. 9 show the effect of temperatures on the adsorption of biosorbent from banana peels at two different values (27 and 50°C). When the temperature raises, the biosorbent can adsorb more vitamin C at all tested concentrations due to higher kinetic energy, so more adsorbate molecules can move onto the surface of the adsorbent (Aksu et al., 2008). This result is similar to the methylene blue adsorption behavior of microcrystalline cellulose from banana pseudo-stem (Nguyen et al., 2021). This indicates that the adsorption of vitamin C on the surface of the biosorbent from banana peels is an endothermic process (Ahmad & Kumar, 2010).

Table 2 Effect of temperature for the adsorption of vitamin C on biosorbent from banana peels at temperatures of 27°C and 50°C

Concentration of vitamin C (% w/v)	q_e (mg/g)	
	27 °C	50 °C
0	0	0
0.5	125	179
1.0	247	312
2.0	390	450
3.0	463	545
4.0	512	602
5.0	545	650

Remark: Results are mean of triplicate analysis

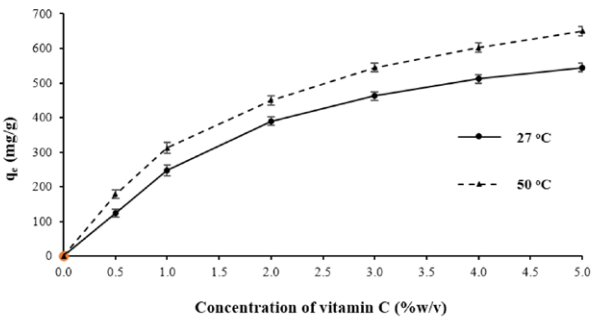

Fig. 9 Effect of temperature for the adsorption of vitamin C on biosorbent from banana peels at different temperatures: (●) 27°C and (▲) 50°C. Data points are the mean of the results of three replicates and error bars show standard deviation

4.4 Adsorption Isotherm

Adsorption isotherms shows the relationship between the amounts of vitamin C adsorbed per unit weight of biosorbent at constant temperature. The adsorption behavior of vitamin C on the biosorbent can be described by Langmuir and Freundlich isotherms as in equation (3) and (4), respectively.

$$\frac{1}{q_e} = \left(\frac{1}{q_{max} K_L C_e}\right) + \left(\frac{1}{q_{max}}\right) \qquad \text{.....(3)}$$

$$\log q_e = \log K_F + \left(\frac{1}{n} \log C_e\right) \qquad \text{.....(4)}$$

When q_e is the equilibrium loading of adsorbate per unit mass of adsorbent (in mg/g)

C_e is the equilibrium concentration of vitamin C solution (mg/L)

q_{max} is the maximum adsorbed vitamin C amount on monolayers (mg/g)

K_L is the Langmuir constant related to adsorption capacity (mg/L)

1/n is the adsorption constant (strength of adsorption) (mg/g)

K_F is the Freundlich constant related to rate of adsorption (mg/g)

The adsorption isotherms of vitamin C adsorbed on the biosorbent are shown in Fig. 10 and 11 for the Langmuir and Freundlich mechanism, respectively. The adsorption isotherm is important to describe how the adsorbate interacts with the adsorbent. It was found that the correlation coefficient (R^2) of the Langmuir isotherm came closer to 1 than the Freundlich isotherm. That means the adsorption behavior of vitamin C on the biosorbent corresponds slightly better with the Langmuir than the Freundlich isotherm. It presents a monolayer surface adsorption mechanism without dissociation of vitamin C and confirmed by XRD of vitamin C from the released solution. Therefore, the behavior of vitamin C molecules is best described as a monolayer adsorption process on the surface of biosorbent from banana peels (Bharathi & Ramash, 2013). This adsorption isotherm is similar to the adsorption of methylene blue in aqueous solutions by biosorption from waste biomaterial (Chowdhury & Saha, 2012) and the adsorption of methyl orange (MO) of asphaltene (Siddiqui, 2017). All of them are adsorption of organic adsorbents with organic sodium salts adsorbates. This corresponds to the adsorption mechanism with weak intermolecular interactions such as electrostatic attraction and hydrogen bonding (Olivito et al., 2021).

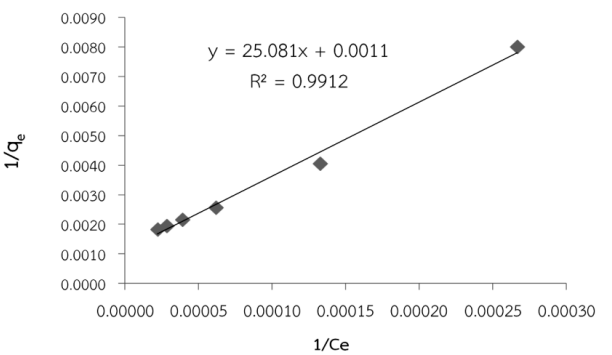

Fig. 10 The correlation curve according to Langmuir adsorption isotherm of vitamin C adsorption by biosorbent from banana peels

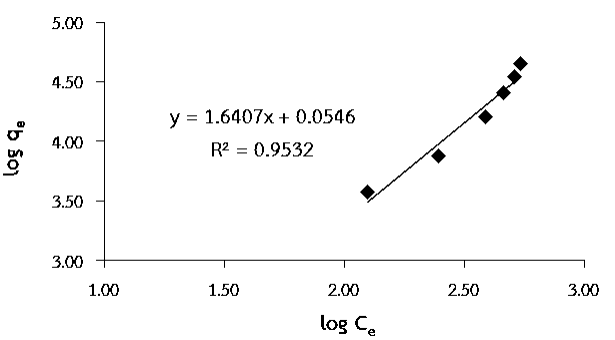

Fig. 11 The correlation curve according to Freundlich adsorption isotherm of vitamin C adsorption by biosorbent from banana peels

5. Desorption of vitamin C from the biosorbent

To study the release of vitamin C in the presence of water as a solvent, different time intervals (0 - 90 min) at constant temperature of 27°C were chosen. It was found that the biosorbent (vitamin C bead from banana

peel) can release vitamin C in an amount equal to 200 milligrams (80%) per gram of biosorbent from a total of 247 mg/g (Fig. 12).

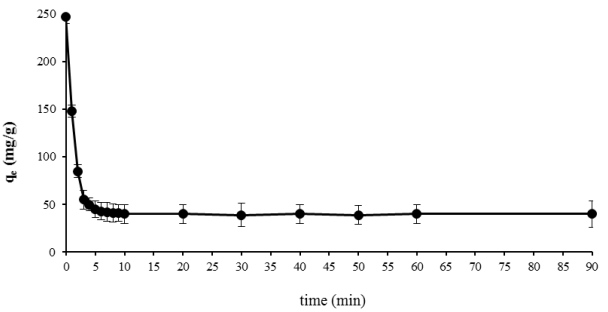

Fig. 12 Effect of contact time for the release of vitamin C from the biosorbent from banana peels. (at temperature of 27 °C). Data points are the mean of the results of three replicates and error bars show standard deviation

The results of a study on the release of vitamin C from vitamin beads from banana peels by analyzing the X-ray diffraction of the dried powder of the substance as released from the vitamin beads. The results are shown in Fig. 13. The XRD pattern of vitamin C (Fig. 13c) confirms the vitamin C release of the beads from banana peels.

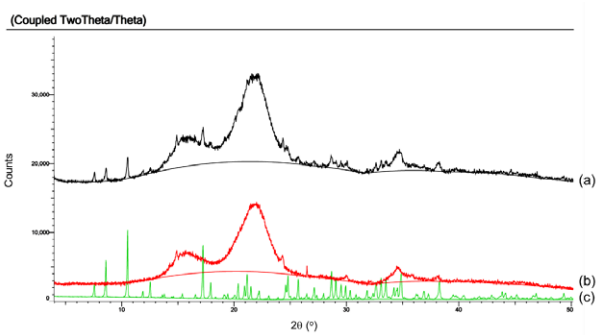

Fig. 13 X-ray diffraction patterns of (a) Vitamin C beads from banana peels (b) Residue from release (c) Vitamin C released from the vitamin beads

A comparison of the applications of biosorbent vitamin C beads prepared from banana peels and commercial polyethylene vitamin C beads for cosmetic products revealed that: The biosorbent vitamin C beads contained 247 mg/g of adsorbent (prepared with 0.25 g of the biosorbent adsorb vitamin C in a solution with a concentration of 1.0%w/v) have a release efficacy of vitamin C (200 mg/g of biosorbent) similar to commercially available polyethylene capsule beads (Vitamin C solution with the concentration of 250 mg/mL, T.P. Drug Laboratories (1969) Co., Ltd.), but differ in the release mechanism. Vitamin C beads prepared from banana peels have a mechanism that gradually releases vitamins from the biosorbents by rubbing, so the use of beads from banana peels is not likely to cause allergic reactions or irritation from the concentrated active ingredient. In contrast, vitamins in polyethylene beads have a single release mechanism of the concentrated active ingredient by bursting the beads. Therefore, these biosorbents are more suitable for use in massaging cosmetic products such as massage creams or gels than for use in liquid products. However, environmental safety is an important aspect which should be realized along with the benefits. Vitamin beads prepared from banana peels consist mainly of natural cellulose fibers from waste material which are degradable in the environment, while polyethylene vitamin beads contain microplastics that are harmful to the environment and are banned for usage globally (Habib et al., 2022).

Conclusion

Banana peel is a waste material from banana product processing that can be used to prepare a microcellulose biosorbent. Physical combined with chemical processes using hydrothermal techniques to improve extraction efficiency saves energy consumption and environmentally friendly methods were used in the preparation of a biosorbent from banana peels with a yield of 8.85%. The adsorption equilibrium of vitamin C by the biosorbent corresponds to a Langmuir isotherm ($R^2 = 0.9912$). Microcellulose biosorbents from banana peels could be used as natural vitamin beads that could store up to 500 mg of vitamin C per gram of banana peel adsorbents. The efficiency of vitamin C release was 80%. However, the safe use of products obtained from this study as ingredients in natural cosmetic products should take into account the regulatory dosage of the active ingredient. A suggestion for further research is a hydrothermal extraction technique to prepare biosorbent from banana peel, in addition to exploring its advantages in terms of energy saving, use of chemicals and controlled emissions into the environment. There is also the potential to collect waste from the process in order to extract by-products such as lignin that provides added value to the process.

Acknowledgments

This work (research project No. C10F630039) was financially supported by Office of National Higher Education Science Research and Innovation Policy Council through Program Management Unit for Competitiveness, Thailand. The authors also thank the Department of Chemical Technology and Department of Cosmetic Science, Faculty of Science, Suan Dusit University, Thailand, for instrumental support.

References

Ahmad, R., & Kumar, R. (2008). Adsorption study of patent blue VF using ginger waste material. *J. Iran. Chem. Soc., 1,* 85-94.

Ahmad, R., & Kumar, R. (2010). Adsorption studies of hazardous malachite green onto treated ginger waste. *J. Environ. Manag., 91,* 1032-1038.

Aksu, Z., Tatli, A., & Tunc, O. (2008). A comparative adsorption/biosorption study of acid blue: Effect of temperature on equilibrium and kinetic parameters. *Chem. Eng. J., 142,* 23-39.

Andrady, A.L. (2011). Microplastics in the marine environment. *Mar. Pollut. Bull., 62*(8), 1596-1605.

AOAC. (2000). *Official methods of analysis* (17th ed.). Washington, DC.: USA.

Arora, S., Siddiqui, S., Gehlot, R., & Ahmed, N. (2018). Effects of anti-browning pretreatments on browning of banana pulp. *Int .J. Curr. Microbiol. App. Sci., 7*(4), 242-249.

Bharathi, K.S., & Ramash, S.T. (2013). Removal of dyes using agricultural wastes as low-cost adsorbents: A review. *Appl. Water. Sci., 3,* 773-790.

Bhasney, S.M., Kumar, A., & Katiyar, V. (2020). Microcrystalline cellulose, polylactic acid and polypropylene biocomposites and its morphological, mechanical, thermal and rheological properties. *Composites Part B: Engineering, 184,* 107717.

Chairgulprasert, V., Japakeya, A., & Samaae, H. (2013). Phytoremediation of synthetic wastewater by adsorption of lead and zinc onto *Alpinia galangal* Willd. *Songklanakarin Journal of Science and Technology, 35*(2), 227-233.

Chowdhury, S., & Saha, P.D. (2012). Biosorption of methylene blue from aqueous solutions by a waste biomaterial: Hen feathers. *Applied Water Science, 2,* 209–219.

Constant, S., Barakat, A., Robitzer, M., Renzo, F.D., Dumas, C., & Quignard, F. (2016). Composition, texture and methane potential of cellulosic residues from Lewis acids organosolv pulping of wheat straw. *Bioresource Technology, 216,* 737-743.

Dufresne, A., Cavaille, J.-Y., & Vignon, M. R. (1997). Mechanical behavior of sheets prepared from sugar beet cellulose microfibrils. *Journal of Applied PolymerScience, 64*(6), 1185–1194.

Duis, K., & Coors, A. (2016). Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. *Environ. Sci. Eur., 28*(2), 1-25.

Garcia, M.E., García, M.C.G., Gracida, J., Hernández, H.M.H., Arvizu, J.A.G., Pierro, P.D., & González, C.R. (2022). Properties and biodegradability of films based on cellulose and cellulose nanocrystals from corn cob in mixture with chitosan. *International Journal of Molecular Sciences, 23,* 10560.

Gowthaman, S., Nakashima, K., & Kawasaki, S. (2018). A State-of-the-art review on soil reinforcement technology using natural plant fiber materials: Past findings, present trends and future directions. *Materials, 11*(4), 553.

Habib, R.Z., Aldhanhani, J.A.K., Ali, A.H., Ghebremedhin, F., Elkashlan, M, Mesfun, M, …Thiemann, T. (2022). Trends of microplastic abundance in personal care products in the United Arab Emirates over the period of 3 years (2018–2020). *Environmental Science and Pollution Research, 29,* 89614–89624.

Jimenez, A., Fabra, M.J., Talens, P., & Chiralt, A. (2012). Edible and biodegradable starch films: *A review. Food and Bioprocess Technology, 5*(6), 2058-2076.

Khawas, P., & Deka, S.C. (2016). Isolation and characterization of cellulose nanofibers from culinary banana peel using high-intensity ultrasonication combined with chemical treatment. *Carbohydrate Polymers, 137,* 608-616.

Kumar, R., Kumari, S., Surah, S., Rai, B., Kumar, R., Sirohi, S., & Kumar, G. (2019). A simple approach for the isolation of cellulose nanofibers from banana fibers and characterization. *Materials Research Express, 6*(10), 1088.

Li, W., Zhang, Y., Li, J., Zhou, Y., Li, R., & Zhou, W. (2015). Characterization of cellulose from banana pseudo-stem by heterogeneous liquefaction. *Carbohydrate Polymers, 132,* 513–519.

Madhushani, W.H., Priyadarshana, I., Kaliyadasa, E., Ranawana, C., & Senarathna, C. (2020). Preparation and characterization of nanofibers extracted from banana pseudostems. *Conference: GARI Winter Multidisciplinary Symposium 2020,* 17th December 2020 (pp.39-46). Global Academic Research Institute, Colombo, Sri Lanka.

Menon, M.P., Selvakumar, R., Kumar, P.S., & Ramakrishna, S. (2017). Extraction and modification of cellulose nanofibers derived from biomass for environmental application. *RSC Advances, 7,* 42750–42773.

Mohapatra, D., Mishra, S., & Sutar, N. (2010). Banana and its by-product utilisation: An overview. *J. Sci. Ind. Res., 69*(5), 323-329.

Nagarajaganesh, B., Ganeshan, P., Ramshankar, P., & Raja, K. (2019). Assessment of natural cellulosic fibers derived from Senna auriculata for making light weight industrial biocomposites, *Industrial Crops and Products, 139, 111546.*

Nguyen, T.T., Thi, B.T.N., Phan, P.T., Le, T.T., Thi, Q.A.N., Bach, L.G., …Nguyen, N.H. (2021). Synthesis of microcrystalline cellulose from banana pseudo-stem for adsorption of organics from aqueous solution. *Engineering and Applied Science Research, 48*(4), 368-378.

NYS AOG. (2015). *Discharging microbeads to our waters: An examination of wastewater treatment plants in New York.* New York: Environmental Protection Bureau, New York State Office of the Attorney General.

Olivito, F., Algieri, V., Jiritano, A., Tallarida, M.A., Tursi, A., Costanzo, P., … Nino, A.D. (2021). Cellulose citrate: A convenient and reusable bioadsorbent for effective removal of methylene blue dye from artificially contaminated water. *RSC Advances, 11,* 34309–34318.

Pelissari, M.F., Sobral, P.J.A., & Menegalli, F.C. (2014). Isolation and characterization of cellulose nanofibers from banana peels. *Cellulose, 21*(1), 417–432.

Phanthong, P., Reubroycharoen, P., Hao, X., Xu, G., Abudula, A., & Guan, G. (2018). Nanocellulose: Extraction and application. *Carbon Resources Conversion, 1*(1), 32-43.

Saleh, T.A. (2021). Protocols for synthesis of nanomaterials, polymers, and green materials as adsorbents for water treatment technologies. *Environmental Technology & Innovation, 24,* 101821.

Segal, L., Creely, J.J., Martin, A.E., & Conrad, C.M. (1959). An empirical method for estimating the degree of crystallinity of native cellulose using the x-ray diffractometer. *Textile Research Journal, 29,* 786-794.

Sharma, R., Oberoi, H.S., & Dhillon, G.S. (2016). *Agro-Industrial Wastes as Feedstock for Enzyme Production: Chapter 2 - Fruit and vegetable processing waste: Renewable feed stocks for enzyme production.* San Diego, CA, USA: Academic Press.

Shi, S., Zhang, M., Ling, C., Hou, W., & Yan, Z. (2018). Extraction and characterization of microcrystalline cellulose from waste cotton fabrics via hydrothermal method. *Waste Management, 82,* 139-146.

Siddiqui, M.N. (2017). Developing an effective adsorbent from asphaltene for the efficient removal of dyes in aqueous solution. *Desalination and Water Treatment, 67,* 371–380.

Singanusong, R., & Sodchit, C. (2011). Production of cellulose from banana peels. *Agricultural Science Journal, 42*(3), 741-744.

Tang, F., Li, Y., Huang, J., Tang, J., Chen, X., Yu, H.-Y., … Tang, D. (2021). An environmentally friendly and economical strategy to cyclically produce cellulose nanocrystals with high thermal stability and high yield. *Green Chemistry, 23,* 4866-4872

Tibolla, H., Pelissari, F.M., Martins, J.T., Vicente, A.A., & Menegalli, F.C., (2018). Cellulose nanofibers produced from banana peel by chemical and mechanical treatments: Characterization and cytotoxicity assessment. *Food Hydrocolloids, 75,* 192-201.

Wanprakhona, S., Pattaraphutanon, P., Borvornsudhasin, P., & Choocherd, N. (2021). Chemical and surface properties of activated carbon from banana peel by dry chemical activation. *Journal of Materials Science and Applied Energy, 10*(3), 96-105.

Yiying, Y., Jingquan, H., Guangping, H., Quanguo, Z., French, A.D., & Qinglin, W. (2015). Characterization of cellulose I/II hybrid fibers isolation from energycane bagasse during the delignification process: Morphology, crystallinity and percentage estimation. *Carbohydrate Polymers, 133,* 438-447.

Yunus, M.A., Raya, I., Maming, & Tuara1, Z.I. (2019). Extraction cellulose from rice husk. *Indonesia Chimica Acta, 12*(2), 79-83.

Journal of Food Health and Bioenvironmental Science

Journal homepage : http://jfhb.dusit.ac.th/

Effects of Sangyod Rice as a Substitute for Wheat Flour on the Physical Properties of Butter Cake Products

Racharat Yampuang[a], Noppamas Poolcharoensil[a], Natthaporn subanmanee[a], Sasiruk Klangwijit[a], Krittika Norajit[a] Phatraya Srabua[b] & Nuttanun Thawerattanon[a]*

[a] *Faculty of Home Economics Technology, Rajamangala University of Technology Krungthep, Bangkok, 10120 Thailand*
[b] *Scientific and Technological Research Equipment Center (STREC), Chulalongkorn University, Bangkok, 10330 Thailand*

Article info

Article history:
Received : 17 August 2022
Revised : 1 December 2022
Accepted : 9 December 2022

Keywords:
Scanning electron microscopy, Texture profile analysis, Thai rice, Wheat flour substitution

Abstract

Sangyod is a native rice of Thailand and is used in many types of food owing to its high nutrient value. Indeed, it is used in bakery products to increase their nutritional value. In this study the effect of using Sangyod rice flour as a substitute for wheat flour in butter cakes was assessed. The physical properties of the butter cakes were produced using Sangyod rice flour 50%, 75% and 100% (by wheat flour weight). Increasing the amount of Sangyod rice flour resulted in a higher density batter and cake but lower specific volume. Scanning electron microscopy revealed that the microstructure of the butter cakes contained fewer air bubbles when the amount of Sangyod rice flour was increased, due to Sangyod rice not containing gluten, which traps air bubbles. Regarding the texture profile, the hardness and chewiness of the butter cakes were significantly higher when they were made with Sangyod rice rather than the control recipe ($p \leq 0.05$). Furthermore, when the amount of Sangyod rice flour in the butter cakes was increased, the L* and b* values decreased and the a* value increased significantly ($p \leq 0.05$).

Introduction

Sangyod rice (*Oryza sativa* L.) is a local rice from Phatthalung Province in the southern region of Thailand and the first rice variety that has been registered as a geographical indication product of Thailand. Sangyod rice grains and the rice grains of other varieties are different, with the seed of the former possessing a reddish-white or dark-red seed coat. Furthermore, Sangyod rice possesses higher quantities of protein, dietary fiber, iron, niacin and phosphorus than other rice varieties as well as higher antioxidant and vitamin B1 and B2 levels. Sangyod rice is high in niacin, oryzanol and γ-aminobutyric acid (GABA), which prior research found to reduce the risk of cancer and prevent Alzheimer's disease (Phantuwong, 2017). Thus, Sangyod is a native rice variety with a high nutritional value (Ministry of Agriculture and Cooperatives, Rice Department, Division of Rice Research and Development, 2016). The products obtained from the

* Corresponding Author
e-mail: nuttanun.t@mail.rmutk.ac.th

rice milling process consisted of 62% whole kernels, 1.5% bran, 29.2% husks and 7.3% broken rice. Broken rice is classified as a low-priced product owing to its unacceptable characteristics from the perspective of consumers. However, it has a high nutritional value (Bunrat, 2016). Therefore, processing broken rice into products will increase the value of agricultural raw materials in the southern region of Thailand (National Bureau of Agricultural Commodity and Food Standards, 2017). Currently, processed Sangyod rice is used as a raw material in the production of various foods, including Kanom Kleeb Lamduan (Nooniam & Wongsudaluk, 2014) and other baked goods such as cookies (Wangpankhajorn et al., 2020), crackers (Sumkam et al., 2019) and brownies (Somboondumrongkul et al., 2016). Bakery products are popular in Thailand (Ministry of Industry, National Food Institute, 2020) and the main raw material in such products is wheat flour, which is accompanied by ingredients such as sugar, eggs, fresh milk, baking powder, salt and butter. Although wheat flour is an important ingredient in bakery products, Thailand only cultivates small amounts of wheat and the quality is unsuitable for such products. Therefore, Thailand imports 9.5 billion baht worth of wheat flour annually from other countries (Soontrunnarudrungsri, 2002). Importantly, wheat flour is less nutritious than Sangyod rice and the consumption of gluten-containing foods such as wheat flour triggers hypersensitivity reactions. These include wheat allergy and celiac disease.

In this study, butter cakes were made by processing the broken kernels of Sangyod rice into flour, which was then used as a substitute for wheat flour that would otherwise need to be imported. Moreover, as Sangyod rice flour is half the price of wheat flour and is a domestically cultivated raw material, its use in bakery products could help reduce production costs. In addition, Sangyod rice flour will add nutritional value to the bakery products.

Materials and methods

1. Preparation of Sangyod rice flour

Broken Sangyod rice from Phatthalung Province was used in this study. The size of the rice was reduced using a coarse grinder and then a fine grinder (Retsch, ZM 200, GmbH & Co., Germany). The resulting Sangyod rice flour was sifted through a 100-mesh sieve (Sinchaipanit et al., 2017) and packed into a 160 μm thick nylon vacuum bag. The bag was sealed with heat and a vacuum to prevent the accumulation of moisture and development of a rancid smell, after which it was stored at room temperature (30°C ± 2°C).

2. Preparation of butter cakes using a standard recipe

The standard recipe for butter cakes used in this study is shown in Table 1 (Sodchuen, 2017). An electric oven was preheated to 175°C and an aluminum pan (9.0 × 17.0 × 5.5 cm) was greased and lined with baking paper. In a large bowl, flour, salt and baking powder were whisked together. In a stand mixer (KitchenAid, Heavy duty 5KPM5, Whirlpool Corporation, USA) fitted with a paddle attachment, butter, sugar, salt and emulsifier were mixed at medium speed until fluffy before adding eggs, one at a time, which were beaten between each addition. Half the flour was added to the mixture and stirred to combine, after which half the milk was added and stirred. This process was repeated with the remaining flour and milk. Each batter formulation (190 g) was placed on a greased aluminum pan and baked in the electric oven at 170°C for 20 min. After baking, the cakes were removed from the pans, cooled on a wire rack for 30 min at room temperature and placed in plastic bags to prevent drying before their physical properties were measured.

Table 1 Standard butter cake recipe

Ingredients	Amount (g)
Butter	350
Sugar	300
Emulsifier (EC 25 K)	30
Salt	3
Vanilla flavoring	15
Egg	420
Wheat flour	300
Baking powder	10
Fresh milk	112

3. Cake formulation

As stated previously, Sangyod rice flour was used as a substitute for wheat flour in the butter cakes at three levels (50%, 75% and 100% according to wheat flour weight). The standard butter cake recipe was used as a control for assessing the physical properties of the cakes.

4. Physical properties of batter and butter cakes

4.1 Batter density

Batter density (Gómez et al., 2010) was analyzed by weighing the batter against the weight of pure water

in the same container and calculated using the ratio of batter weight to pure water volume (Eq. 1).

$$\text{Batter density } (g/cm^3) = \frac{\text{Batter weight (g)}}{\text{Pure water volume } (cm^3)} \quad \text{(Eq. 1)}$$

4.2 Cake specific volume

Cake volume was analyzed by substituting sesame seeds, according to the AACC method 10-05 (AACC., 2000), and weighing the butter cake. It was calculated using the ratio of cake volume to cake weight (Eq. 2).

$$\text{Cake specific volume } (cm^3/g) = \frac{\text{Cake volume } (cm^3)}{\text{Cake weight (g)}} \quad \text{(Eq. 2)}$$

4.3 Cake density

Cake density was analyzed by substituting sesame seeds, according to the AACC method 10-05 (AACC., 2000), and weighing the butter cake. It was calculated using the ratio of cake weight to cake volume (Eq. 3).

$$\text{Cake density } (g/cm^3) = \frac{\text{Cake weight (g)}}{\text{Cake volume } (cm^3)} \quad \text{(Eq. 3)}$$

4.4 Cake texture

The texture of the butter cake (Gómez et al., 2010) was analyzed using the TA-XT2 texture analyzer (Stable Microsystems, Surrey, UK). The texture profile was analyzed using a cylindrical probe [diameter, 25 mm (P/25)]. The probe was set to press on the sample at a speed of 2 mm/s and it was pressed at 50% of the sample height. The first and second presses were performed 30 sec apart. After cooling the cakes at room temperature for 3 hr, the samples were cut from the center to a size of 4 × 4 × 2 cm (width × length × height). The results were reported as hardness, springiness, cohesiveness and chewiness.

4.5 Scanning electron microscopy

Scanning electron microscopy analysis was conducted using a JEOL JSM-6610LV (Tokyo, Japan). The samples were cut from the center to a size of 0.5 × 1.5 × 0.5 cm (width × length × height) and placed in a glass vial. Acetone was then added to the vial until it completely flooded the sample. This was repeated three to four times for 2 hr for each sample. The samples were then dried and placed in a fume hood for 2 hr, after which they were stored in a desiccator. The samples were removed from the desiccator, mounted on a specimen stub (with the cross-section facing up) using double-sided tape and coated with a thin layer of gold.

4.6 Color measurement

Color was measured using an UltraScan VIS spectrophotometer (Hunter Associates Laboratory, Inc., USA). The instrument was standardized using a light trap and white plate. Results are expressed in the CIE L* a* b* color space in the reflection mode. Color was determined three times on each cake center, with each cake analyzed in three separate locations three times. The three points were positioned in the center of the cake and in the center of four imaginary sectors, for which the cake was divided along the diameter.

5. Statistical analysis

Values reported in tables are the averages of triplicate measurements ($\bar{x}$) ± standard deviation. A completely randomized design was used in this study. ANOVA and Duncan's multiple range test were used to determine statistical significance at $p \leq 0.05$ via the statistical analysis program SPSS version 21 (IBM Corp., Chicago, USA).

Results and discussion

1. Physical properties of batter and butter cake made from Sangyod rice flour

The physical properties of batter and butter cake produced using 50%, 75% and 100% Sangyod rice flour (as a substitute for wheat flour), with a standard butter cake recipe used as the control, are shown in Table 2 and Fig. 1. When higher amounts of Sangyod rice flour were used, batter density and cake density increased but cake specific volume decreased ($p \leq 0.05$).

Table 2 Physical properties of batter and butter cakes made using Sangyod rice flour

Physical properties	Sangyod rice flour substitution			
	0% (Control)	50%	75%	100%
Batter density (g/cm^3)	0.75 ± 0.04^c	0.77 ± 0.01^{bc}	0.80 ± 0.05^b	0.90 ± 0.45^a
Cake specific volume (cm^3/g)	15.3 ± 1.00^a	14.5 ± 1.00^{ab}	14.3 ± 1.00^b	12.7 ± 1.00^b
Cake density (g/cm^3)	0.06 ± 0.04^b	0.07 ± 0.04^b	0.08 ± 0.10^{ab}	0.10 ± 0.10^a

Remark: Different superscript lowercase letters in the same row indicate a statistically significant difference ($p \leq 0.05$)

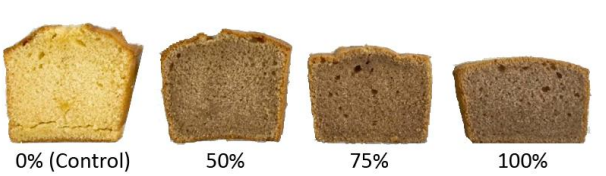

Fig. 1 Butter cake produced using Sangyod rice flour, as a substitute for wheat flour, at four distinct levels (left to right): 0%, 50%, 75% and 100%

The low density or specific gravity of batter determines its ability to hold air (Tinakorn Na Ayutthaya et al., 2018). When the springiness of gluten is reduced, the batter cannot not be fully stretched to retain the air that expands during baking. Increasing the amount of Sangyod rice flour dilutes the amount of gluten, thereby decreasing the ratio of gluten in the batter and interfering with gluten network formation during mixing, which results in a disorganized gluten arrangement. Consequently, the air storage capacity is reduced (Tinakorn Na Ayutthaya et al., 2018). In butter cakes made with Sangyod rice flour, the amount of gluten was insufficient to retain the expanded air during baking. Thus, the specific volume of the cakes decreased but their density increased.

The reduction in the volume and specific volume of the butter cakes was associated with an increase in density, which occurred when the amount of Sangyod rice flour in the cakes was increased. Volume, specific volume and density indicated the amount of air retained in the final product. In addition, because Sangyod rice flour lacks gluten, the structure of the butter cake was not sufficiently stable to support the formed structure (Hera et al., 2012). This finding was consistent with that of Premprasopchok et al. (2014), who studied the effects of brown rice flour as a substitute for wheat flour on the physical and sensory properties of black sesame carrot cake. The produced black sesame carrot cake had a low-volume and significantly increased density.

2. Texture profile analysis and microstructure

Low-volume baked goods have a hard texture as they contain less air, whereas high-volume baked goods contain more air, resulting in a softer texture. Moreover, low-volume cakes have a hard and dense texture (Aydogdu et al., 2018). Therefore, when more Sangyod rice flour was used to make the butter cakes, the hardness was significantly increased ($p \leq 0.05$; Table 3), with 100% Sangyod rice flour resulting in the hardest cake. A high hardness value indicates that a high amount of force is required to deform the product. The hardness of the cakes was due to their reduced gluten ratio i.e. the cakes with 100% Sangyod rice flour contained less air and were less flexible than the other cakes. Weenuttranon (2018) who observed an increase in hardness when wheat flour was replaced with Sangyod rice flour. However, using less than 25% Sangyod rice flour, there was no statistically significant difference.

Regarding the chewiness of the butter cakes, when the Sangyod rice flour substitution rate was higher, chewiness was significantly increased ($p \leq 0.05$).

Table 3 Texture profile analysis of butter cake made using Sangyod rice flour

Texture profile	Sangyod rice flour substitution (%)			
	0% (Control)	50%	75%	100%
Hardness (g)	6,035±26.86^{d}	6,597±136.41^{c}	7,545±78.15^{b}	8,140±71.30^{a}
Springiness (mm)	0.65±0.02^{b}	0.65±0.05^{b}	0.80±0.01^{a}	0.83±0.09^{a}
Cohesiveness	0.36±0.02^{c}	0.40±0.02^{b}	0.41 ± 0.03^{b}	0.45±0.05^{a}
Chewiness (g)	1,467±117.84^{b}	1,811 ± 156.76^{b}	2,576 ± 410.84^{a}	2,986 ± 60.74^{a}

Remark: Different superscript lowercase letters in the same row indicate a statistically significant difference ($p \leq 0.05$)

A higher chewiness value indicates that it is more difficult to crush, deform and chew the sample to the extent that it can be swallowed. The Sangyod rice flour in the butter cakes gave a hard texture with increased density, which also increases the chewiness. This finding is consistent with those of Aydogdu et al. (2018) and Gómez et al. (2010), who studied the effect of dietary fiber on cake quality, finding that an increase in dietary fiber significantly increased the hardness and chewiness of the cake.

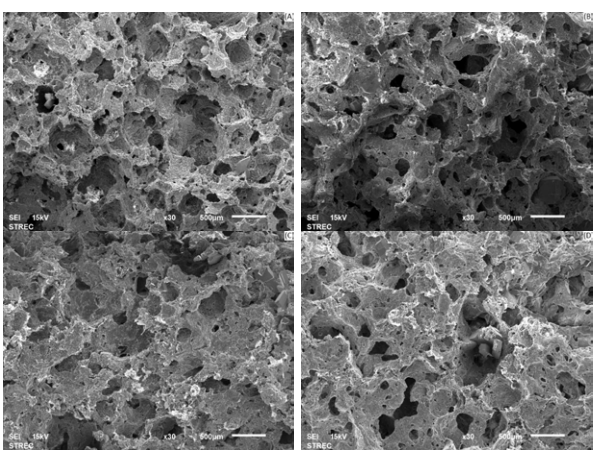

Fig. 2 Scanning electron microscope–derived images of the microstructure of butter cakes produced using Sangyod rice flour, as a substitute for wheat flour, at four distinct levels: (A) 0%, (B) 50%, (C) 75% and (D) 100%

Fig. 2 shows the microstructure of the butter cakes substituted with different amounts of Sangyod rice flour, which differentially affected the porosity and pore size of the butter cakes. For example, the control cake (0% Sangyod rice flour) was highly porous with large pores due to the air-retaining properties of gluten, which expands and increases in volume when heated during baking. When protein binding is complete, the air cells no longer expand and the baked product stops swelling. Most of the water that binds to the protein during mixing is released and evaporated or absorbed by starches. When the protein structure is completely coagulated, the

finished baked product can retain its shape. Thus, the structure of baked goods appears porous and sponge-like. However, replacing wheat flour with Sangyod rice flour deprives the butter cake of its ability to form gluten and store air. Therefore, its texture is compact and lacks porosity. This is shown in Fig. 2B–D, in which the cavities of the butter cakes made with Sangyod rice flour are smaller and fewer in number relative to those of the control cake.

3. Color parameters

The baking process reduces the brightness of a cake (Lang et al., 2020). Indeed, browning occurs during baking due to the melanoidins from the browning reaction (also known as the Mallard reaction) involving amino acids and proteins (Maua et al., 2017), whereas other pigments are due to the oxidation of phenolic compounds. In the butter cakes, increased amounts of Sangyod rice flour produced a darker cake because the flour contains anthocyanin, a flavonoid pigment that gives red, purple and blue colors and antioxidants that can prevent cardiovascular disease, cancer and diabetes (Castañeda-Ovando et al., 2009).

Table 4 Effects of the percentage of Sangyod rice flour substitution on the color characteristics of butter cakes

Color	Sangyod rice flour substitution			
	0% (Control)	50%	75%	100%
L*	83.0 ± 0.63^{a}	58.8 ± 0.70^{b}	54.7 ± 0.77^{c}	50.7 ± 1.57^{d}
a*	3.25 ± 0.14^{b}	7.46 ± 0.25^{a}	7.56 ± 0.18^{a}	7.58 ± 0.32^{a}
b*	27.5 ± 0.39^{a}	13.6 ± 0.67^{b}	10.9 ± 0.41^{c}	8.6 ± 0.60^{d}

Remark: Different superscript lowercase letters in the same row indicate a statistically significant difference ($p \leq 0.05$)

As shown in Table 4, increasing the amount of Sangyod rice flour in the butter cakes increased their redness (a*) but decreased their brightness (L*) and yellowness (b*), indicating that butter cakes made using Sangyod rice flour were darker with more distinct shades of red, likely due to the formation of intramolecular bonds in proanthocyanidin caused by oxidation. Anthocyanin is a monomer that decomposes into chalcones; when chalcones are heated, they degrade into brown products.

Conclusion

In the present study, using 50% Sangyod rice flour as a replacement for wheat flour in butter cakes did not significantly alter the batter density, cake specific volume, cake density, springiness and chewiness relative to the control recipe. However, using Sangyod rice flour produced some unpleasant effects, such as an increase in cake hardness and darkness. The present results suggest that Sangyod rice flour could be used to make other baked goods that add additional economic value to local agricultural products. In future studies, the improvement in the quality of butter cakes made with 100% Sangyod rice flour e.g. in terms of their consumer acceptance, sensory evaluation, physical properties, should be determined.

Acknowledgment

The researchers would like to extend their gratitude for the support given by Rajamangal University of Technology Krungthep, Thailand.

References

AACC. (2000). *Approved methods of the American Association of cereal chemists* (10th ed.). St. Paul, Minnesota, USA: American Association of Cereal Chemists International.

Aydogdu, A., Sumnu, G., & Sahin, S. (2018). Effects of addition of different fibers on rheological characteristics of cake batter and quality of cakes. *Journal of Food Science and Technology, 55*(2), 667–677.

Bunrat, N. (2016). *Quality of Sungyod rice during storage and its instant drink production* (Master's Thesis). Songkhla, Thailand: Prince of Songkla University.

Castañeda-Ovando, A., Pacheco-Hernandez, L., Páez-Hernández, E., Rodríguez, J.A., & Galán-Vidal, C.A. (2009). Chemical studies of anthocyanins: A review. *Food Chemistry, 113*(4), 859-871.

Gómez, M., Moraleja, A., Oliete, B., Ruiz, E., & Caballero, P.A. (2010). Effect of fibre size on the quality of fibre-enriched layer cakes. *LWT – Food Science and Technology, 43*(1), 33–38.

Hera, E.D.L., Ruiz-París, E., Oliete, B., & Gómez, M. (2012). Studies of the quality of cakes made with wheat-lentil composite flours. *LWT - Food Science and Technology, 49*(2012), 48-54.

Lang, G.H., Kringel, D.H., Acunha, T.S., Ferreira, C.D., Dias, A.R.G., Zavareze, E.R., & Oliveira, M. (2020). Cake of brown, black and red rice: Influence of transglutaminase on technological properties, in vitro starch digestibility and phenolic compounds. *Food Chemistry, 318*(2020), 126480.

Maua, J.L., Lee, C.C., Chen, Y.P., & Lin, S.D. (2017). Physicochemical, antioxidant and sensory characteristics of chiffon cake prepared with black rice as replacement for wheat flour. *LWT - Food Science and Technology, 75*(2017), 434-439.

Ministry of Agriculture and Cooperatives, Rice Department, Division of Rice Research and Development. (2016). *Rice knowledge bank.* Retrieved June 22, 2021 from http://webold.ricethailand.go.th/ rkb3/title-index.php-file=content.php&id=93.htm

Ministry of Industry, National Food Institute. (2020). *Bakery product 2019*. Retrieved December 19, 2021 from http://fic.nfi. or.th/ConsumerBehaviorDomesticDetail.php?id=33#

National Bureau of Agricultural Commodity and Food Standards. (2017). *Thai agricultural standard: Thai rice (TAS 4004-2017)*. Retrieved January 31, 2021 from https://www.acfs.go.th/standard/download/Thai-Rice _60.pdf

Nooniam, T., & Wongsudaluk, W. (2014). *Utilization of Sang – Yod rice flour in Thai shortbread cookies*. Retrieved July 31, 2021 from http://172.17.40.11/ebook/pages/title_detail.php?title_code=1344

Phantuwong, N. (2017). *Functional and biological properties of sang yod rice bran hydrolysate prepared by enzymatic hydrolysis and its application in rice pudding product* (Doctor of Philosophy Thesis). Songkhla, Thailand: Prince of Songkla University.

Premprasopchok, T., Kingvi, P., & Cheunjit, P. (2014). *Effects of wheat flour substitution with Sinin brown rice flour on physical and sensory qualities of black sesame carrot cake*. Paper presented at the 52nd Kasetsart University Annual Conference: Agro-Industry, Bangkok, Thailand. Retrieved January 15, 2021 from https://kukr.lib.ku.ac.th/kukr_es/index.php?/ BKN/search_detail/result /13722

Sinchaipanit, P., Budpong, K., Disnil, S., & Twichatwitayakul, R. (2017). Influences of rice berry flour as a wheat flour substitute in brownie: Textural and quality attributes. *SDU Research Journal Science and Technology, 10*(2), 69-79.

Sodchuen, N. (2017). *Basic bakery*. Lampang, Thailand: Lampang Vocational College.

Somboondumrongkul, A., Luekhajon, P., & Srisaya, J. (2016). *The use of Sungyot rice flour in Brownie.* Retrieved January 24, 2021 from https://www.repository.rmutsv.ac.th/bitstream/handle/123456789/331/Fulltext.pdf?sequence=1&isAllowed=y

Soontrunnarudrungsri A. (2002). *Development of butter cake from fragrance rice* (Master's Thesis). Bangkok: Kasetsart University.

Sumkam, P., Chanrit, T., & Wisutthipaet, S. (2019). Development of Sangyod rice (*Oryza sativa* L.) cracker. *Journal of Thai Food Culture, 1*(1), 29–38.

Tinakorn Na Ayutthaya, K., Phota, K., & Nanta, N. (2018). Effect of wheat flour substitution with brown rice flour on sponge cake's qualities. *Journal of Food Health and Bioenvironmental Science, 11*(3), 6-11.

Wangpankhajorn, P., Maneechot, S., Junsi, M., Jannu, T., & Samakradhamrongthai, R.S. (2020). Effect of substitution of wheat flour with Sungyod rice flour on physical and sensory qualities in cookies. *Journal of Science and Technology, 9*(2), 63-70.

Weenuttranon, J. (2018). *Product development of bread with Sangyod rice flour substituted for wheat flour*. [Paper presentation]. The 2018 ICBTS International Academic Multidisciplines Research Conference in Vienna, Vienna, Austria.

Journal of Food Health and Bioenvironmental Science
Journal homepage : http://jfhb.dusit.ac.th/

Quality and Consumer Acceptance of Ready to Drink Horse Mango (*Mangifera foetida* Lour.) Juice

Dudsadee Sapbua*, Thippika Thorarit & Saowapan Palasuwan

Program of Culinary Technology and Service, School of Culinary Arts, Trang Center, Suan Dusit University, Trang, 92130 Thailand

Article info

Article history:
Received : 1 November 2022
Revised : 15 December 2022
Accepted : 19 December 2022

Keywords:
Horse mango, Fruit juice, Consumer acceptance, *Mangifera foetida* Lour., Beta-carotene

Abstract

Horse mango (*Mangifera foetida* Lour.) fruit is rich in nutrition and is characterized by soft pulp-like mango that is suitable to prepare for ready-to-drink juice. The purpose of this study was to examine the physical properties, chemical properties, microbiological test, sensory evaluation and consumer acceptance test of the horse mangoes' pulp and juice. Ready-to-drink Horse mango juice with 3 concentrations of pulp (20, 40 and 60%) was pasteurized and poured into glass bottles. The results showed that the physical properties of Horse mango pulp were observed at L*(68.78), a*(-13.24), b*(50.26), pH (3.37) and Total Soluble Solids (15.17 ^{o}Brix). The content of chemical quality of Horse mango pulp reported carbohydrate, protein, fat, ash, vitamin C per 100 g of pulp as 12.67 g, 0.74 g, 0.48 g, 0.95 g, 29.98 mg, respectively and beta-carotene as 0.86 mg/kg. In reference to the Horse mango juice, the result of the coloring content was shown as b* values (yellowness) increased with a higher amount of Horse mango pulp related to beta-carotene (the yellow pigment). The microbiological content of the 3 concentrations of Horse mango juice aligned to the Thai Community Product Standard (TCPS) 701/2557 because of pasteurization (90 °C 10 min) during the juice productions. The ready-to-drink at 40% of Horse mango juice received the highest score on all attributes (color, odor, flavor, taste and overall liking) and was analyzed for chemical content; carbohydrate, protein, fat, ash, vitamin C per 100 g of juice as 21.50 g, 2.27 g, 0.04 g, 0.33 g, 10.29 mg, respectively and beta-carotene content as 0.61 mg/kg. For the acceptance of 100 consumers, consumer accepted 70% of juice products and decided to buy ready-to-drink Horse mango juice products at 66%. However, many consumers are still unfamiliar with Horse mango therefore adding another juice for example, pineapple and orange juices could obtain more acceptance.

* Corresponding Author
e-mail: dudsadee_sap@dusit.ac.th

Introduction

Thailand is a Southeast Asian country located in the tropics which has a great diversity of fruits (Baimai, 2010). Horse mango (*Mangifera foetida* Lour.) is a plant belonging to the Anacardiaceae family and is in the genus *Mangifera,* the same as mango (*Mangifera indica* L.). Horse mango is a native plant native to Southeast-Asian countries which is found in several countries such as Thailand, Malaysia and Indonesia. For Thailand, it is known as mamujt or malamut or maa-chang or ma chae and in Malaysia and Indonesia as bacang or limus or macang (Orwa et al., 2009). Horse mango fruit is oval-shaped and the peel is green in color and changes to yellow when ripe while the flesh is orange-yellow with a sour and sweet flavor. Horse mango has a strong unique smell and a rough texture (Wong & Ong, 1993). Horse mango fruit is indicated to have rich nutrients and phytochemicals such as carbohydrate (17.9 %), protein (0.8%), vitamin C content (47.4 mg/100 g), calcium (16 mg/100 g), phosphorus (19 mg/100 g), thiamine (0.09 mg/100 g), carotenes (0.255 mg/100 g) and antioxidant capacity (31.53 – 97.30%) (Tyug et al., 2010; Ikram & Khairul, 2009). Mostly, Horse mango is grown in southern Thailand and immature fruit is used as an ingredient for traditional food like yellow curry and salads "Yum Ma-Mut". The ripe fruit can be eaten fresh and to a lesser extent to process for other products. However, previous studies revealed the products from horse mango fruit as horse mango powder product, horse mango fiber products, horse mango jam and horse mango sherbet ice cream (Tyug et al., 2010; Palasuwan & Sapbua, 2020; Nuwongsri et al., 2021). A prior report showed that 55% of horse mango jam received the best on color, odor, flavor and overall liking and 97% of consumers accepted the jam while 76% of consumers decided to buy horse mango jam (Palasuwan & Sapbua, 2020). The consumer acceptance is important to note that the method of the study allows for evaluating from the consumer point of view about new products that a high level of acceptance leads to rationalization, causing consumers to be more likely to approve of new products (Bos et al., 2013).

Fruit juice is the liquid contained in fruit which is prepared by squeezing fruit flesh. The fresh fruit juice is safe for drinking within a day but when the juice is heated or pasteurized, it can prolong product shelf-life by inhibiting microbial and enzyme activity (Deak, 2014). In addition, fruit juices are an important source of vitamins and minerals (Islam et al., 2015). After the COVID-19 Pandemic, the trends in fruit juice consumption reported that fruit juice consumption increased to support health and immunity. During the 10-year period (2012 – 2021), fruit juice products were produced in Thailand and sold second in the world after China (Trade Policy and Strategy Office, Ministry of Commerce, 2022). The report revealed carotenoids boost immunity facing COVID-19 and related symptoms (Khalil et al., 2021) According to research the advantage of vitamin C as a primary prevention of COVID-19 due to vitamin C being essential for the proper functioning of the immune system (Colunga Biancatelli, et al., 2020). Therefore, the consumption of fruits or fruits juice which have carotenoids and vitamin C has positive affects for health. Ready-to-drink horse mango juice is another alternative for consumers who are concerned about health. The development of ready-to-drink horse mango juice adds value to local fruits which are highly nutritious as well as helping to conserve local plants. Thus, the objective of this study was to examine the physical properties, chemical properties, microbiological test, sensory evaluation of the horse mango pulp and Horse mango juice and consumer acceptance test for commercial usage in the future.

Materials and methods

1. Plant materials preparation

Horse mango used in this study were sampled from local markets and farms located in Trang, Phatthalung, and Nakhon Si Thammarat provinces in Southern Thailand. Fruits were collected in size (8-10 cm.) and at physiological maturity (20% yellow peel) shown in Fig. 1. The whole fruit was washed under running tap water to remove dirt and insects, peeled and washed again to clean Horse mango pulp from rubber. The Horse mango pulp was cut into small pieces by a knife and

Fig. 1 The characteristics of horse mango's 20 % yellow peel and pulp

blended with a blender (HR2195, PHILIPS, China) until smooth and filtered by 60-mesh sieve to become pulp for drinking.

2. Preparation of Horse mango juice

Ready-to-drink Horse mango juice was subjected to different levels of concentration 20%, 40% and 60% and the ingredients are presented in Table 1. The process of juice from Horse mango pulp are shown in Fig. 2.

Table 1 The ingredients of horse mango juice

Ingredient (g)	Horse mango juice		
	20 %	40%	60%
Horse mango pulp	20.0	40.0	60.0
Water	65.4	45.4	25.4
Sugar	14.4	14.4	14.4
Citric acid	0.2	0.2	0.2

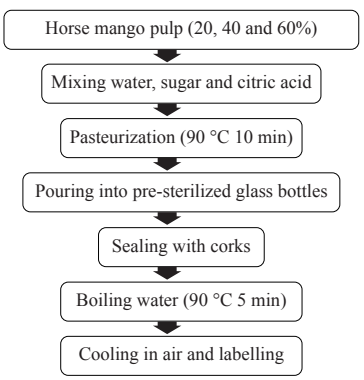

Fig. 2 The process of horse mango juice

3. Quality of Horse mango pulp and Horse mango juice

3.1 Physical properties

3.1.1 Determination of color

Reflected color measurement of pulp was measured using Colorimeter (Minalta CR-410 Series, Konica Minolta, lnc., Japan). For all 3 levels of Horse mango juice was performed using Colorimeter (Minalta CR-410 Series, Konica Minolta, lnc., Japan) in combination with the sample holder CR-A505 and specimen holder CM-A96 and a glass cell 10 mm CM-A98.This method followed by Guzel-Seydim et al. (2021) by The CIELAB L* a* b* system. Results were reported as an average of individual values as L* (lightness), a* (+a =red, -a = green) and b*(+b = yellow, -b = blue).

3.1.2 Determination of TSS

Total soluble solid (TSS) of Horse mango used a hand refractometer (ATAGO MASTER-M, China) (AOAC., 2000).

3.1.3 Determination of pH

The pH value of Horse mango was measured using the digital pH meter (Seven Compact, Mettler Toledo, Switzerland).

3.2 Chemical quality

3.2.1 Determination of carbohydrate, protein, fat and ash content

The major compound content of Horse mango pulp as protein, fat and ash were determined by AOAC (2016), official method 920.152, 954.02 and 923.03, respectively and carbohydrate was determined by method of Sullivan & Carpenter (1993).

3.2.2 Determination of vitamin C content

Determination of ascorbic acid or vitamin C content was modified from Furusawa & Kishida (2001) and Mazurek & Jamroz (2015). Each standard ascorbic acid solution was prepared using 10 mg, mixed in 100 mL of distilled water and using the HPLC mobile phase for dilution of the desired solution. HPLC system was equipped with a photo-diode array detector (Shimadzu, Kyoto, Japan), Josco PU-980 HPLC pump (Jasco, Tokyo, Japan) and a column thermostat. Separations were made using a column (250 x 4.6 mm^2 i.D.). The mobile phase was set as flow rate of 1.0 mL/min at room temperature. The injection volume was 20 µL using 1 mL of a sample and the samples are expressed as vitamin C.

3.2.3 Determination of Beta-carotene content

Beta-carotene content was analyzed by high-performance liquid chromatography (HPLC) with a diode-array detector (DAD) (HPLC-DAD) (Britton et al., 2004). Two grams of horse mango samples were homogenized and ethanolic KOH was added for saponification. After that, the samples were extracted with hexanes, hexanes were washed with water for 2-3 times until neutralize, then dried with a vacuum on the water bath and reconstituted with buffer solution. The samples solution was measured for beta-carotene by HPLC-DAD. The beta-carotene content was calculated from the area under the peak of the absorbance and compared with the standard solution.

3.3 Microbiological test

Microbiological test of Horse mango juice was determined according to Thai Community Product Standard; TCPS (ICS 67.160.20) by total plate count, *Salmonella* spp., *Staphylococcus aureus, Bacillus cereus, Clostridium perfringens* and yeast and mold by FAD

BAM, *Escherichia coli* and coliform by MPN (Maturin & Peeler, 2001).

3.4 Sensory evaluation

The sensory evaluation of horse mango juice was performed using the 9-point hedonic scale. Fifty untrained panelists evaluated their preference of quality attributes including color, odor, flavor, taste and overall liking. The 9-point hedonic scale was used as 9 like extremely, 8 like very much, 7 like moderately, 6 like slightly, 5 neither dislike nor like, 4 dislike, 3 dislike moderately, 2 dislike very much and 1 dislike extremely (Resurreccion, 1998).

4. Consumer acceptance test

The best score of the horse mango juice from the sensory test was evaluated for consumer acceptance test using the 5-point hedonic scale in terms of color, odor, taste and overall liking (Lawless & Heyman, 2010). The 100 consumers were chosen randomly at Huay Yod District, Trang Province.

5. Statistical analysis

Completely randomized design (CRD) was performed to study the quality of Horse mango pulp and Horse mango juice on the physical and chemical properties. The experiments were done in triplicate. The randomized completely block design (RCBD) was performed to study the quality of Horse mango juice on sensory properties. The results were presented as mean values ± standard deviations (S.D.). The statistical analysis used SPSS software (SPSS Version 17; SPSS Inc., Chicago, USA). Analysis of variance (ANOVA) with Duncan's New Multiple's Range Test (DMRT). The differences were determined to be statistically significant at $p < 0.05$.

Results and discussion

1. Quality of Horse mango pulp and Horse mango juice

1.1 Quality of Horse mango pulp

The appearance of Horse mango pulp appeared a yellow color when it was ripening, the texture of Horse mango pulp was similar to the mango pulp as illustrated in Fig. 3 and the physical properties of Horse mango pulp are shown in Table 2. The pH value and the TSS content of Horse mango pulp were 3.37 and 15.17 °Brix, respectively. According to previous studies, the pH value and the TSS content of pulp of six mango varieties were in the range of 3.33-4.75 and 11.90 – 17.06 °Brix (Bekele et al., 2020). The color expressed L*(lightness), a*(redness) and b*(yellowness) value as 68.78, -13.24 and 50.26, respectively. The Horse mango pulp appeared yellow because of the ripening process of the fruit. The chlorophyll (green) was degraded while the carotenoids (yellow) exposed more vivid (Sriwimon & Boonsupthip, 2011).

The chemical properties of horse mango pulp reported as carbohydrate, protein, fat, ash, vitamin C and beta-carotene content as 12.67 g/100 g, 0.74 g/100 g, 0.48 g/100 g, 0.95 g/100 g, 29.98 mg/100 g and 0.86 mg/kg, respectively (Table 2). In a prior study, bacang or Horse mango (*Mangifera foetida*) grown in Malaysia was analyzed with 100 g of edible portion, it contained carbohydrate, protein, fat, ash, vitamin C and carotene content as 17.9, 0.8, 0.21, 0.66, 0.047 and 0.0003 g, respectively (Tyug et al., 2010; Salma et al., 2008). In this study, vitamin C and beta-carotene content of Horse mango pulp reported lower than the prior study due to the variation of growing condition of each country such as climate, soil, water and light exposure that influence the antioxidant component (vitamin C and beta-carotene) (Ikram & Khairul, 2009). Thepyotin et al. (1999) reported vitamin C and beta-carotene content of *Mangifera indica* L. cv. 'Mahachanok' mango pulp as 0.469 g/100 g and 7.830 µg/g which is more than this study's vitamin C and beta-carotene content of horse mango pulp because it is a different species of fruit although it is a same genus of planet.

Fig. 3 Characteristics of Horse mango pulp

Table 2 Physical and chemical properties of horse mango pulp

Quality	Quantity
Physical	
Total soluble solid (°Brix)	15.17 ± 0.29
pH	3.37 ± 0.12
L*	68.78 ± 0.21
a*	-13.24 ± 0.99
b*	50.26 ± 0.97
Chemical	
Carbohydrate (g /100g)	12.67 ± 0.06
Protein (g /100g)	0.74 ± 0.03
Fat (g /100g)	0.48 ± 0.04
Ash (g /100g)	0.95 ± 0.02
Vitamin C (mg /100g)	29.98 ± 0.34
Beta-carotene (mg /kg)	86.00 ± 0.0

Table 3 Physical and chemical properties of Horse mango juice

Horse mango juice	Physical properties			Chemical properties	
	L*	a*	b*	Vitamin C (mg /100g)	Beta-carotene (mg/kg)
Horse mango 20%	70.80 ± 0.68[a]	-1.95 ± 0.05[a]	15.3 ± 0.46[c]	3.13 ± 0.13[c]	0.48 ± 0.01[c]
Horse mango 40%	61.97 ± 0.53[b]	-2.10 ± 0.08[ab]	27.43 ± 0.26[b]	10.29 ± 0.04[b]	0.61 ± 0.01[b]
Horse mango 60%	56.21 ± 0.31[c]	-2.31 ± 0.22[b]	35.93 ± 0.08[a]	17.35 ± 0.05[a]	1.08 ± 0.01[a]

Remark: The results were expressed as average ± standard deviation. The difference letters represented significant difference at $p < 0.05$

1.2 Quality of horse mango juice

1.2.1 Physical and chemical properties

The physical and chemical properties of horse mango juice are shown in Table 3, lightness (L* values) decreased and yellowness (b*) increased which were significantly different at $p < 0.05$ with a higher concentration of Horse mango pulp. Beta-carotene content of all Horse mango juice increased with a higher amount of Horse mango pulp and was significantly different at $p < 0.05$. This result was similar to the result of Thepyotin et al. (1999) study on consumer acceptance and quality of ready-to-drink Mahajanaka mango juice. In that study the result showed mango juice had beta-carotene content and it increased with a higher amount of mango pulp. The content of beta-carotene related to b* values (yellowness) since the yellow pigment in fruit pulp was carotenoid (Sriwimon & Boonsupthip, 2011). The vitamin C content of juice with Horse mango pulp at 20% 40% 60% were 3.13, 10.29 and 17.35 mg /100g, respectively. This result infers that as added Horse mango pulp increased in the juice then the vitamin C content also increased. However, the vitamin C content of all Horse mango juice is lower than the fresh Horse mango fruits (29.98 mg /100g). Ascorbic acid or vitamin C is known to be sensitive to heat treatment. This was found when the fresh Chokanan mango juice showed ascorbic acid as 8.91 mg/mL and when heat was transferred to the juice the ascorbic acid was 3.10 mg/ml (Santhirasegaram et al., 2013). In this study, the Horse mango juice was treated with heat (90°C 10 min) to increase the ascorbic acid losses. In addition, vitamin C is beneficial to human health because it is an antioxidant that prevents humans from oxidative stress (Stevens et al., 2007).

1.2.1 Microbiological examination

Microbiological examination of Horse mango juice was inspected according to Thai Community Product Standard (TCPS) 701/2557. The result showed that the amount of micro-organism in all 3 concentrations of the pasteurized horse mango juices products were not exceeding the standard (Table 4).

Table 4 Microbiological quality of Horse mango juice

Microbiological	TCPS standard 701/2557	Horse mango juice		
		20%	40%	60%
Total plate count (CFU/mL)	Less than 1	Less than 1	Less than 1	Less than 1
Salmonella (CFU/mL)	Not detected	Not detected	Not detected	Not detected
Staphylococcus aureus (CFU/mL)	Less than 1	Less than 1	Less than 1	Less than 1
Bacillus cereus (CFU/mL)	Less than 1	Less than 1	Less than 1	Less than 1
Clostridium perfringens (CFU/mL)	Less than 1	Less than 1	Less than 1	Less than 1
Escherichia coli (Per 100 mL)	Not detected	Not detected	Not detected	Not detected
Coliform (Per 100 mL)	Less than 1	Less than 1	Less than 1	Less than 1
Yeast and mold (CFU/mL)	Less than 1	Less than 1	Less than 1	Less than 1

1.2.2 Sensory test

Sensory evaluation of Horse mango juice on color, odor, flavor, taste and overall liking and the final score of 50 panelists are illustrated in Table 5. The Horse mango juice that received the highest score for all attributes of juice was the juice at 40%. However, there was no significant difference among all concentrations. As observed from the sensory score, the flavor attribute received the lowest score for all attributes due to the unique smell of Horse mango that affected the flavor liking.

Table 5 Sensory test of Horse mango juice

Sensory attribute	Horse mango juice		
	20 %	40%	60%
Color[ns]	6.9 ± 1.4	6.9 ± 1.6	6.8 ± 1.6
Odor[ns]	6.9 ± 1.6	7.2 ± 1.6	6.9 ± 1.6
Flavor[ns]	6.7 ± 1.6	6.9 ± 1.8	6.8 ± 1.6
Taste[ns]	7.2 ± 1.8	7.2 ± 1.5	7.1 ± 1.5
Overall liking[ns]	7.2 ± 1.9	7.3 ± 1.6	7.2 ± 1.1

Remark: The results were expressed as average ± standard deviation. ns represented non-significant difference at $p < 0.05$

2. Consumer acceptance test

2.1 Information of Horse mango juice with 40%

The best concentration of Horse mango juice from the sensory test was 40% (Fig. 4). So, this concentration of juice was used in the next step for the consumer acceptance test. The 40% Horse mango juice products were analyzed for chemical content; moisture, carbohydrate, protein, fat, ash, vitamin C and beta-carotene content as 77.90 g/100g, 21.50 g/100g, 2.27 g/100g, 0.04 g/100g, 0.33 g/100g, 10.29 mg/100g and 0.61 mg/kg, respectively (Table 6). The beta-carotene content of juice (0.61 mg/kg) slight decreased compared to the beta-carotene content of fresh Horse mango (0.86 mg/kg) due to the pigment stability of beta-carotene that decreased with high temperature (Thakur, 2018). According to research regarding fresh Chokanan mango juice which showed carotenoid content as 82.03 µg/100 mL but when heat was transferred to the juice then the carotenoid content became 48.92 µg/100 mL (Santhirasegaram et al., 2013). Beta-carotene is a natural antioxidant related to reduce risk of cancer and is a precursors of vitamin A (Grune et al., 2010).

Fig. 4 Characteristics of 40% drink Horse mango juice

Table 6 Chemical properties of 40% Horse mango juice

Chemical quality	Quantity
Moisture (g /100g)	77.90 ± 0.0
Carbohydrate (g /100g)	21.50 ± 0.01
Protein (g /100g)	2.27 ± 0.01
Fat (g /100g)	0.04 ± 0.01
Ash (g /100g)	0.33 ± 0.01
Vitamin C (mg /100g)	10.29 ± 0.04
Beta-carotene (mg/kg)	0.61 ± 0.01

2.2 Consumer acceptance test

The 40% Horse mango juice was tasted for acceptance by 100 consumers (47 men and 53 women) who accepted the unique smell of Horse mango juice. Most of the consumers were aged under 20 years old (25%) and between the ages of 20-30 years (25%), most had education levels below a bachelor's degree (45%). While 34% were students that had an average monthly income less than or equal to 10,000 baht (41%). Most consumers decided to buy juice products with taste attributes (66%), frequency of drinking juice 1-2 times a week (36%) and bought juice products at department stores (32%) and convenience stores (32%).

The results of consumer acceptance scores on color, odor, taste and overall liking were equal to 4.1, 4.0, 4.0 and 4.0 as shown in Table 7 and which all attributes had a high level of liking. Consumer acceptance at 70% of Horse mango juice products and 66% of consumers decided to buy Horse mango juice products. However, the consumers acceptance of Horse mango juice were lower than 80% that might be due to the strong and unique smell of Horse mango. Therefore, mixing another fruit juice such as pineapple and orange juices might improve the consumers acceptance. According to research the mixed fruit juice by 35% mango juice, 40% orange juice and 25% pineapple was the best consumer acceptance and showed the highest score on sensory evaluation (Begam et al., 2018). Currently, most consumers are still unfamiliar with fresh local mango fruits so adding well known fruit juice such as pineapple and orange juices that have rich nutrients is a positive method to promote the local fruit.

Table 7 Acceptance score of 40% of Horse mango juice

Sensory attribute	Score
Color	4.1 ± 0.7
Oder	4.0 ± 0.8
Taste	4.0 ± 0.7
Overall liking	4.0 ± 0.6

Remark: The results were expressed as average ± standard deviation

Conclusion

The Horse mango (*Mangifera foetida* Lour.) flesh revealed L*(68.78), a*(-13.24) and b*(50.26), pH (3.37), and Total Soluble Solids (15.17 ^{o}Brix) Carbohydrate, protein, fat, ash, vitamin C and beta-carotene content were 12.67 g/100 g, 0.74 g/100 g, 0.48 g/100 g, 0.95 g/100 g, 29.98 mg/100 g, and 0.86 mg/kg, respectively. Horse mango are underutilized fruits. Therefore, ready-to-drink Horse mango juice products are an alternative juice that has benefits for health. Beta-carotene of all concentrations of horse mango juice increased with a higher

amount of Horse mango pulp, the same as b* value (yellowness) of juice. The 40% Horse mango juice received the highest score on all attributes (color, odor, flavor, taste and overall liking) and this concentration of juice was analyzed for chemical properties: moisture, carbohydrate, protein, fat, ash and beta-carotene content as 77.90 g/100 g, 21.50 g/100 g, 2.27 g/100 g, 0.04 g/100 g, 0.33 g/100 g 10.29 mg/100 g and 0.61 mg/kg, respectively. Microbiological tests of the products passed the Thai Community Product Standard (TCPS) 701/2557 and 70% of consumer accepted the ready-to-drink Horse mango juice products. Most consumers are still unfamiliar with fresh local mango fruits so adding well known fruit juice such as pineapple and orange juices can aid in promoting the local fruits which have rich nutrients.

Acknowledgments

The authors would like to express gratitude to the Research and Development Institute, Suan Dusit University for funding support as well as the Trang Center, Suan Dusit University for providing the facilities for this research.

References

AOAC. (2000). *Official methods of analysis* (17th ed.). Washington, DC: Association of Official Analytical Chemists.

AOAC. (2016). *Official methods of analysis* (20th ed.). Washington, DC: Association of Official Analytical Chemists.

Baimai, V. (2010). Biodiversity in Thailand. *The Journal of the Royal Institute of Thailand, 2,* 107-118.

Begam, S., Das, P., & Karmoker, P. (2018). Processing of mixed fruit juice from mango, orange and pineapple. *Fundamental and Applied Agriculture, 3*(2), 440-445.

Bekele, M., Satheesh, N., & Jemal, S. (2020). Screening of Ethiopian mango cultivars for suitability for preparing jam and determination of pectin, sugar, and acid effects on physico-chemical and sensory properties of mango jam. *Scientific African, 7*, 1-10.

Bos, C., Van der Lans, I.A., Van Rijnsoever, F. J., & Van Trijp, H. (2013). Understanding consumer acceptance of intervention strategies for healthy food choices: A qualitative study. *BMC Public Health, 13,* 1073.

Britton, G., Liaaen-Jensen, S., & Pfander, H. (2004). *Carotenoids handbook (Vol.1B)*. Basel, Switzerland: Birkhauser Verlag.

Colunga Biancatelli, R.M.L., Berrill, M., Catravas, J.D., & Marik, P.E. (2020). Quercetin and vitamin C: An experimental, synergistic therapy for the prevention and treatment of SARS-CoV-2 related disease (COVID-19). *Frontiers in Immunology, 11,* 1451.

Deak, T. (2014). Chapter 17-thermal treatment A2-motarjemi, yasmine. In H. Lelieveld (Ed.), *Food Safety Management* (pp. 423–442). San Diego: Academic Press.

Furusawa, N, & Kishida, K. (2001) High-performance liquid chromatographic procedure for routine residue monitoring of seven sulfonamides in milk. *Fresenius J Anal Chem, 371*(7),1031-1033.

Grune, T., Lietz, G., Palou, A., Ross, A.C., Stahl, W., Tang, G., Biesalski, H.K. (2010). β-Carotene is an important vitamin a source for humans. *The Journal of Nutrition, 140*(12), 2268S–2285S.

Guzel-Seydim, Z.B., Seydim, A.C., & Greene, A.K. (2021). Effect of brewing method on quality parameters and antioxidant capacity of black tea. *Functional Food Science, 1*(8), 1-13.

Ikram, E., & Khairul, H. (2009). Antioxidant capacity and total phenolic content of Malaysian underutilized fruits. *Journal of Food Composition and Analysis, 22*(5), 388-393.

Islam, M., Morshed, S., Saha, S., Quader, F., & Alam, M. (2015). Evaluation of nutritive value of mango juices found in Bangladeshi markets. *Journal of Environmental Science and Natural Resources, 8*(1), 95–98.

Khalil, A., Tazeddinova, D., Aljoumaa, K., Kazhmukhanbetkyzy, Z.A., Orazov, A., & Toshev, A.D. (2021). Carotenoids: Therapeutic strategy in the battle against viral emerging diseases, COVID-19: An overview. *Preventive Nutrition and Food Science, 26*(3), 241–261.

Lawless, H.T., & Heymann, H. (2010). *Sensory evaluation of food Ithaca.* New York: Springer Science Business Media.

Maturin, L.J., & Peeler, J.T. (2001). Chapter 3 - Aerobic plate count. in Food and Drug Administration (FDA), *Bacteriological analytical manual online* (8th ed.). Retrieved January, 21, 2022 from https://www.fda.gov/food/laboratory-methods-food/bam-chapter-3-aerobicplate-count

Mazurek, A., & Jamroz, J. (2015). Precision of dehydroascorbic acid quantitation with the use of the subtraction method–validation of HPLC–DAD method for determination of total vitamin C in food. *Food Chemistry, 173,* 543–550.

Nuwongsri, S., Thorarit, T., & Sapbua, D. (2021) Effect of horse mango (*Mangifera foetida*) pulp on qualityand consumer acceptance of sherbet ice cream. *Journal of Food Health and Bioenvironmental Science, 14*(1), 20-25.

Onwa, C., Mutua, A., Kindi, R., Jamnadass, R. A., & Simon, A. (2009). *Agroforestree database: A tree reference and selection guide version 4.0.* Retrieved April 18, 2022 from http://www.worldagroforestry.org/af/treedb.

Palasuwan, S., & Sapbua, D. (2020). Development of horse mango (*Mangifera foetida*) jam and consumer acceptance. *Journal of Food Health and Bioenvironmental Science, 13*(2), 46-52.

Resurreccion, A.V.A. (1998). *Consumer sensory testing for product development.* Gaitherburg, MD: Aspen.

Salma, I., Kahdijah, A., Masrom, H., Mohd, N.A., & Halimah, S. (2008). *Underutilized fruit with potential: Bacang.* Poster presented at Conference: Biodiversity and National Development, Kuala Lumpur, Malaysia. DOI:10.13140/RG.2.2.12240.40968. Retrieved April 11, 2022 from https://www.researchgate.net/publication/325893594_Underutilized_fruit_with_potential_Bacang

Santhirasegaram, V., Razali, Z., & Somasundram, C. (2013). Effects of thermal treatment and sonication on quality attributes of Chokanan mango (*Mangifera indica* L.) juice. *Ultrasonics Sonochemistry, 20,* 1276–1282.

Stevens, R., Buret, M., Duffe, P., Garchery, C., Baldet, P., & Rothan, C. (2007). Candidate genes and quantitative trait loci affecting fruit ascorbic acid content in three tomato population. *The Journal of Plant Physiology, 143,* 1943–1953.

Sriwimon, W., & Boonsupthip, W. (2011). Utilization of partially ripe mangoes for freezing preservation by impregnation of mango juice and sugars. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 44*(2), 375-383.

Sullivan, D. and Carpenter, D. (1993) *Method of Analysis for Nutrition Labeling.* Arlington: AOAC International.

Thakur, N. (2018). Heat stability and antioxidant potential of beta-carotene isolated from a fungal isolate. *Bulgarian Journal of Agricultural Science, 24*(5), 891–896.

Thepyotin, W., Rungtrakulthai, W., & Bootphrom, S. (1999). Consumer acceptance and quality of ready to drink Mahajanaka mango juice. *RMUTP Research Journal Special Issue,* 5, 131-140.

Tyug, T.S., Johar, M.H., & Ismail, A. (2010). Antioxidant properties of fresh, powder, and fiber products of mango (*Mangifera foetida*) fruit. *International Journal of Food Properties, 13*(4), 682-691.

Wong, K.C., & Ong, C.H. (1993). Volatile components of the fruits of bachang (*Mangifera foetida* Lour.) and kuini (*Mangifera odorata* Griff.). *Flavour and Fragrance Journal, 8*(3), 147-151.

Journal of Food Health and Bioenvironmental Science
Journal homepage : http://jfhb.dusit.ac.th/

The Use of Fruit Yeast as a Substitute for Instant Yeast in Sweet Bread Products

Nuttanun Thawerattanon, Noppamas Poolcharoensil, Narissara Uthai, Rungtip Wongtom, Doungtip Kaikaw & Racharat Yampuang*

Faculty of Home Economics Technology, Rajamangala University of Technology Krungthep, Bangkok, 10120 Thailand

Article info

Article history:
Received : 20 August 2022
Revised : 18 December 2022
Accepted : 21 December 2022

Keywords:
Fruit yeast, Natural yeast, Sweet bread

Abstract

This study aimed to solve the problem of fruit wastage through the following objectives: (1) create usage for food waste materials from the food industry, (2) study the process of yeast fermentation from fruits and (3) study the physical properties of sweet bread. The ability to form yeasts was studied using a total soluble solid (TSS) (°Brix). TSS decreased, indicating the amount of yeasts formed. Fermented sugarcane contained more yeast than fermented apple based on the TSS of sugarcane and apple fermented broths on the third day, which decreased by 50.85% and 34.5% ($p \leq 0.05$), respectively, while instant yeast decreased by 79.2%. The culture broth yeast was used as a substitute for instant yeast in sweet bread products. The physical properties of the bread were studied by volume, specific volume, density and texture profile analysis. The use of yeast from sugarcane and apple had a decreased volume that resulted in a decrease in specific volume by an average of 13.29% and 28.33%, respectively and had more density and more rigid texture than instant yeast ($p \leq 0.05$). Thus, consumers who are allergic to instant yeast powder now have an alternative with the bread produced from fermented fruit yeast.

Introduction

Food waste includes leftover food from meals and industrial plants. This accounts for 1.3 billion tons of food waste annually or 30% of the world's food production (Gustavsson et al., 2011). In 2017, food wastage in Thailand was as high as 6.96 million tons (FAO, 2017) and fruits and vegetables comprised around 40%-50% of these losses. Currently, food waste from production is used to produce additional products. For instance, insect food (Halloran et al., 2017) and biofermented water (Chanvichit, 2019) are used as ingredients for animal food and bread product development from fruit waste (Horthong et al., 2014). Yeasts are organisms that can be beneficial or harmful to food. Yeast fermentation is critical in many foods, including bread, beer, wine and vinegar. The most widely used species is *Saccharomyces cerevisiae*. Bread yeast is used to flavor food, with some yeast extracts having a distinctive smell and high sodium content. Yeasts should only be eaten in small quantities (Horthong et al., 2014). Some yeast grows in cultured media. The

* Corresponding Author
e-mail: racharat.y@mail.rmutk.ac.th

production costs of yeast cultures are quite high and there are many necessary production steps. Instant yeasts are also expensive.

This study aimed to introduce natural yeast obtained from fruit fermentation. This involved using ripe fruits or fruit peels as a substitute for instant yeasts in sweet bread products. Prior research showed that fruit yeasts have the same ability to leaven bread as instant yeasts (Tsegaye et al., 2018). Bread made from fermented fruit yeasts is an alternative for consumers who are allergic to instant yeast powder. It increases the amount of probiotics in bread and reduces the cost of bread production.

Materials and methods

1. Preparation of the Bread Recipe

The ingredients to make a bread consisted of the following (Iampitakkit, 2019): bread flour (1,000 g, White swan, Thailand), evaporated milk (200 g, Carnation, Thailand), water (300 g), sugar (125 g, Mitrphol, Thailand), butter (175 g, Orchid, Thailand), gluten (20 g, Bangkok flour & food trading, Thailand), instant yeast (15 g, Fermipan, Canada), salt (15 g, Prungthip, Thailand), emulsifier (10 g, Bacom A100, Bakels, Malaysia) and two eggs. The ingredients were kneaded in a dough-kneading machine for 20 min. The prepared dough was made into 40 g, round buns and stored in a temperature-controlled cabinet at 35°C for 75 min. The buns were then baked at 170°C for 20 min.

2. Fruit yeast preparation

Yeast fermentation was studied using two fruits: bagasse and apple. The fruits were prepared by washing and trimming the rotten parts before soaking in water for 24 h and then dried. Raw materials were prepared for fruit yeast fermentation. The water, fruit and sugar ratios for fermentation were 50:25:8, respectively. Water and sugar were placed in a sterilized jar. Then, the total soluble solids (TSS) were measured at 20 °Brix, adding fruits. Finally, the jar was shaken and moved to a temperature-controlled cabinet set at 32°C for three days.

3. Study of yeast fermentation

Fruit yeast was used to study the characteristics of yeast produced by yeast count using the spread plate technique on the surface of sterile potato dextrose agar. The potato dextrose agar was incubated at 37°C for two days to study the morphology of colony growth on solid food cell shape, following the method of Barnett et al. (2000). Next, the amount of yeasts produced was determined by measuring the TSS in comparison with soluble solids in the initial solution. Comparatively, the amount of TSS decreased and bubbles formed in the container.

4. Physical properties of sweet bread made from fruit yeast

The use of fruit yeast as a substitute for instant yeast in sweet bread was studied. Culture broth yeasts were replaced by all water and instant yeasts in sweet bread recipes (100%). Then, the physical properties of natural yeast bread were studied. Finally, it was compared with instant yeast bread.

4.1 Bread specific volume

The volume was analyzed by replacing sesame seeds, following the AACC method 10–05 (AACC., 2000). Sesame seeds were poured into an empty container (V1) to determine its capacity. Then, whole bread was placed inside it and sesame seeds were added to the remaining space in the container (V2). A graduated cylinder was used to calculate V1 and V2. The difference between the two was used to determine the bread volume. The specific volume was calculated from the bread volume to bread weight ratio (Eq. 1).

$$\text{Specific Volume } (\text{cm}^3/\text{g}) = \frac{\text{Bread volume } (\text{cm}^3)}{\text{Bread weight (g)}} \quad \text{(Eq. 1)}$$

4.2 Bread density

Product density was analyzed by replacing sesame seeds, following the AACC method 10–05 (AACC, 2000) and weighing the bread. Density was calculated from the bread weight to bread volume ratio (Eq. 2).

$$\text{Density } (\text{g}/\text{cm}^3) = \frac{\text{Bread weight (g)}}{\text{Bread volume } (\text{cm}^3)} \quad \text{(Eq. 2)}$$

The texture of bread was analyzed using texture profile analysis (TPA) according to the method of Huttner & Arendt (2010).

Huttner & Arendt (2010) analyzed the texture of bread using texture profile analysis (TPA). A 100-mm diameter cylindrical probe (P/100) was used. A sample size of 25 × 25 × 25 mm (width × length × height) was cut. The operating conditions of the machine were set to a pre-test speed of 1.00 mm/s, test speed of 1.00 mm/s, post-test speed of 1.00 mm/s and distance of 40% of its original height. Hardness, cohesiveness, gumminess and chewiness were reported.

5. Statistical analysis

A completely randomized design was used in the analysis of variance and Duncan's new multiple range

tests. A confidence level of 95% was considered. The statistical analysis program SPSS V. 21 (IBM Corp., Chicago, USA) was used to run the analyses.

Results and discussion

1. The study of yeast morphology

Yeast counts of 1.8×10^4 and 1.3×10^4 colonies were found in sugarcane and apples, respectively. The colonies that grew on the solid surface were round, convex and had a smooth surface, smooth edges, opaque white color and elliptical shape with unilateral arrangement (Fig. 1). This unilateral arrangement was predominantly observed in yeast strains *S. cerevisiae* and consistent with the results obtained by Salem et al. (2016), which showed that yeast cells could be seen under a 40 magnification microscope. However, in addition to *S. cerevisiae*, other microorganisms that contaminate with the fruits during fermentation may also be presented.

Fig. 1 Yeast colonies (left to right: instant yeast, sugarcane yeast and apple yeast)

2. Sugar utilization of various fruit yeasts

Fermentation happens naturally in any sugar-containing mash made from fruits. When exposed to a warm environment, airborne yeasts converts sugar into carbon dioxide and ethyl alcohol (Saranraj et al., 2017). The amount of yeast from different fruits are shown in Table 1. Instant yeast had the highest significant reduction in TSS (°Brix), followed by sugarcane and apple, which decreased by 50.85% and 34.5%, respectively. The TSS of instant yeast was reduced by 79% on the third day, indicating that sugarcane could produce more yeast than apples. On days 1–3, instant yeast, sugarcane and apple showed significantly different decreases in TSS ($p \leq 0.05$). This was because yeast uses sugar as an energy source. Sugar can be converted into carbon dioxide and ethyl alcohol during fermentation. However, from day three onwards, there was no statistically significant decrease in TSS ($p > 0.05$) since the three fruit yeast types began to convert to alcohol on day four. This was observed as foaming on the surface of the yeast solution. However, fruits can generate less yeast than instant yeast because the latter is purer compared to fruit yeast, which is an injured cell. Therefore, it uses less sugar than instant yeast.

Table 1 Utilization of yeast sugar from three different sources

Day	Total soluble solid (°Brix)		
	Instant yeast	Sugarcane yeast	Apple yeast
1	12.40 ± 0.40^{aC}	15.57 ± 0.25^{bC}	18.40 ± 0.20^{cC}
2	8.10 ± 0.20^{aB}	11.57 ± 0.40^{bB}	15.47 ± 0.25^{cB}
3	4.16 ± 0.15^{aA}	9.83 ± 0.30^{bA}	13.10 ± 0.30^{cA}
4	3.90 ± 0.30^{aA}	9.10 ± 0.20^{bA}	12.70 ± 0.40^{cA}
5	3.20 ± 0.40^{aA}	8.95 ± 0.45^{bA}	12.14 ± 0.20^{cA}

Remark: Superscripts in English letters (A–C) in the same column indicate a significant difference ($p \leq 0.05$)
Superscript in English letters (a–c) in the same row indicates a significant difference ($p \leq 0.05$)

3. Study of the physical properties of bread

The results are shown in Fig. 2, Tables 2 and 3. It was found that fruit yeast reduced the bread volume and as a result the specific volume of bread. Density increased ($p \leq 0.05$) compared to instant yeast bread because fruit produces less yeast compared to instant yeast (Table 1).

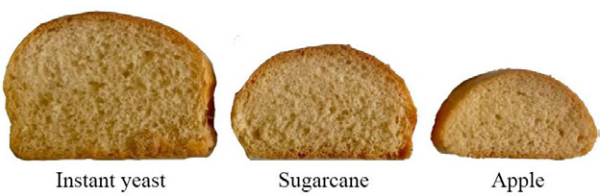

Fig. 2 Sweet bread produced using different yeasts (left to right: instant yeast, sugarcane yeast and apple yeast)

Table 2 Physical properties of sweet bread made from fruit yeasts

Physical Properties	Type of yeast		
	Instant yeast	Sugarcane yeast	Apple yeast
Volume (cm^3)	394.33 ± 4.04^{a}	336.66 ± 3.21^{b}	274.33 ± 3.05^{c}
Specific volume (cm^3/g)	4.85 ± 0.07^{a}	4.27 ± 0.03^{b}	3.53 ± 0.02^{c}
Density (g/cm^3)	0.21 ± 0.03^{c}	0.25 ± 0.03^{b}	0.29 ± 0.04^{a}

Remark: Superscripts in English letters (a–c) in the same row indicate a statistically significant difference ($p \leq 0.05$)

In baked goods, yeasts are responsible for raising the product through fermentation. Sugar is used as an energy source and carbon dioxide is produced in the process. Air expands at higher temperatures during fermentation. The gluten from kneading the mixture expands and stretches to envelop those gases, thus making the product rise. When heated in an oven, yeast

stops working and the resulting alcohol evaporates during baking (Gisslen, 2016). However, if there is insufficient yeast, the bread will not increase as it should. Using fruit yeasts as a substitute for all instant yeasts in sweet bread products reduces breads volume and as a result the specific volume. The volume and specific volume of bread made with instant yeast, sugarcane yeast and apple yeast were 394.33/4.85, 336.66/4.27 and 274.33/3.53, respectively. This is because fruit yeasts are injured cells. Fermentation efficiency is reduced and the ability to use sugar decreases. As a result, the ability to generate carbon dioxide is reduced accordingly and bread will rise less. Apple yeasts had the lowest specific volume due to insufficient yeast production during bread production. When used in bread production, the yeast was less capable of emitting carbon dioxide, reducing the rise in bread. Due to small air cavities, the bread was very dense after baking.

Bread density is inversely related to volume and specific volume. The density of a bread increases when its volume and specific volume are reduced. The bread made with apple yeasts was the densest, corresponding to a more significant hardness, gumminess and chewiness that were directly related to each other. Thus, when the hardness and gumminess of bread increased significantly ($p \leq 0.05$), it was harder to chew.

Different types of yeast resulted in different amounts of yeast and varying bread quality. It decreased the bread volume and specific volume but increased the density, affecting the texture profile in terms of increasing hardness, gumminess and chewiness (Table 3). This corresponds to decreased texture profile test scores.

Table 3 Texture profiles of fruit yeast bread

Texture	Type of yeast		
	Instant yeast	Sugarcane yeast	Apple yeast
Hardness (N)	66.95 ± 2.43^{a}	76.87 ± 6.73^{b}	143.57 ± 1.77^{c}
Springiness ^{ns}	0.69 ± 0.23	0.79 ± 0.28	0.88 ± 0.01
Cohesiveness	0.71 ± 0.01^{a}	0.75 ± 0.06^{b}	0.80 ± 0.02^{c}
Gumminess	44.04 ± 0.75^{a}	60.57 ± 1.62^{b}	103.33 ± 2.40^{c}
Chewiness	37.2 ± 1.88^{a}	40.32 ± 17.22^{b}	90.83 ± 2.47^{c}

Remark: Superscripts in English letters (a–c) in the same row indicate a significant difference ($p \leq 0.05$)
ns means that there was no significant difference ($p > 0.05$)

Conclusion

This study examined fruit yeasts as a substitute for instant yeasts in sweet bread products by comparing sugarcane yeast and apple yeast with instant yeast as a control recipe. Sugarcane yeasts produced significantly more yeast than apple yeast ($p \leq 0.05$) based on the amount of TSS reduced. The bread made with sugarcane and apple yeasts had less bread volume and specific volume, but was denser compared with bread from instant yeast. This was due to fermented fruit yeasts cannot make the bread rise as much as instant yeasts. Consequently, the bread produced from sugarcane and apple yeasts was dense, gummy and chewy. However, the study of microscopy and biochemistry should be performed in the future.

Acknowledgment

This research was supported by Rajamangala University of Technology Krungthep, Thailand.

References

AACC. (2000). *Approved methods of the American Association of cereal chemists* (10th ed.). St. Paul, Minnesota, USA: American Association of Cereal Chemists International.

Barnett, J.A., Payne, R.W., & Yarrow, D. (2000). *Yeasts: Characteristics and identification* (6th ed.) Cambridge: Cambridge University Press.

Chanwichit, P. (2019). The effects of bio-extract and chemical fertilizer on growth and yield of spinach (*Spinacia oleracea* L.) under a hydroponic growing system. *Naresuan Phayao Journal, 12*(3), 51-54.

FAO. (2017). *New food balances.* Retrieved June 21, 2021 from https://www.fao.org/faostat/

Gisslen, W. (2016). *Professional baking* (6th ed). New Jersey, USA: John Wiley & Sons.

Gustavsson, J., Cederberg, C., Sonesson, U., Van Otterdijk, R., & Meybeck, A. (2011). *Global food losses and food waste.* Retrieved May 17, 2021 from https://www.fao.org/3/i2697e/i2697e.pdf

Halloran, A., Hanboonsong, Y., Roos, N., & Bruun, S. (2017). Life cycle assessment of cricket farming in north-eastern Thailand. *Journal of Cleaner Production, 156,* 83-94.

Huttner, E.K., & Arendt, E.K. (2010). Recent advances in gluten-free baking and the current status of oats. *Trend in Food Science and Technology, 21*(6), 303-312.

Horthong, T., Jumponsatien, T., & Wannarak, T. (2014). *Bread form fruit yeast.* [Unpublished manuscript]. Special Project in Foods and Nutrition, Rajamangala University of Technology Krungthep.

Iampitakkit, N. (2019). *Bakery.* Bangkok: Bangkok Vocational Training Center (Wat Worachanyawas).

Salem, A.Z.M., Kholif, A.E., & Puniya, A.K. (2016). *Yeast additive and animal production.* Services. Occidental, India: PubBio Med Central Research Publishing Services.

Saranraj, P., Sivasakthivelan, P., & Naveen, M. (2017). Fermentation of fruit wine and its quality analysis: A review. *Australian Journal of Science and Technology, 1*(2), 85-97.

Tsegaye, Z., Tefera, G., Gizaw, B., & Abatenh, E. (2018). Characterization of yeast species isolated from local fruits used for bakery industrial application. *Journal of Applied Microbiological Research, 1*(1), 21-26.

Journal of Food Health and Bioenvironmental Science
Journal homepage : http://jfhb.dusit.ac.th/

Crisp Bhutan Oyster Mushroom (*Pleurotus pulmonarius*): A Potential Innovative Product for Commercialization

Ubol Chuensumran[a] & Att Khunsee[b]*

[a] *Division of Food Processing Technology, School of Culinary Arts, Suan Dusit University, Bangkok, 10300 Thailand*
[b] *Division of Culinary Technology, School of Culinary Arts, Suan Dusit University, Lampang Center, Lampang, 52100 Thailand*

Article info

Article history:
Received : 27 November 2022
Revised : 26 December 2022
Accepted : 28 December 2022

Keywords:
Food innovation, Bhutanese Oyster mushrooms, Medicinal mushroom products

Abstract

This research was undertaken to create therapeutic mushroom products and disseminate knowledge to entrepreneurs. The researchers along with a community created product design and distribution channels and developed a brand for medicinal mushroom products based on the community's identity. Bhutanese oyster mushrooms were used to create mushroom products that consisted of Bhutanese oyster mushroom (88%), sugar (5%), vegetable oil (3%), soy sauce (2%), coriander seeds (1%) and salt (1%).The production method was conducted by to washing the mushrooms thoroughly, squeezing the water out and setting them aside; then mixing all ingredients together and dry in a hot air oven at 60°C for 90 min. Then the batter was fried in hot oil at 90-95°C for 5 min, and then placed in the oven at 150°C for 20 min to crisp and reduce the surface moisture of the product. The sensory preference (7.80 – 8.80 points) was at a high level. Moreover, the knowledge can be transferred to entrepreneurs to implement in real production. It is a product that has the potential to be marketed for distribution as a health food with an emphasis on semi-premium marketing which can be differentiated by demonstrating the value and nutrients of Bhutanese oyster mushrooms. This research included designing aesthetic, outstanding and unique packaging to create the identity for the community. Furthermore, a brand of medicinal mushroom products called "Hed Khik Khak or Giggling Mushrooms" was created. The feedback acceptance (99%) was at a high level. The product has been registered under the trademark for Klong Chik Sub-district Community Enterprise to be used with therapeutic mushroom products in the future.

Introduction

Bhutanese oyster mushrooms are similar to grey oyster mushrooms as both are able to adapt to the environment as well as resistant to a relatively wide temperature range. Bhutanese oyster mushrooms grows and blooms well, produces high yields, can be planted in every season and is popular among consumers and

* Corresponding Author
e-mail: Att_khu@dusit.ac.th

mushroom growers. According to this study's findings on a model mushroom farm the production rate consisted of 300-500 kilograms of fresh mushrooms per week that were usually sold in the market as fresh mushrooms. However, the shelf life of fresh oyster mushrooms is relatively short, causing a high rate of perishable fresh mushrooms and effecting the income of sellers to earn less. Therefore, there is a need to develop products from fresh mushrooms in order to add value and help generate income. Prior research on developing healthy food products from mushrooms focused on three types of mushrooms: shiitake, straw mushrooms and oyster mushrooms. It was found that oyster mushrooms increased the proportion of protein and dietary fiber while reducing the amount of carbohydrates (Chantarakan, 2012). It is important information for product development of oyster mushrooms. Furthermore, previous research on oyster mushrooms has developed a variety of food products such as shredded mushrooms, crispy oyster mushrooms and deep-fried oyster mushrooms. Products also include mushrooms that are processed into foods such as mushroom chili paste, mushroom juice and mushroom buns, which are considered fresh foods and have a short or limited shelf life. This research was undertaken in order to aid mushroom farmers to develop products made from mushrooms that have a long shelf life that could be sold as souvenirs and are unique to the community. The purpose of this study was to research and develop medicinal mushroom products and transfer the knowledge to entrepreneurs to acquire the capability to produce the products in actual production, suggest patterns and distribution channels for Medicinal Mushroom Products, design therapeutic mushroom products to be aesthetic, outstanding and unique to the community and create a brand of therapeutic mushroom products.

Materials and methods

1. The development of the traditional flavored therapeutic mushroom recipe(s)

The ingredients consisted of the following: Bhutanese oyster mushroom (88%), sugar (5%), vegetable oil (3%), soy sauce (2%), coriander seeds (1%) and salt (1%). The production method started by washing the mushrooms thoroughly and then squeezing the water out and set aside. Mixed the oyster mushroom meat, sugar, vegetable oil, soy sauce, coriander seeds and iodized salt together. The mixture was dried in a hot air oven at 50 - 60°C for 60, 90, 120 and 150 min. The dehydrated mushrooms were fried in hot oil at 90 - 95°C for 5 min. The cooked mushrooms were baked to make them crispy and reduce the moisture on the surface of the products. The samples were oven-dried at 150°C for 20 min.

The time taken to dry mushrooms before frying at 60 ± 2°C for 60, 90, 120 and 150 min were studied and the physical quality i.e. color value was analyzed. Color value was evaluated by Hunter lab colorimeter (Chroma Meter CR 300 Series, Japan) by measuring L* a* b* C* (Chroma) and h_o (Hue angle). The replication experiment was done twice. The values were evaluated 3 times and in each replication, 10 positions of color values were analyzed. Chemical quality analysis consisting of water activity value (a_w) was conducted. a_w was randomly sampled from the dried shredded mushrooms. The water activity value (a_w) was measured by using the water activity meter (Aqua Lab, USA). The total moisture content (Infrared Moisture Analyzer, MA150) was used to find the weight loss due to the evaporations of the water and volatile matter at the specified temperature by randomly selecting the fried and dried shredded mushrooms from the experimental samples (AOAC., 2000). Two replicates of the experiment were performed and each replicate was measured 3 times.

The dried therapeutic mushroom samples were analyzed at 60 ± 2°C, fried and dehydrated. The color values were analyzed by sampling the fried and dried shredded mushrooms. According to the mushroom samples, color values were analyzed with a Hunter lab colorimeter (Chroma Meter CR 300 Series, Japan) by measuring L* a* b* C* (Chroma) and h_o (Hue angle). The replication experiment was done twice. The values were evaluated 3 times. In each replication, 10 positions of color values were analyzed. The quality was analyzed, i.e. Water Activity (α_w) (Aqua Lab, USA). According to the mushroom samples, α_w was analyzed using a water activity meter. The fat value was evaluated for fat content analysis according to the method (AOAC., 2000). The amount of oil absorbed during frying reduction (% oil reduction) was calculated by comparing it with the fat content of shredded mushrooms (Kaikaew et al., 2016). Total moisture content (Infrared Moisture Analyzer, MA150) was conducted to find the weight loss due to evaporation of water and volatile matter within the specified temperature by randomly selecting the fried and dried shredded mushrooms from the experimental samples. Fat oxidation analysis was evaluated by the TBARS (Thio barbituric acid reactive

substances) method. Fried and dried shredded mushrooms were randomly sampled for lipid oxidation analysis by the TBARS (Thio barbituric acid reactive substances) method, adapted from Wei et al. (2011) and Wrostad et al. (2005) by measuring the amount of Malondialdehyde substance with Thiobarbituric acid (TBA). Absorbance was measured with a spectrophotometer (Libra S11 Biochrom, USA) at a wavelength of 532 nm, reported in milligrams of malondialdehyde per 1 g of each sample. Two replicates of the experiment were performed and each replicate was measured 3 times.

The selected food experts had experience and direct positions in food processing and developments from 10 community enterprises to assess the characteristics of the Medicinal mushroom products in the terms of appearance, color, scent, taste and texture by using the focus group method with the scaled scores from 1 to 9 (i.e. 1 is the most disliked and 9 is the most liked).

2. The study of the nutritional values (selected recipe(s))

A centrifuge was used to grind the chosen samples of shredded mushrooms. Then, using the contents of moisture, lipids, proteins, fiber, ash and carbohydrates were examined according to the experiment (AOAC., 2000) the experiments were done twice with each replicate being measured three times.

3. The study of consumer acceptance (selected recipe(s))

The selected shredded mushrooms samples (Bhutanese oyster mushroom (88%), sugar (5%), vegetable oil (3%), soy sauce (2%), coriander seeds (1%), and salt (1%) or the samples of the traditional flavored shredded mushrooms that had been fried and dehydrated for 90 min) were tested for consumer acceptance by the Central Location Test (CLT) method through the questionnaire which was conducted with 200 consumers (market test) using non-purposive sampling, that is, the research team recruited consumers who had consumed shredded mushrooms. They were designated as a sample group. Data Collection in Lampang Province was conducted in August 2021. According to testing the confidence of the questionnaire, it was discovered that Cornbrash's alpha was at 0.8816 (Cronbach, 1951). It was greater than 0.70. It infers that the questionnaire was reliable and could be used to collect data.

4. The study on shelf life (selected recipe(s)) after 6 months of storage

In the study of the shelf life of the product at the room temperature, the analyzed qualities were as follows: (1) physical quality such as color value, solubility and viscosity, (2) chemical quality such as pH value, a_w value, moisture content, (3) microbial quality such as total microbial count, numbers of yeasts and molds, *E. coli, S. aureus* and *Salmonella* spp. according to the method (AOAC., 1995).

5. The study of the packaging design and marketing channels of therapeutic mushroom products

Two parts were considered regarding package design: (1) Aesthetic aspect by including the identity of the community in order to make it stand out and recognizable, such as the local tree, local culture, etc. and selecting the color group and art components to suit the consumer group and (2) Functionality aspect by choosing materials that are suitable for products and food storage and designing packaging to make it easy to eat and clearly communicates the brand's value and uniqueness.

6. Statistical analyses

All experiments were triplicated, and the data was analyzed by using IBA SPSS Statistics for Windows version 20 (IBA, Armonk, NY, USA.). The differences between the values were considered significant at $p \leq 0.05$. The averages were calculated by Duncan's new multiple range test. The completely randomized design was applied for the determination of the physical quality and chemical properties of the mushroom recipe, while the randomized complete block design was applied for the sensory evaluation. All analyses were also executed by using IBM SPSS Statistic version 20 software.

Results and discussion

1. Researching and developing the medicinal mushroom products, and transferring knowledge to entrepreneurs to enable the capability of production

The ingredients are as follows: Bhutanese oyster mushroom 88%, sugar 5%, vegetable oil 3%, soy sauce 2%, coriander seeds 1% and salt 1%. The production method are as follows; wash the mushrooms thoroughly, squeeze the water out, set them aside, then take oyster mushroom meat, sugar, vegetable oil, soy sauce, coriander seeds and iodized salt and mix them together, dry in a hot air oven at 50-60°C for 60, 90, 120 and 150 mins. Take the dehydrated mushrooms and fry them in hot oil at 90-95°C for 5 min. Bake the cooked mushrooms to make them crispy and reduce the moisture on the surface of the product. The samples were oven-dried at 150°C for 20 min. The results of physical and chemical analysis of dried mushroom samples at 60 ± 2 °C are shown in Table 1.

Table 1 Results of physical and chemical analysis of dried mushroom samples at 60 ± 2 °C

Baking time (mins)	Water activity (α_w)	Humidity (% w.b.)	Color value (CIE-Lab)				
			L*^{ns}	a*	b*^{ns}	C*^{ns}	h^{ns}
60	0.79±0.01^a	44.49±1.56^a	54.62±1.84	3.95±0.55^c	12.20±1.25	17.71±0.50	65.80±0.84
90	0.58±0.01^b	28.00±0.17^b	51.92±1.11	6.11±0.31^b	12.93±0.41	18.03±0.11	66.83±1.46
120	0.55±0.00^b	24.62±0.33^c	51.19±1.88	6.28±0.25^a	12.49±0.37	17.82±0.45	67.45±1.49
150	0.55±0.02^b	22.22±0.40^c	51.49±1.20	6.40±0.55^a	12.85±0.40	17.92±0.60	67.58±1.20

Remark: Mean ± Standard Deviation was calculated from the analysis of two replicates
Different letters in columns indicate significant differences ($p \leq 0.05$)
ns means no significant difference ($p > 0.05$) in each column

From the results of samples prepared at the medicinal mushroom products preparation step to analyze the physical values after drying at 60°C for 60, 90, 120, and 150 min, respectively, it was found that samples dried for a long time had a higher amount of humidity and α_w were statistically significantly decreased. The samples incubated for 60 minutes had the highest 44.49% of moisture content and α_w of 0.79, which are the levels of water content that are easy for microbial growth and chemical changes, causing easy deterioration due to chemical reactions. The preservation process before frying the mushrooms is to store them in a sealed container that helps prevent cross-contamination during refrigeration to prevent spoilage and deterioration. The samples that had been incubated for 90, 120 and 150 mins had moisture content and α_w between 22.22 and 28.00 percent and 0.55 to 0.58, respectively; the values are the amount of free water in the food that is within the dry food standard. While waiting for the frying process, the mushrooms could be stored at room temperature. However, storing in a sealed container protects the mushrooms from oxygen humidity and light, and allows the raw material to be stored for a longer time during the frying process (Fellow, 2000).

When considering the color values (CIE-Lab) of the aforementioned samples, namely L*, a*, b* C*, and h, the results showed that with the longer drying time, the brightness (L*) value decreased which was inversely proportional to a redness (a*) due to a shift to more brown color which comes from the non-enzymatic browning reaction, that is, Maillard reaction; it is a reaction caused by the combination between reducing sugars and amino acids in mushrooms. The product was a ring derivative that polymerizes rapidly, yielding a nitrogen-containing and insoluble brown substance. This reaction needed to take place in the condition that had been heated with water at the α_w level greater than 0.20. This reaction not only resulted in browning and a reduction in lightness (L*) but also affected the scent and taste of the food in both positive and negative ways (Rattananon, 2008; Fennema, 1996). This reaction could also occur during storage, resulting in the darkening of the product. No significant changes were observed from the b*, C*, and h values.

The drying of mushroom samples were prepared at 60, 90, 120 and 150 min to fry in oil at 150°C until they were well-cooked. The physical and chemical values were then analyzed (Tables 2 and 3). The results showed that the samples fried for longer tended to have lower moisture content and α_w values. For the samples that were baked for 120, 90 and 60 mins. The moisture content was 11.29, 11.41 and 15.45% and the α_w values were 0.37, 0.38 and 0.39 respectively. In order to consider the α_w value compared to the standard of ready-to-eat seasoned mushroom community products, the experimental results showed that all 3 samples of

Table 2 Results of physical analysis of therapeutic mushroom samples dried at 60 ± 2°C and then fried and dehydrated

Baking time (min)	Water activity (a_w)	Humidity (% w.b.)	Color value (CIE-Lab)				
			L*^{ns}	a*	b*^{ns}	C*^{ns}	h^{ns}
60	0.39±0.01^a	15.45±0.12^a	32.89±1.44	17.14±0.27^b	12.99±1.34	18.64±0.81	41.98±0.20
90	0.38±0.00^a	11.41±0.04^a	30.91±0.49	18.46±0.14^a	13.50±0.72	19.45±1.29	42.56±0.79
120	0.37±0.01^b	11.29±0.01^b	30.64±b0.02	18.99±0.15^a	13.64±0.31	18.98±0.58	42.64±0.67
150	0.36±0.01^b	11.90±0.01^b	31.40±b0.05	18.89±0.18^a	12.84±0.52	18.65±0.55	42.44±0.87

Remark: Mean ± Standard Deviation was calculated from the analysis of two replicates
Different letters in columns indicate significant differences ($p \leq 0.05$)
ns means no significant difference ($p > 0.05$) in each column

therapeutic mushroom products were produced within the standard level, that is, having a_w value not more than 0.60, which the humidity at this level is a safe level for dry food due to its low chemical transformation and microbial growth (Rattananon, 2008) allowing the mushrooms to be stored at room temperature. It is a condition that reduces the burden of storage and transportation and is suitable for businesses without the requirement of high investment in product management.

Based on the values of colorimetric analysis (CIE-Lab), the results showed no difference in b*, C*, and h values. Only the illuminance (L*) values in the samples had a higher tendency when baked for a shorter time. This is inversely proportional to redness (a*) as when products were baked longer, redness (a*) value increased as a result of the change by non-enzymatic browning as in the baking process. However, differences appeared in the preparation step at temperature 60°C, resulting in the browning change by the Maillard reaction. In the frying process, the high heat (about 160°C) caused the Maillard reaction at the beginning of frying. When the temperature increases, the sugar in the sample will gradually change to brown. In this phenomenon, the water will be removed from the structure and there will be caramel formation in this process which helps improve the flavor of food products. However, if there is too much chemical reaction generated, it will cause mushrooms to have a bitter taste and burn. Moreover, there are reports that substances obtained from the process of caramel formation produce substances with antioxidant effects. If there is too much of this process, it can cause carcinogens. Hence, it is crucial to regulate the production process in order to be the most appropriate (Tolgahan & Vural, 2019; Cedric et al., 2021).

Table 3 Chemical analysis results of therapeutic mushroom samples dried at 60 ± 2°C and then fried and dehydrated

Baking time (minutes)	Oil reduction (% w.b.)	Oil reduction (% d.b.)	TBA (mg/kg)
60	48.24^{a}±6.94	52.91^{a}±7.54	0.48^{a}± 0.00
90	41.83^{ab}±2.11	44.34^{ab}±2.35	0.47^{a}± 0.00
120	26.62^{b}±4.92	27.74^{b}±5.27	0.45^{b}± 0.01
150	25.22^{b}±5.20	26.50^{b}±6.70	0.45^{b}± 0.01

Remark: Mean ± standard deviation was calculated from the analysis of two replicates
Different letters in columns indicate significant differences ($p \leq 0.05$)
ns means no significant difference ($p > 0.05$) in each column

When the samples were analyzed for oil content, it was discovered that the samples prepared with the shortest baking time of 60 min, had the highest oil reduction, followed by the samples baked at 90, 120 and 150 min, respectively. These results are consistent with the work of Rattanathammawat et al. (2003) which was caused by different initial water loss rates and different moisture content reduction rates (Sirilert & Silalai, 2016). During frying, water is lost from the product and replaced by the absorbed oil. These results varied with the trend of fat oxidation analysis results (Thiobarbituric acid, TBA). The oil content that was examined was abundant and entirely from the frying process. Lipid oxidation is accelerated by light during storage, high heat from frying, and oxygen concentration. More peroxides of aldehydes obtained from unsaturated fatty acids were obtained from unsaturated fatty acid, causing the mushroom to rapidly and easilybe rotten (Rattananon, 2008; Noiduang et al., 2015; Fennema, 1996) As a result, the product needs to be kept in a container that is sealed as a method to ensure no light penetration, oxygen, or water vapor and as a method to extend the product's shelf life.

For the sensory quality assessment, 10 food experts were used as testers. They all participate in community enterprises. This was to evaluate the characteristics of medicinal mushroom products in terms of appearance, color, scent, taste and texture using a focus group method by tasting 4 samples of therapeutic mushroom products (where 9 = most liked and 1 = most disliked). The results showed that 90 min of traditional flavored medicinal mushroom products that were fried and dehydrated had the highest overall liking preference score of 8.5 ± 0.4. Regarding the evaluation of the sensory quality test of the medicinal mushroom products dried at 60°C for 60, 90, 120 and 150 min, respectively, the results showed that samples that were dried longer time of 90 min had the characteristics of the medicinal mushrooms in the terms of appearance, color, smell, taste, texture and liking. The overall liking scores were 7.9±0.3, 7.9±0.5, 7.8±0.5, 8.8±0.8, 8.4±0.5 and 8.5±0.4, respectively. The flavored mushroom products had consistent colors and no rancid smell. The products had the aroma of the coriander seeds. The natural colors of the ingredients were used. The products had a sweet and salty taste. The texture was not sticky or hard. In addition, there are the recommendations for product developments from the experts. That is, the products should have light color in order to make it appealing. White sesame seeds and cannabis leaves should be added to the products in order to add more value and nutrients. The scores are shown in Table 4.

Table 4 Sensory quality assessment scores

Attributes	Samples of traditional flavored medicinal mushroom products that have been fried and dehydrated			
	1 (60 Min)	2 (90 Min)	3 (120 Min)	4 (150 Min)
Appearance	6.4±0.5^{c}	7.9±0.3^{a}	6.9±0.5^{b}	6.8±0.5^{b}
Color	6.8±0.8^{c}	7.9±0.5^{a}	6.8±0.3^{c}	7.3±0.5^{b}
Odor	7.1±0.7^{b}	7.8±0.5^{a}	6.9±0.4^{c}	7.0±0.5^{b}
Taste	7.1±0.8^{c}	8.8±0.8^{a}	7.7±0.4^{b}	7.2±0.6^{c}
Texture, such as soft, crispy	7.0±0.5^{c}	8.4±0.5^{a}	7.4±0.5^{b}	7.6±0.6^{b}
Overall liking	7.0±0.4^{d}	8.5±0.4^{a}	7.6±0.7^{b}	7.4±0.8^{c}

Remark: Differences letters in rows indicate significant differences ($p \leq 0.05$)

2. Nutrition analysis results (selected recipe(s))

The centrifuge was used to grind the chosen samples of medicinal mushroom products. Then, the (AOAC., 2000) technique was used to find the contents of moisture, fat, protein, fiber, ash and carbohydrate. The findings are shown in Table 5.

Table 5 Nutrient content test results

Test item	Result	Unit
Ash 3.75	g/100g	
Moisture	5.90	g/100g
Total Energy	450.90	Kilocalories/100g
Energy from fat	161.10	Kilocalories/100g
Total Fat	17.90	g/100g
- Total saturated fatty acid	7.58	g/100g
Cholesterol	Not Detected	mg/100g
Protein	7.30	g/100g
Total carbohydrate (include fiber)	65.15	g/100g
Total dietary fiber	12.85	g/100g
Total sugar	49.7	g/100g
Sodium (Na)	850	mg/100g
Calcium (Ca)	33.4	mg/100g
Iron (Fe)	1.94	mg/100g
Total Vitamin A	Not Detected	µg/100g
Vitamin B1	0.21	mg/100g
Vitamin B2	0.20	mg/100g

3. Consumer acceptance study results (selected recipe(s))

The selected shredded mushrooms samples were tested for consumer acceptance by the Central Location Test (CLT) through the questionnaires that were administered with 200 consumers (market test) by using the non-purposive sampling. It was found 67.50% (135 people) of the respondents were females and the majority were between the ages of 31 and 45, representing 34.50% (or 69 people). They had a bachelor's degree or above, making up 52.50% (or 105 people), 42.50 percent were business unit employees (85 people). According to the sample's occupation, their monthly income ranged between 3,001 and 10,000 baht (84 people). When considering the therapeutic mushroom purchasing behavior of consumers, it was discovered that 97 percent of respondents answered that they loved medicinal mushroom products, but almost half of them had a frequency of consumption only 1-2 times a month (42%), showing a small purchase volume.

Seventy-two Percent of consumers typically purchase 1 to 5 packs, whereas 74% of the sample group purchases medicinal mushroom products for personal use, followed by souvenir shopping. Few respondents purchased with the intention of resale. Therapeutic mushroom products are most often bought in convenience stores, followed by department stores, markets, souvenir shops, exhibition halls, direct producers and others such as internet or vegetarian eateries, respectively. The participants would purchase medicinal mushroom products based on taste and nutritional value at a similar level, followed by price, packaging, product brand, being recommended by someone to buy therapeutic mushroom products and marketing promotion, respectively. The marketing strategy for healthy drinks needs to focus on nutritional value. However, the taste also needs to be delicious. The price of the product must be suitable for the quality as well.

When evaluating factors that affect product purchases, it was found that the majority of the sample group approved of the products at the highest level of preference (The average score was 8.12). The majority of customers concentrated on safety and cleanliness with the highest preference level (the average score was 9.28). The taste was the second most important factor for consumers to buy at the highest level of preference (The score was 8.89). The findings of the testing on factors influencing the purchase of therapeutic mushroom products, however, received the most favorable level in all aspects. These results displayed how items have evolved and improved over time in the research as shown in Table 6.

Table 6 Factors affecting the purchase of therapeutic mushroom products

Attributes	Liking score
Color	7.67±2.29
Visual appearance	8.19±2.02
Taste	8.89±2.29
Odor	8.10±2.29
Texture	7.95±2.01
Packaging, Labeling	8.52±2.03
Price	8.36±1.82
Promotional advertising	7.83±2.31
Brand	7.89±2.49
Safety, Hygiene	9.28±1.42

When considering the characteristics that the participants liked from the therapeutic mushroom product tasting test, it was discovered that overall preference was at a high level. According to Table 7, they preferred the taste the most, followed by the appearance, scent, color and texture.

Table 7 Test results of therapeutic mushroom products

Sensory attributes	Liking score
Physical appearance	7.91±0.93
Colour	7.70±1.14
Scent	7.73±1.26
Taste	8.23±1.02
Texture	7.28±1.59
Total preference	8.12±0.98

Ninety-nine Percent of the sample group approved of the entrepreneurs' therapeutic mushroom products. For the price level that the sample group expected to pay for the item, more than half of the respondents selected the price of 35 baht per size of 40 grams, which was followed by 30 baht and 40 baht. The sample group believed that the pricing would be in line with similar products available in the market. Given the range of such pricing, the respondents may not yet be fully aware of the product's benefits. Within such a price range of 30-35 baht for each size of 40 grams, there is still a possibility in the market as shown in Fig. 1.

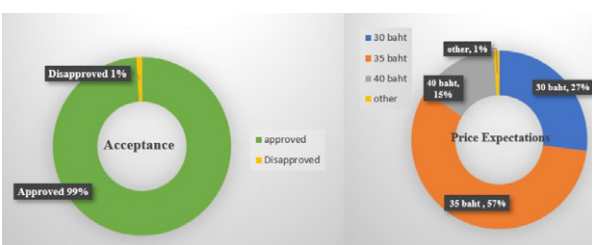

Fig. 1 Product acceptance and product price expectations of the sample group

4. Finding the product shelf life

The study of the product shelf life was conducted within 6 months and the product quality was analyzed. It was found that it was in accordance with the criteria specified by the law as shown in Table 8.

Table 8 Product quality test results after 6 months storage

Test Item	Result
Potassium sorbate	Not detected
Sodium benzoate	Not detected
Lead (Pb)	Not detected
pH	5.97
Water activity (at 25°C)	0.448 (threshold <0.6)
Net weight	40.06 g
Total Plate Count at 35°C	3.2 x 10^2 (Criteria Compliant) CFU/g
Coliforms	<3.0 (Criteria Compliant) MPN/g
Staphylococcus aureus	Not detected
Salmomella spp.	Not detected
Yeast and Molds	10 (Criterion 100) CFU/g

Consequently, the knowledge gained from the study and development of therapeutic mushroom products can be transferred to local entrepreneurs who have the ability to manufacture the products. The research team developed traditional flavored therapeutic mushroom products that resulted from the community participation of the community enterprise of Klong Chik Sub-district, Bang Pa-in District, Phra Nakhon Si Ayutthaya Province as souvenirs to generate income for the community and to create the community's identity.

5. The study of the packaging design and marketing channels of therapeutic mushroom products

An envelope made of aluminum foil was chosen for the package design to keep the product fresher and readily available for consumption. The front's graphic emphasizes the community enterprise's pride in the business that cultivates fresh Bhutanese mushrooms and turns them into medicinal mushroom products to be consumed with rice which is good for health and can be easily consumed by just opening the sachet.

A checklist to highlight the product's advantages was used in green and yellow vegetarian icons to convey the message directly to consumers who are health-conscious and added a premium look with a silver line. On the back, nutrition tables were arranged for easy reading and in order, including picking a font that is smooth and large. The icons and details were added to convey the details per category. The 'Hed Khik Khak or Giggling Mushrooms' logo is on the side which was designed to have natural tones with fun lined designs with a hidden smiley meaning to boost sweetness and create a memorable identity along with the amusing brand named 'Hed Khik Khak or Giggling Mushrooms' that includes numerous varieties which are to be invented in the future, as shown in Fig. 2.

Fig. 2 Therapeutic mushroom products

In terms of the branding, the trademark of therapeutic mushroom products has been registered with the Department of Intellectual Property with the product name 'Hed Khik Khak or Giggling Mushrooms' by designing a mark/symbol (Logo) as shown in Fig. 3.

Fig. 3 Trademark symbol

In addition, for therapeutic mushroom products' branding, the community and the researcher(s) collaborated to perform an acceptance survey for the created brand of Giggling Mushrooms. It was approved with a high score (7.89 points). The trademark has been registered for Khlong Chik Community Enterprise to be used to develop new products under the brand name.

In the aspect of marketing channels and guidelines for marketing the products, the channels for health stores and restaurants are suitable for the product characteristics. However, when considering the main distribution channels for health foods that focus on herbs, it was found that online marketing and contemporary shops that house regional civic organizations need to be targeted by highlighting the range of products to entice customers to purchase. Customers who purchase goods through these platforms typically acquire the products in small volumes but they may purchase them more frequently. Additionally, entrepreneurs like to organize promotional activities through these large retailers. Convenience stores serve as a distribution route for small goods which customers can opt to purchase and eat right away. Also, most consumers tend to consume mushroom products with steamed rice or boiled rice. Distributing the products in convenience stores satisfies customer demand in this sense.

The distribution channels directed to the target market could be divided into 3 ways, namely through sales representatives through a health store and through restaurants. For each channel, appropriate distribution costs need to be taken into consideration. The entrepreneurs must consider the appropriateness, namely the cost of sales through various stores using the company's salesperson, the cost of distribution through health stores and the cost of distribution through restaurants. Furthermore, a proper marketing plan must be planned for the implementation of marketing activities using marketing mix strategies to create awareness and to meet the needs of customers in order to achieve marketing results appropriately and achieve the market goals as specified. In addition, the design of therapeutic mushroom products needs to be aesthetic, outstanding and unique to represent the identity of the community. The researcher(s) worked on product design with the community along with conducting an acceptance questionnaire on the designed packaging and label with the highest acceptance score (8.52 points).

Conclusion

It was determined through the analysis of conventionally flavored therapeutic mushroom products that they could be successfully marketed and distributed. The utilization of locally cultivated Bhutanese oyster mushrooms is the product's standout feature. It is healthy for the body because it is organic and delightfully seasoned and it is ideal for those who care about their health. It is good for the working/school age group who wants nutritious food and the elderly who wants the benefit as it can help them relax. It is a healthy food that can be consumed daily. Based on these benefits, the community can create good popularity for the product with an emphasis on semi-premium marketing using

social media channels, health stores and restaurants, including various booths to showcase products by concentrating on marketing strategies regarding product differentiation (Differentiated strategy) to demonstrate the value and nutrients of the Bhutan oyster mushrooms. The products are available in sachet sizes, priced at 30-35 baht per sachet per 40 grams and come in a box size of 7 sachets with the price of 199 baht per box. Another strategy is to allow people to taste the products at product exhibitions. The promotion at the point of sale is for boosting the interest in the products.

Acknowledgments

This research study was granted by Program Management Unit for Competitiveness (PMUC). The support provided by the Suan Dusit University are gratefully acknowledged.

References

AOAC. (1995). *Official methods of analysis the association of official analysis chemistry.* Washington, D.C.: Association of Official Analytical Chemists.

AOAC. (2000). *Official methods of analysis the association of official analysis chemistry.* Washington, D.C.: Association of Official Analytical Chemists.

Chantarakan, S. (2012). *Development of healthy snack products from mushrooms* (Master's thesis). Bangkok: Kasetsart University.

Cedric, M., Cecile, G., Constance, D., Ferderic, J.T., Chantal, F., Emmanuelle, P., … Henri, N. (2021). Investigation of the antioxidant capacity of carmels: Combination of laboratory assays and *C. elegans* model. *Journal of Function Foods, 78,* 04308.

Cronbach, L.J. (1951). Coefficient alpha and the internal structure of tests. *Psychometrika, 16* (3), 297-334.

Fellow, P. (2000). *Food processing technology: Priciples and pactrice* (2nd ed.). Washington, DC: Woodhead Publishing.

Fennema, O.R. (1996). *Food chemistry* (3th ed.). New York: Marcel Dekker.

Kaikaew, D., Wongpaisanlit, R., & Wongtom, R. (2016). Effect of sucrose syrup content on oil absorption in fried banana chip production. *Food Journal, 45*(2), 73-79.

Noiduang, P., Tapaothong, N., & Masileerungsri, K. (2015). Use of dried aril of gac fruit powder as an antioxidant in mayonnaise product. *Journal of Food Technology, Siam University, 10*(1), 9-18.

Rattananon, N. (2008). *Food chemistry* (2nd ed.). Bangkok: Odiantro Publishing.

Rattanathammawat, R., Suwonsichon, T., Chompreda, P., Sriroth, K., & Haruthaithanasan, V. (2003). Effect of moisture content of half-snacks and frying time on physicochemical properties of taro flour snack. *Proceedings of 41st Kasetsart University Annual Conference* (pp. 45-52). Bankok: Kasetsart University.

Sirilert, T., & Silalai, N. (2016). Effect of pre-frying treatments on moisture content, effective moisture diffusivity and oil uptake content of deep-fat fried shitake mushrooms (Lentinusedodes). *Journal of Food Technology, Siam University, 11*(1), 57-66.

Tolgahan, K., & Vural, G. (2019). Caramelization in food quality and safety perspective. *Food Chemistry, 2019,* 18-29.

Wei, Z., Li, X., Thushara, D., & Y, Liu. (2011). Determination and removal of malondialdehyde and other 2-thiobarbituric acid reactive substances in waste cooking oil. *Journal of Food Engineering, 107*(3-4), 379–384.

Wrolstad, R.E., Acree, T.E., Decker, E.A., Penner, M. H., Reid, D.S., Schwartz, S.J., ... & Sporns, P. (2005). Lipids. In *Handbook of food analytical chemistry* (pp. 467-564). USA: John Wiley & Sons.

Journal of Food Health and Bioenvironmental Science

Journal homepage : http://jfhb.dusit.ac.th/

Total Phenolics, Flavonoids, DPPH Radical Scavenging and Tyrosinase Inhibition Activities of Sacha inchi (*Plukenetia volubilis* L.)

Sasamol Phasuk*, Virapong Saeng-xuto & Katevadee Vonpian

Science and Innovation for Development Program, Valaya Alongkorn Rajabhat University under the Royal Patronage, Pathum Thani, 13180 Thailand

Article info

Article history:
Received : 24 October 2022
Revised : 23 December 2022
Accepted : 28 December 2022

Keywords:
Sacha inchi, Phenolics, Flavonoids, DPPH radical scavenging, Tyrosinase inhibition

Abstract

Sacha inchi (*Plukenetia volubilis* L.) or Inca star is a medicinal plant whose fruits and seeds are mainly used in processing. The objectives of this research were to analyze the total phenolic and flavonoid contents of Sacha inchi from leaves, vines and shells and to study the efficiency of DPPH radical scavenging activities and tyrosinase inhibition activities of Sacha inchi. The leaves, vines and shells were extracted with 95% ethanol using the maceration technique. The crude extracts of leaves, vines and shells were analyzed in terms of total phenolic contents using the Folin-Ciocalteu method, whereas the total flavonoid contents were analyzed using aluminium chloride colorimetric method. The results of the percentage yield in leaves, vines and shells were found to be 19.4815 ±0.0617, 4.81 ± 0.0482 and 2.51 ± 0.0943, respectively. The total phenolic contents in leaves, vines, and shells were 26.3441±0.8895, 37.0925±0.8898 and 30.5202±1.9938 mg of gallic acid/g sample, respectively. The total flavonoid contents were 407.6190±2.5036, 171.2516±3.6696, and 179.0318±3.7771 mg of rutin/g sample, respectively. DPPH radical scavenging activities in leaves, vines and shells were shown with EC_{50} as 0.0092±0.0090, 0.0803±0.0063 and 0.2527±0.1105 mg/mL, respectively, compared to standard BHT with EC_{50} as 0.1296±0.0528 mg/ml. The tyrosinase inhibition activities in leaves, vines and shells were shown with IC_{50} as 0.0016±0.0107, 0.4924±0.1500 and 0.5986±0.2751 mg/mL, respectively, compared to standard kojic acid with IC_{50} as 0.0002±0.0011 mg/mL. All selected parts of the Sacha inchi had phenolic and flavonoid contents. The DPPH radical scavenging activities in Sacha inchi extracts were found in leaves, vines and shells. In addition, the tyrosinase inhibition activities were found mainly in leaves, although to a lesser degree in vines and shells of Sacha inchi.

* Corresponding Author
e-mail: sasamol@vru.ac.th

Introduction

Sacha inchi (*Plukenetia volubilis* L.) is a medicinal as well as rubber plant in the family Euphorbiaceae. This novel vegetable has been researched for its health benefits in terms of its characterizations, phytochemical contents, mineral forms and antioxidant activities. Most of the previous research mainly involves crude extraction from the seed. The efficacy of this herb's active ingredients in other parts such as vines and shells is still, unknown.

The seed is considered an important dietary source of health-promoting phytochemicals that are rich in high contents of total phenolics, total carotenoids and hydrophilic and lipophilic antioxidant capacities (Chirinos et al., 2013; Maurer et al., 2012). The beans of Sacha inchi are roasted, heated and eaten as a snack. The seeds are fragrant, crunchy and have a delicious nutty flavor. Sacha inchi beans can be processed into snack products such as salted roasted nuts and fried beans. They can also be processed into food products such as sauce, soy sauce, soybean paste, processed into Sacha inchi bean flour for cooking and baking. In addition, they can be extracted into oil that can be used for many purposes (Norhazlindah et al., 2022). Sacha inchi seeds could be processed into edible oil as dietary supplement products (Suwanangul et al., 2022). For the leaves, the young shoots are boiled or eaten with chili paste or bamboo shoot soup. The dark old green leaves are chopped into small pieces and dried in the sun to make tea. The bright green leaves are extracted from chlorophyll or extracted to make chlorophyll water. The pod and seed husks are used to make compost or to be compressed into fuel sticks for cooking (Juntawieng, 2020). Kittibunchakul et al. (2022) found that the leaves provide antioxidant effects and phenolic compositions and are a source of health promotion. This will prevent damage to DNA, protein and lipid, which decreases the risk of heart disease and cataracts and results in people's better health (Ames et al., 1993).

A community group is currently using various parts of the Sacha inchi including leaves, vines and shells. The processing community enterprise group of Sacha inchi is named Kru Cheun Farm, which is located at 1 Village No. 14, Ban Kaeng Sub-district, Mueang District, Sa Kaeo Province and whose chief entrepreneur is Ms. Patma Sidawong. Leaves are dried and packed in containers for sale as healthy tea while vines and shells of the seeds are dismissed as waste. According to the World Health Organization's Good Agricultural and Collection Practice (GACP) standard of the traditional medicine industry, the safety and quality of raw medicinal plants, materials and finished products depend on factors that may be classified as intrinsic (genetic) or extrinsic (environment, collection method, cultivation, harvest, post-harvest processing, transport and storage practices). Inadvertent contamination by microbial or chemical agents during any of the production stages can also lead to deterioration in safety and quality. Medicinal plants collected from the wild population may be contaminated by other species or plant parts through misidentification, accidental contamination, or intentional adulteration, all of which may have unsafe consequences (Zhang, 2003). The community has grown these plants without using insecticide to prevent the effects of chemicals. Sacha inchi is provided with enough water and kept away from any source of chemicals. Weeds are removed every 1-2 months. At 5-6 months after planting, Sacha inchi trees begin to blossom and bear pods. Then 3 months later, the pods begin to age and are ready to be picked. These should be ripe and drying pods, that is, black and then brown. For leaves, green rather than brown ones should be chosen, which are the younger leaves. The personnel of planting, nursing and harvesting communities must receive training from community government agencies to acquire technical knowledge and expertise. After harvest, the community will keep the crops and pack them in a clean and ventilated greenhouse. They must not be affected by chemicals and insects. In this study, the researchers selected the green leaves, vines and shells of the seeds of Sacha inchi. All of these parts would be discarded by the community members and were not used for their business purpose. Sacha inchi parts needed to be transported to researchers by a temperature-controlled vehicle (Sidawong, 2021). After that, they were extracted and studied in terms of their biological activities.

To meet the needs of the community members who are concerned about the plant safety, they requested an investigation into the activities and properties of the various parts of Sacha inchi, including leaves, vines and shells. Other parts of this medicinal plant that are aforementioned can be considered as an important dietary source that has a high level of phenolics, flavonoids, antioxidants and tyrosinase inhibition activities.

The effect of oxidative activities is related to and correlates with total phenolics (Velioglu et al., 1998). In

this study, the research explored this effect by using the basis from the scavenging of DPPH free radicals. Moreover, the researchers investigated the performance of inhibition tyrosinase activities because prior studies have found that medicinal plants in this same family have anti-tyrosinase activities (Meechai et al., 2010; Momtaz et al., 2010). Baurin et al. (2002) ran a preliminary screening of tropical plants and found many families including Euphorbiaceae having antityrosinase activities. Plants in the same family have the possibility to have the same biological effect. These activities in this research could be considered as an important dietary or raw material source for promoting community natural products.

Materials and methods

1. Preparation of crude extracts by the maceration method

Leaves, vines and shells of Sacha inchi were washed thoroughly and dried at 45°C until they were dry. They were crushed and separated. Five hundred grams of Sacha inchi leaves, vines and shells were baked and crushed. They were wrapped in a thin white cloth, soaked in 95% ethanol 3,000 mL and shaken gently. They were separated and stored in a dark place for five days. Each type was filtered to remove residues. All parts were re-extracted with ethanol and left for 5 days. Then the extracts were stored in the flask for evaporation of the solvent. The solvent was evaporated with a rotary vacuum evaporator and freeze-dryer (triplicates).

The researchers recorded the results and calculated the percentage yield (% yield) of the crude extracts obtained from the formula.

$$\% \text{ yield of crude extracts} = \frac{\text{weight of crude extracts X 100}}{\text{weight of dry plants before extraction.}}$$

The crude extracts of each part were stored in the refrigerator at 5°C to wait for the determination of phytochemical and DPPH radical scavenging and anti-tyrosinase activities (Kotpoohtorn, 2016).

2. Determination of total phenolic and total flavonoid contents in leaves, vine and shells of Sacha inchi extracts

2.1 Total phenolic contents (TPC)

Following a modified method by Wattanuruk et al. (2020b), the total phenolic contents (TPC) were determined by Folin-Ciocalteu method. Ten milligrams of extracts from Sacha inchi leaves, vines and shells were dissolved with 99.99 % ethanol, an adjusted volume of 5 mL. After that, 100 µL of diluted extracts in 8.4 mL distilled water was mixed with 500 µL of freshly prepared diluted Folin-Ciocalteu. One minute after, 1 mL of 20% Na_2CO_3 was added. Mixtures were incubated at room temperature for two hours in the dark. The standard solution of gallic acid (10 mg) was dissolved with 99.9% ethanol, adjusted volume 5 mL and diluted in various concentrations (1.0, 0.8, 0.4, 0.2, 0.1 and 0.05 mg/mL.). The absorbance was measured at a wavelength of 760 nm by UV-Vis spectrophotometer (triplicates).

The basis of the calibration curve of gallic acid was brought to calculate the total phenolic contents, which were expressed as gallic acid equivalent (GAE) in milligrams per gram of the sample (mg GAE/g dried extract).

2.2 Total flavonoids contents (TFC)

Following a modified method by Wattanuruk et al. (2020b), the total flavonoids contents (TFC) were determined using rutin as a standard. Ten milligrams of extracts from Sacha inchi leaves, vines and shells were dissolved in 80% ethanol and adjusted to volume 10 mL. After that, 1 mL of extracted samples/standard solution was put in a 10 mL volume metric flask, with an addition of 4 mL of distilled water for 0 min, a mixing of 0.3 mL of 5% $NaNO_2$ for 5 min, an addition of 0.3 mL of 10% $AlCl_3$ for 6 min, an addition of 2 mL of 1 M NaOH, an addition of 10 mL distilled water and everything was shaken well. The absorbance was measured at a wavelength of 510 nm using a UV-Vis spectrophotometer (triplicates).

The qualification was done using rutin as the standard and the results were expressed as milligrams of rutin equivalent (mg RE) per gram of the sample (mg RE/g dried extract).

3. Determination of DPPH radical scavenging activities in leaves, vine and shells of Sacha inchi extracts

Following a modified method by Senajuk et al. (2020) and Sharma & Bhat (2009), the DPPH radical scavenging activities were determined. One milligram of extracts from Sacha inchi leaves, vines and shells with concentrations of 500, 250, 125, 62.5 and 31.25 mg/mL was pipetted into a test tube and 3 mL of 0.2 mM DPPH (1,1-diphenyl-2-picrylhydrazyl) was added. Everything was shaken well. After that, it was incubated at room temperature for 30 min in the dark. The absorbance was measured at a wavelength of 517 nm by a UV-Vis

spectrophotometer, using 95 % ethanol as blank, 0.2 mM DPPH as control and BHT (butyl hydroxytoluene) as a standard (triplicates). The efficiency of antioxidant activities is as follows (Pukumpuang et al., 2012):

$$\% \text{ DPPH Scavenging} = \frac{(\text{Abs control} - \text{Abs sample} / \text{Abs control}) \times 100}{\text{Abs Control}}$$

Graphs were plotted for EC_{50} values and were compared to BHT for each extraction of leaves, vines and Shells of Sacha inchi. Values were reported as EC_{50}, obtained by graphing the relationship between % DPPH inhibitions and sample concentrations.

4. Determination of tyrosinase inhibiting activities in leaves, vines and Shells of Sacha inchi extracts

Following a modified method by Kleangjan & Ponmai (2017), the tyrosinase inhibiting activities were determined. The sample solution was prepared by weighing extracts from leaves, vines and shells of Sacha inchi (0.01 g each), dissolved with 99.99% ethanol, shaken for 30 min. The extract volume was then adjusted to 10 mL and diluted in various concentrations (500, 250, 125 and 62.5 mg/mL). The samples were compared with kojic acid standard solution.

A, B, C and D types of solution were added into a multi-well dish of microplate leader.

A (control): tyrosinase solution 50 µL, 0.02 M sodium phosphate buffer solution (pH 6.8) 150 µL, and 99.99% ethanol 50 µL.

B (blank of A): 0.02 M sodium phosphate buffer solution (pH 6.8) 150 µL and 99.99% ethanol 50 µL.

C (test sample): tyrosinase solution 50 µL, 0.02 M sodium phosphate buffer solution (pH 6.8) 150 µL and sample/standard in 99.99% ethanol 50 µL.

D (blank of C): 0.02 M sodium phosphate buffer solution (pH 6.8) 150 µL and sample solution 50 µL.

The samples were compared with kojic acid standard solution. After the substance was added to a multi-well dish and shaken to mix well, the tests were incubated at room temperature (25°C) for 10 min. After that, 50 µL L-DOPA solution was added into a multi-well dish and shaken to mix well. The absorbance was measured at a wavelength 492 nm with a UV-Vis spectrophotometer. Then they were incubated at the room temperature for 2 min and absorbance was measured again at the same wavelength (triplicates). The calculation of % tyrosinase inhibition is as follows:

$$\% \text{ Tyrosinase Inhibition} = \frac{(A-B)-(C-D) \times 100}{A-B}$$

A. B, C and D were different types of absorbance at 492 nm wavelength between the measured values before and after 2 min of the incubation period.

5. Statistical analysis

All treatments and determination were implemented in triplicates and data were analyzed in terms of mean ± standard deviation.

Results and discussion

1. The extraction of substances in leaves, vines, and shells of sacha inchi

It was found that the percentage yield in leaves, vine and shells of sacha inchi extracts was 19.4815±0.0617, 4.8100 ± 0.0482 and 2.5100 ± 0.0943, respectively. The percentage yield and other physical properties of Sacha inchi extracts are shown in Table 1.

Table 1 Yield and other physical properties of Sacha inchi extracts

Sample (from different parts of Sacha inchi)	Yield (%)	Color and consistency
Leaves	19.4815±0.0617	Deep green, Gummy solid
Vines	4.8100± 0.0482	Light brown, solid in form of powder
Shells	2.5100± 0.0943	Light brown, solid in form of powder

2. Total phenolic contents (TPC) and total flavonoid contents (TFC) of Sacha inchi extracts

The total phenolic contents in Sacha inchi leaves, vines and shells were 26.3441±0.8895, 37.0925±0.8898 and 30.5202±1.9938 of gallic acid/g sample, respectively. The total flavonoid contents were 407.6190±2.5036, 171.2516±3.6696 and 179.0318±3.7771 mg of rutin/g sample, respectively. Total phenolic and total flavonoid contents of Sacha inchi extracts are shown in Table 2.

Table 2 The amounts of total phenolics and total flavonoids of Sacha inchi extracts

Sample (from different parts of Sacha inchi)	Phenolic (mg GAE/g)	Flavonoid (mg Rutin/g)
Leaves	26.3441±0.8895	407.6190±2.5036
Vines	37.0925±0.8898	171.2516±3.6696
Shells	30.5202±1.9938	179.0318±3.7771

Chirinos et al. (2013) investigated Sacha inchi seeds and found a high amount of nutrients in them including phenolic properties. Other parts also had large amounts of phenolics, namely vines, followed by shells and leaves, respectively. The total flavonoid contents were highest in leaves, followed by shells and vines, respectively. Both phenolic and flavonoid compounds

are great activities of antioxidants (Atoui et al., 2005; Velioglu et al., 1998).

3. DPPH radical scavenging activities

Regarding the evaluation of hydrogen donation of the antioxidants, EC_{50} values represented the concentration of antioxidants that decreased the DPPH radicals to half of their initial concentration (Wattanuruk et al., 2020a). The results of this measure in leaves, vines and shells of Sacha inchi showed EC_{50} values of 0.0092±0.0090, 0.0803±0.0063 and 0.2527±0.1105 mg/mL, respectively, compared with the BHT standard with an EC_{50} of 0.1296±0.0528 mg/mL. The DPPH radical scavenging activities are shown in Table 3.

Table 3 DPPH radical scavenging activities of Sacha inchi extracts

Sample (from different parts of Sacha inchi) and standard	EC_{50} (mg/mL)
Leaves	0.0092±0.0090
Vines	0.0803±0.0063
Shells	0.2527±0.1105
BHT (standard)	0.1296±0.0528

Sacha inchi seeds were reported to have antioxidant capacity (Chirinos et al., 2013). Kittibunchakul et al. (2022) studied antioxidant activities in Sacha inchi leaves and found Sacha inchi young leaves had significantly higher antioxidant activities than mature leaves, exhibiting 1.1-1.2-fold. No studies have reported that other parts such as vines and shells have this effect. In this research, the results of EC_{50} showed that leaves of sacha inchi were rich in flavonoids and their phenolic compounds and had the greatest amounts of antioxidants, followed by vines, BHT (standard test) and shells, respectively. In this research, the results are in line with those in Senajuk et al. (2020). They argued that the extracts with a low EC_{50} have high free radical scavenging activities. The relationship between total phenolic contents and antioxidant activities shows that the amounts of total phenolics vary with antioxidant activities (Yan & Asmah, 2010). It is very interesting to develop a product related to anti-aging that will prevent the body from oxidative stress.

4. Tyrosinase inhibition activities of sacha inchi extracts

The inhibition of tyrosinase activities in leaves, vines and shells of sacha inchi extracts is shown in Table 4.The IC_{50} were 0.0016±0.0107, 0.4924±0.1500 and 0.5986± 0.2751 mg/mL, respectively, compared to standard kojic acid, IC_{50} as 0.0002±0.0011 mg/mL.

Table 4 Tyrosinase inhibition activities of sacha inchi extracts

Sample (from different parts of sacha inchi) and standard	IC_{50} (mg/mL)
Leaves	0.0016+0.0107
Vines	0.4924±0.1500
Shells	0.5986±0.2751
Kojic acid (standard)	0.0002±0.0011

Leaves of Sacha inchi showed high efficacy of tyrosinase inhibition, followed by vines and shells. Kojic acid, which is a standard solution, showed better efficacy than others in terms of inhibitory effects. Even though there is no research about the tyrosinase inhibition of Sacha inchi plants, the three parts of Sacha inchi explored in this study might have the effects of anti-tyrosinase activities.

Even when Meechai et al. (2010) studied the anti-tyrosinase activities of 77 Thai medicinal plant extracts and found about 11 plant extracts, including plants in the Euphorbiaceae family, showed the potential for these activities, Sacha inchi was not mentioned in this study. However, if the fact that the extracts of Sacha inches leaves, vines and shells contain phenolics and flavonoids is taken into consideration, then there are a number of studies confirming that plants containing phenolics and flavonoids usually have an inhibitory effect on tyrosinase activities. (Tidchai, 2019; Chumchaiyapurk, 2015; Momtaz et al., 2010)

As revealed in this study, the leaves of Sacha inchi have inhibitory effects on tyrosinase activities even if these effects are lesser than those of kojic acids. Therefore, the effects of leave extract are more interesting if compared to vines and shells. What has been found in this study will be useful for future research that involves the development of whitening products.

Conclusion

The extracts of Sacha inchi leaves, vines and shells have phenolic and flavonoid contents. The obtained results of each sample extract have shown that phenolic and flavonoid compounds are a source of antioxidant effects. All of them have some anti-tyrosinase effects. The leaf extracts have the highest content of flavonoids and a medium of phenolics. They exhibit a high amount of-enriched antioxidant activities. The extracts of leaves also have the effects of inhibitory tyrosinase activities. Vines and shells are the parts that showed the second and third highest amounts of antioxidants, respectively. Vine and shell extracts have a much lower tyrosinase

inhibitory effects than leaf extracts. All these properties can be further researched in the future to examine more efficacy and safety in vivo and do clinical studies. This would allow the local medicinal plant to be a new source of commercial value as it can be the main ingredient in the creation of new products related to anti-aging, health, and beauty.

Acknowledgments

The authors would like to express their gratitude to the Research and Development Institute, the Science Center, Valaya Alongkorn Rajabhat University under Royal Patronage, and Dao Inca Herb Processing Community Enterprise Group "Kru Cheun Farm" Ban Kaeng Subdistrict, Mueang District, Sa Kaeo Province for their support.

References

Ames, B.N., Shigenaga, M.K., & Hagen, T.M. (1993). Oxidants, antioxidants, and the degenerative diseases of aging. *Proceedings of the National Academy of Sciences, 90*(17), 7915-7922.

Atoui, A.K., Mansouri, A., Boskou, G., & Kefalas, P. (2005). Tea and herbal infusions: their antioxidant activity and phenolic profile. *Food Chemistry, 89*(1), 27-36.

Baurin, N., Arnoult, E., Scior, T., Do, Q.T., & Bernard, P. (2002). Preliminary screening of some tropical plants for anti-tyrosinase activity. *Journal of Ethnopharmacology, 82*(2-3), 155-158.

Chirinos, R., Zuloeta, G., Pedreschi, R., Mignolet, E., Larondelle, Y., & Campos, D. (2013). Sacha inchi (*Plukenetia volubilis*): A seed source of polyunsaturated fatty acids, tocopherols, phytosterols, phenolic compounds, and antioxidant capacity. *Food Chemistry, 141*(3), 1732-1739.

Chumchaiyapurk, T. (2015). Development of a lotion made from crude extracts of *Carthamus tinctorius* Linn. flowers and *Psidium guajava* Linn.leaves as a tyrosinase inhibitor (Master's Thesis). Pathumtani: Valaya Alongkorn Rajabhat University Under the Royal Patronage, Pathumtani.

Juntawieng, U. (2020). Development of soap product of Sacha inchi. *Journal of Innovative Technology and Research, 4*(2), 70-79.

Kittibunchakul, S., Hudthagosol, C., Sanporkha, P., Sapwarobol, S., Suttisansanee, U., & Sahasakul, Y. (2022). Effects of maturity and thermal treatment on phenolic profiles and in vitro health-related properties of Sacha inchi leaves. *Plants, 11*(11), 1515.

Kleangchan, C., & Ponmai, S. (2017). Anti-tyrosinase activity of Sago palm (*Metroxylon sagu Rott.*) (Research report). Nakhonsithamarat: Rajamongala University of Technology Srivijaya.

Kotpoohtorn, J. (2016). Extraction, phytochemical screening, antioxidant and antibacterial of crude extract from *Oroxylum Indicum* (Doctoral dissertation). Chonburi: Burapha University.

Maurer, N.E., Hatta-Sakoda, B., Pascual-Chagman, G., & Rodriguez-Saona, L.E. (2012). Characterization and authentication of a novel vegetable source of omega-3 fatty acids, Sacha inchi (*Plukenetia volubilis L.*) oil. *Food Chemistry, 134*(2), 1173-1180.

Meechai, I., Puripattanavong, J., & Dej-adisai, S. (2010). Screening of anti-tyrosinase activity from Thai medicinal plants. In *The 9th NRCT-JSPS Joint Seminar, Natural Medicine Research for the Next Decade: New Challenges and Future Collaboration* (pp. 119-121). Bangkok: Chulalongkorn University.

Momtaz, S., Lall, N., Hussein, A., Ostad, S. N., & Abdollahi, M. (2010). Investigation of the possible biological activities of a poisonous South African plant; Hyaenanche globosa (Euphorbiaceae). *Pharmacognosy Magazine, 6*(21), 34.

Norhazlindah, M.F., Jahurul, M.H.A., Norliza, M., Shihabul, A., Islam, S., Nyam, K.L., & Zaidul, I.S.M. (2022). Techniques for extraction, characterization, and application of oil from Sacha inchi (*Plukenetia volubilis L.*) seed: A review. *Journal of Food Measurement and Characterization,* 1-12.

Pukumpuang, W., Thongwai, N., & Tragoolpua, Y. (2012). Total phenolic contents, antibacterial and antioxidant activities of some Thai medicinal plant extracts. *Journal of Medicinal Plants Research, 6*(35), 4953-4960.

Senajuk, W., Phasuk, S., Nilsang, P., & Takolpuckdee, P. (2020). Free radical scavenging activity of riceberry and Pathum Thani rice extracts for developing face powder. *Journal of Food Health and Bioenvironmental Science, 13*(3), 9-15.

Sharma, O.P., & Bhat, T.K. (2009). DPPH antioxidant assay revisited. *Food Chemistry, 113*(4), 1202–1205.

Sidawong, P. (2021). *Kru Chuen Farm*. Retrieved from https://Web.Facebook.Com/KruChuen33.

Suwanangul, S., Senphan, T., & Ruttarattanamongkol, K. (2022). Antioxidant and biochemical properties of protein hydrolysates prepared from Sacha inchi meal. *Journal of Agricultural Research and Extension, 39*(2), 55–67.

Tidchai, W. (2019). Efficiency of *Feronia limonia* (L.) Swing crude extract as tyrosinase activity inhibition (Master's Thesis). Pathumtani: Valaya Alongkorn Rajabhat University Under the Royal Patronage.

Velioglu, Y.S., Mazza, G., Gao, L., & Oomah, B.D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry, 46*(10), 4113–4117.

Wattanuruk, D., Phasuk, S., Nilsang, P., & Takolpuckdee, P. (2020a). Cytotoxicity activity of crude extracts of Leum Phua Khaow-Mak (*Oryza Sativa* L. variety Leum Phua) against fibroblast cell. *Journal of Food Health and Bioenvironmental Science, 13*(2), 28-35.

Wattanuruk, D., Phasuk, S., Nilsang, P., & Takolpuckdee, P. (2020b). Total phenolics, flavonoids, anthocyanins and antioxidant activities of Khaow-Mak extracts from various colored rice. *Journal of Food Health and Bioenvironmental Science, 13*(1),10-18.

Yan, S., & Asmah, R. (2010). Comparison of total phenolic contents and antioxidant activities of turmeric leaf. Pandan leaf and torch ginger flower. *International Food Research Journal, 17*(2), 417-423.

Zhang, X. (2003). WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants. Geneva: World Health Organization.

Journal of Food Health and Bioenvironmental Science
Journal homepage : http://jfhb.dusit.ac.th/

The Concentration of Syrup in Making Foi Thong, Med Kanoon, Thong Yip and Thong Yod

Porndara Ketthongkam*

Department of Home Economics, Faculty of Education Ramkhamhaeng University, Bangkok, 10240, Thailand

Article info

Article history:
Received : 2 May 2022
Revised : 18 July 2022
Accepted : 2 August 2022

Keywords:
Foi Thong, Med Kanoon, Thong Yip, Thong Yod

Abstract

The concentration of syrup that uses egg yolk, boiled into the syrup, as the main ingredient requires the right amount of thickness to make Thai desserts. In this article, Foi Thong, Med Kanoon, Thong Yip and Thong Yod are used and arranged according to the concentrations of syrup with low to high. Starting with Foi Thong which is at 55-57°Brix is round, sticky and glazed. Med Kanoon at 65-66°Brix and is glazed and non-crystalline sugar. Thong Yip is at 70-72°Brix is fluffy, sticky and juicy with syrup. The last one, Thong Yod is at 72-74°Brix has droplet-shape and is non-flat, fluffy, soft and juicy with syrup. The clear syrup is used for soaking Thong Yip and Thong Yod as well as to use to dilute the Foi Thong's sweetness in cases where the usage of condensed syrup occurs. The above clear syrup should have a concentration of 36-38°Brix. In addition, white sugar applies to this clear syrup. Moreover, using syrup that had a concentration of 60 to 70 g of white sugar to 100 mL of syrup will help enhance osmotic pressure and reduce water activity (α_w) which could prevent microbial growth. Most people prefer to use duck egg yolk rather than hen egg yolk for preparing the desserts, due to the greater luster of color, viscosity and oiliness. However, some prefer both duck egg yolk and hen egg yolk mixed, to add a soft texture and lessen the fishy smell of duck egg yolk.

Introduction

Thai egg dessert is usually called "Golden Dessert" during the Ayutthaya period. In the reign of King Narayana the Great, there were many foreigners who migrated to Ayutthaya and served as courtiers, including a Portuguese named Maria Guyomar de Pinha (Tao Thong Keep Mah), who followed her husband (Constantine Phaulkon or Chao Phra Ya vichayen) who migrated to Thailand and was promoted to a courtier. She served as the head chef in the department of dessert in the royal court. At that time, she created desserts from a fusion between European desserts and Thai desserts. Hence, several desserts acquired their various original tastes and changes in appearance. Moreover, she innovated new methods in making desserts. As a result, she was honored as a dame and played a major role in influencing the development of Thai desserts.

Tao Thong Keep Mah introduced innovative methods in making Thai desserts by introducing baking as an addition to other primary methods: boiling, steaming, toasting, coating in sugar and stirring.

* Corresponding Author
e-mail: porndara.kk@gmail.com

Additionally, she introduced egg white and egg yolk into the preparation of the Thai desserts. As a result, these desserts developed into desserts used in important ceremonies. These egg-based desserts include Foi Thong, Med Kanoon, Thong Yip, Thong Yod, Thong Ek, Jah Monggud and Saneh Chand.

Various Thai desserts need different concentrations of syrup in their preparation. The concentration of syrup will affect the characteristics of the desserts like glossiness, softness, sweetness and shape, along with maintaining their sponginess. Hence, Thai desserts require appropriate concentrations of syrup to achieve the desirable characteristics.

When the concentration of syrup is inappropriate, it will affect the texture and the characteristics of the desserts. If the concentration of syrup is too high, it will cause early coagulation in the egg before the syrup diffuses into it which makes the dessert's texture rough. In contrast, too low concentrated syrup causes a low rate of diffusion of sugar into the desserts, which is less than a highly concentrated syrup, making the desserts flat and non-fluffy. Practically, the diffusion of the solution will diffuse from high to low concentration. (Mongkonworawan et al., 2002). The concentration of syrup at 60-70% will prevent microbial growth which can extend the shelf life of the desserts, due to the sugar gathering with the free water. The above process causes the water to evaporate, resulting in not enough moisture for microbial growth in food. Moreover, the sugar can remove water from the microbial cell.

This article focuses on Foi Thong, Med Kanoon, Thong Yip and Thong Yod because these desserts apply the same concentration of syrup theory and have been popular among Thai and foreigners. According to the Suan Dusit's Poll, the survey indicated that the top 5 Thai desserts Thai people would like to learn to make are Thong Yod with 22.89%, followed by Thong Yip with 21.76% (Suan Dusit Poll, 2021). This type of dessert needs knowledge and understanding of the syrup used because the concentration of the syrup will not be the same for each dessert. Therefore, syrup for making Foi Thong is not suitable for making Med Kanoon, Thong Yip and Thong Yod. Some people may ask why these desserts are too sweet. The author will answer that these desserts require white sugar and the taste is supposed to be very sweet. However, there are methods to reduce the sweetness.

The author demonstrates the concentration level of syrup in making egg desserts or "Golden Desserts" from low to high. Firstly, Foi Thong, uses the lowest concentration of syrup. Followed with Thong Yip and Thong Yod because the remaining syrup from making Foi Thong can be used to make Thong Yip and Thong Yod, again as the concentration of the syrup will increase from the boiling process. The author has always used this concentration sequence in making Thai desserts throughout 22 years of experience as an instructor. Lastly, although Med Kanoon uses less concentrated syrup than Thong Yip and Thong Yod, it is still possible to prepare it at the end by adding water to the remaining syrup from making Thong Yip and Thong Yod, which will become diluted by water and is now suitable for making Med Kanoon. However, some people may arrange the sequence by starting with Foi Thong, Thong Yod, Thong Yip and Med Kanoon. (A Cuisine, 2019; Chemistry of Textiles, Food and Surroundings, 2016). However, this type of dessert is not popular among people who are concerned about health, due to its low nutrition, most of them come from carbohydrates, protein and a little fat. It has high cholesterol, so it is not recommended for oldery and those who have atherosclerosis.

Water

Water is a macronutrient making up a large percentage of meat (up to 75%), fruits and vegetables (up to 95%). In food, it is a medium for chemical reactions and transportation of other food components. It also adds structure by imparting turbidity to cells. In the food industry, water content is generally referred to as moisture. A number of functional properties of water are important in food processing such as the ability to dissolve and disperse other food components, the ability to be bound by other food components, the ability to transfer heat, the ability to sublimate, and its ability to transport materials (Simons, 2022).

Sugar

Sugar for cooking, scientifically named "Sucrose", is classified as a disaccharide which consists of 2 kinds of monosaccharide, glucose and fructose, which are connected through glycosidic linkages. A syrup infused desserts have white sugar as the main ingredient to provide a sweet taste. It performs various functions in different foods and desserts. However, this article will only discuss how sugar functions in syrup-infused desserts. White sugar, which is used to prepare Med Kanoon, Thong Yip and Thong Yod, refers

to highly purified sucrose crystals of clear white to light yellow with slight moisture content. It has granulated particles which do not stick together and a small amount of molasses mixed within. White sugar is manufactured by bleaching sugarcane with sulfur or carbon dioxide. It is used mainly for household consumption and sugar-related industry.

1. Properties in Sugar

1.1 Sweetness is a distinctive property of sugar, which is the primary purpose of its use in cooking. The sweetness occurs from taste receptors stimulation in the tongue and other areas within the mouth. The perceived sweetness depends on the type of sugar. Sugar's sweetness has a standard form of comparison based on sucrose which has 100 points of sweetness. The other types of sugar that are sweeter than sucrose will have more than 100 points of sweetness, while less sweet sugar, on the other hand, will have less than 100 points of sweetness.

1.2 Solubility - Each type of sugar will dissolve differently by nature. Fructose has the best water-soluble properties, followed by sucrose and the lowest soluble is lactose. In cooking, most of the sugar used is sucrose. Hence, when sugar is involved in a solubility experiment, sucrose is often used in the test. When sugar is put into the water, the point at which sugar can be dissolved at maximum is called the "saturation point"; sucrose at 25°C will dissolve by 68%. If you raise the temperature, the solubility increases, becoming a supersaturated solution, for example, if you raise the temperature to 100°C, the sugar will dissolve by 82% and when left to cool down at room temperature, without any interference or vibration, you can get a concentrated solution that is in an unstable state. However, if there is shaking or disturbing in other ways, such as having a residue falling into it, the sugar part which occurred by the saturation point will immediately crystallize.

1.3 The boiling point of the sugar solution-The dilution of the sugar solution results in a higher boiling point of the syrup. When the temperature increases, the solution becomes more soluble since the dilution does not reach the saturation point. As a result, the boiling point of the syrup is used to measure the concentration level of the sugar solution (as shown in Tables 1 and 2). As the concentration increases, the water in the sugar solution evaporates, the ratio of sugar to water increases further and the syrups respectively become stickier. Therefore, a principle is applied to prepare syrup at different concentrations for making candy and Thai desserts according to the level of the syrup boiling temperature. Consistent control of the syrup boiling temperature is required because, at different temperatures, the syrup will have physical characteristics difference, due to the control of the temperature of the syrup. Therefore, control of the syrup temperature will produce diverse types of products with unique characteristics, so it is always necessary to have a varied set of temperature levels.

1.4 Sugar crystallization happens when a reduction in the saturated syrup's temperature is lower than the saturated point resulting in the syrup being in an extreme saturated state. As the temperature drops, the more dissolved sugar will exceed the saturated point and begins to crystallize. In contrast, if the syrup is in an oversaturated state after it has cooled down, it would harden immediately without crystallization. As a result, the above principle was applied to produce many kinds of desserts, such as crystalline candy, which contains sugar crystals within its texture. The candy uses syrup that is saturated at the time of crystallization, while non-crystalline candy (hard candy) syrup must be highly saturated and not crystallized when cooled.

Table 1 Boiling points of sugar solutions at different concentrations

Concentration (percent by weight)		Boiling point (degrees Celsius)
Percentage of sugar	Percentage of water	
30	70	100
50	50	102
70	30	106
90	10	123
95	5	140
97	3	151
99.5	0.5	166
99.6	0.4	171

Source: Dhovitayakhun (2013)

1.5 Sugar melting-When sugar was melted with fire, it was found that each type of sugar has a different melting point. Sucrose will melt at 160°C, giving a liquid with a clear appearance, but when the temperature is raised to 170°C or higher, caramelization (burning of sugar) occurs producing a unique smell and color and if heated further, the substance will turn a brown color. Melting of sugar can be used to make various products such as food coloring in black-colored drinks, making caramel-scented candy, etc.

1.6 Sugar gives volume and weight to the product, for example, foods that contain sugar around 70% of the total weight, such as candy, concentrated fruit juice and many more. Sugar helps the product gain weight and provides larger body volume. The use of a large amount

of sugar in a solvent helps the product gain more viscosity and meatiness, which are generally appreciated by consumers. When sweetening agents are used instead of sugar, the amount used will be small because they have 100 to 1,000 times more sweetness than sugar. The product will lose viscosity and volume, so a bulking agent must be used instead of sugar.

1.7 Osmotic pressure-A high concentration sugar solution can draw water out from cells of vegetables, fruits, and meats. It is found in the preservation of foods that utilize sugar. When fruit is covered in a high-concentration syrup, the fruit will become wrinkled as the juice in the fruit is pulled out of the cell by osmotic pressure, which depends on the concentration of the sugar used. The property of osmotic pressure is applied to remove moisture from the food, before being dried by applying sugar mixtures and stored for about 24 hr. A portion of the fruit meat is spread out until the concentration of the syrup is the same as the amount of sweetness of the fruit, so the fruit dries faster.

1.8 Decomposition with acid, When disaccharide is mixed with acid, breakage occurs, especially when exposed to high temperatures (speed up the process). Sucrose breaks down to glucose and fructose, causing changes in sugar properties. Decomposition using acid will depend on the heat used. The type of sugar like monosaccharide often has little to no effect on the decomposition using acid. Decomposition will occur earlier and faster when the concentration of acid is high. Heating time also plays a factor in this process. When the heat is low but given a long heating duration, the decomposition will happen more thoroughly than when the heat is high but given a shorter time.

2. Function of sugar in food

2.1 As a sweetener-Sugar is used commonly in desserts such as Thai desserts, various drinks, etc. Proper use of sugar as a sweetener has many factors to consider, for example, sugar type, concentration, temperature, the acidity of food and other ingredients added to the food, which will affect sugar properties.

2.2 Give food a tasteful smell-Adding sugar to the can make the food smell different, and sugar helps enhance the taste of the food. As seen in the past, it was popular to use sugar instead of MSG to help the food have a rounder taste and reduce undesirable flavors, such as using sugar in sour food to counteract the acidic taste, offering a rounder flavor. The caramelly aroma in milk chocolate products is from the Maillard reaction between the protein in milk and the reducing of sugar in the food.

2.3 It gives texture to the food (body)-Sugar gives a meaty sensation when eating the food, called “mouth feel”, pertaining to the feeling in the mouth. It is often present in drinks that contain sugar.

2.4 Color the food in the so-called caramel color, which is light brown to dark brown. It is formed by heating the sugar until it reaches a temperature of 170°C or higher.

2.5 It allows food to be stored longer-Putting 60-70% sugar concentrated solution will prevent microbial growth. When sugar and “Free” water are combined in the food, there will not be enough water or moisture for microorganisms to grow and sugar can also draw water out of microbial cells. Therefore, sugar was classified as one of the most popular methods of preserving food with fruits and juices such as yam, juice, fruit in syrup., Meanwhile, Foi Thong, Thong Yip and Thong Yod have a shelf life of 3 days at room temperature. However, if you refrigerate it, it can be extended for more than 7 days.

2.6 As a dispersant-many foods use sugar as a dispersant, such as fruit drinks or powdered herbs, powdered gelatin.

2.7 As a lubricant-The sugar solution is an excellent lubricant in food such as salad dressing or a mixture of cake dough. When sugar is added, it makes it easier for food to flow. Similarly, syrup helps act as a lubricant to prevent desserts such as Foi Thong, Med Kanoon, Thong Yip and Thong Yod from sticking together.

3. Factors that may affect the properties of sugar-based food

3.1 The type of sugar used-the difference in sugar’s structure will give different characteristics to the end products. For example, sucrose, a crystalline sugar, should be used to prepare foods that require crystallization, for instance, crispy jelly, pumpkin in syrup, Foi Thong Krob, and Thong Yod Krob. On the other hand, foods that do not require crystallization should utilize fructose because it is difficult to crystallize or may cause sucrose to break down into invert sugar (glucose and fructose) which has reduced crystallinity.

3.2 Food flavor-Each sugar has a unique scent and flavor suitable for different products. Jaggery, for example, is ideal for Thai desserts with a mixture of coconut milk, but not suitable for mixing with bakery products.

3.3 Food color-Some foods do not need an opaque look or distorted color, such as Foi Thong, Thong Yip, Thong Yod, Med Kanoon, candy, juice, ice cream., Therefore, it is preferable to use sugar that does not cause a change in the color of the product, such as white sugar.

However, some products, such as ginger ale tofu, require a caramelly brown color to look more appetizing. Additionally, ginger ales often obtain an appealing color from red sugar.

3.4 The temperature used for cooking-In Maillard reactions, the relatively reactive carbonyl group belonging to the reducing sugar undergoes a chemical reaction with the nucleophilic groups of the amino acid. This reaction triggers the change in colour and facilitates the formation of many flavour compounds. The use of high temperature will result in darkened color and an increase in susceptibility to being burnt. Therefore, it is suitable for some foods that require brown coloration, such as roasted coffee and fried food. However, it may not be ideal for foods that do not require color changes, such as powdered milk.

The temperature which can cause brown coloration is 160-170°C. However, making Foi Thong, Med Kanoon, Thong Yip and Thong Yod will not cause this reaction, due to their temperatures are less than 160°C.

3.5 The amount used-Using large quantities of sugar will help preserve foods or increase their storage time. However, if used in small amounts, it may accelerate the degradation because microorganisms consume sugar as their main energy source.

3.6 Food ingredients - Acidic and basic (pH value) properties in food ingredients, if they are high, may result in sugar breaking, causing changes in food characteristics. In making mango jam, if the usage of conserved mango is the main ingredient, then the resulting product is non-crystallized., While using other mangoes with sourness or acidity, the resulting product is similar but crystallized. Syrup for making Thong Yip and Thong Yod, have a similar result. Some manufacturers add lemon juice to the syrup to prevent crystallization due to syrup making these two desserts have a concentration of 72°Brix (Ketthongkam, 2014), which is high in concentration.

Table 2 The physical characteristics of sugars at boiling points are different

Boiling point (°C)	Physical characteristic name	The appearance of syrup
103	Thread (gloss)[1]	A thin, clear line
104	Large thread[1]	The lines are thicker and harder.
105	Small pearl[2]	Sugar gathers in small drops.
106	Large pearl[2]	Sugar gathers in large drops.
110	Blow (soufflé)[3]	There's a syrup bubble.
111	Feather[2]	It has a feather-like split line.
116	Soft ball[2]	Sugar lumps together into soft lumps.
120	Hard ball[2]	A hard lump of sugar
129	Light crack[2]	It's thin.
133	Medium crack[2]	It's a fragile sheet.
143	Hard crack[2]	It's a hard disk very fast, easily broken.
168	Extra hard crack[2]	The hard plate is brown.
180	Caramel[2]	A crisp brown solid, very fragile.

Remark: 1 Use a test method by using your thumb and index finger to soak it in water, grasp the boiled sugar, and then stretch out your fingers

2 Use a test method by using a spoon soaked in water, dipping the boiled sugar, and dipping it in cold water

3 Use a test method by using a spoon to scoop the boiled sugar and bring it up to blow

Source: Dhovtayakhun (2013)

3.7 Time spent heating-If food containing sugar content is cooked for a long time, the resulting product will become darkened in color. On the other hand, Cooking starch-based food products containing sugar content, for a long time will result in reduced viscosity in the food.

3.8 Product storage period-Sugar can enhance osmotic pressure and reduce water activity (α_w) which affects the inhibition of microbe.

a) Enhancement of osmotic pressure: If the concentration of syrup is low, the microbe will grow by using sugar as a carbon resource. But, if it is high, the microbe cannot grow further because its cell will lose water and cause the cell to die. If it is a spore, the microbe will neither grow nor die. When the osmotic pressure decreases to an appropriate level, it can continue its growth. After the sugar dissolves in water, it causes an increase in osmotic pressure depending on the type of sugar's concentration, whereas monosaccharides will give the osmotic pressure value greater than disaccharides at a higher 10% concentration.

Furthermore, an acidity without nitrogen in sugar solution can enhance bacteriostatic and mycostatic, which are attributes that prevent and inhibit the growth of bacteria and fungi. A concentration of 60-65% is enough for preserving food such as jam, scrambled fruit and acidic fruit syrup. But, non-acidic syrup such as malt extract and honey need 75-80% of sugar concentration. So, the food will be safe from osmophilic yeast and mold (Kyzlink, 1990).

b) attribute of water activity (α_w) when adding sugar into the water, sugar will capture with water's molecule by hydrogen bond, then the α_w decrease. The sugar solution which has α_w = 0.90, will affect normal microbe not to grow, but yeast and mold can do well. Increasing of sugar concentration make α_w decrease which cause microbe to grow less.

However, some microbes can endure high concentration of sugar, such as *Aspergillus glaucus, Saccharomyces roaxii* and *Torulopsis* sp. Moreover, a

yeast in the Zygosaccharomyces group can endure and grow well in high concentration of sugar.

Brix Refractometers

When light enters a liquid at an angle, it changes direction. This phenomenon is called refraction. Light will refract more when travelling through a liquid with dissolved or suspended solids. Therefore, refraction can be used to measure the concentration of dissolved or suspended solids within a solution. Refractometers are scientific instruments that measure refraction angles and correlate them to an already established refractive index.

Degrees Brix (°Bx) is the sugar content of an aqueous solution. One degree Brix is 1 gram of sucrose in 100 g of solution and represents the strength of the solution as percentage by mass. If the solution contains dissolved solids other than pure sucrose, then the °Bx only approximates the dissolved solid content. There is a direct relationship between the refractive index and the final Brix percentage. The Brix Scale calibrates the number of grams of pure cane sugar (sucrose) contained in 100 mL. of water. A refractometer uses the refractive index to convert the raw Brix scale measurement into a weight percentage of sucrose content, and this displays as % Brix (Anonymous, 2020).

The raw materials used to make Foi Thong, Med Kanoon, Thong Yip and Thong Yod

The raw materials are the egg yolk and watery part of egg whites in duck eggs, which are thick, dark, and greasier than hen eggs. However, it has a strong fishy smell compared to hen eggs, which is a disadvantage when using duck eggs. The following paragraphs will explain several things related to eggs in the mentioned desserts.

1. Egg yolk

Its volume is about 31% of the total egg weight and has different parts: the vitelline membrane, the germinal disc, the Latebra, and the yolk, which divide into dark layers alternating with the pale layers. Essential proteins in the yolk are ovovitellin, which is about ¾ of the total protein in the yolk., Glyceride, lecithin, and cholesterol make up the fat within the egg yolk. The yolk color is mostly xanthophylls from green plants and yellow corn the hen ingested. The yolk contains various crucial vitamins, everything except vitamin C. The yolk membrane is a protein similar to egg white protein, and the water can pass in and out of it well enough. The yolk's concentration allows outside water to enter and causes it to expand. When an egg is stored for a long time, the yolk inside will become less swollen. The older the yolk, the flatter and the thinner it becomes. All the minerals in the egg are essential nutrients for the human body, these include iron, phosphorus, sulfur, copper, potassium, sodium, magnesium, calcium, choline, and manganese.

When making Thong Yip and Thong Yod the egg has to be beaten until foam appears. The force from beating will insert air into the egg, it makes the structure change from liquid to a little foam embody together. This foam is the air which enters the protein's structure and other components of the egg. When the beaten egg is bought to a boil in high concentrated syrup or bake, the heat will make the air in the foam expand and give the dessert fluffiness. Mostly, beating the egg causes foam for the dessert to be soft and fluffy. In addition, when the foam is formed a quantity is increased and can rise to 3 times before beating.

2. Egg White (Albumen)

raw egg whites have a slightly yellow-green color. The word "egg whites" comes from when the egg white is slitted and the above slit has a white color (Allus-White). Hence it is called "egg whites". In addition, the egg white may be divided into five categorized layers.

2.1 Outer liquid layer is the outermost layer of the egg white, attached to the eggshell membrane, and is transparent and runny. It is also called "Thin egg white". This part is used as an ingredient to make Foi Thong but uses only a little bit. The purpose is to reduce the yolk's viscosity so that the yolk flows through the small cavity of the sprinkled container and makes it look like a long line.

2.2 The thick egg white (middle dense layer) is light gray, the most common part, next to the outer liquid layer, and it is wrapped around the outer liquid layer and the yolk, preventing the danger of external concussion to the yolk.

2.3 Inner liquid layer is the layer adjacent to the yolk as the part where the yolk membrane holds the yolk in the middle of the egg.

2.4 The chalaziferous membrane is a part of the egg white, which acts as a wrap around the egg yolk and where the yolk attachment pole holds the ligament. It keeps the yolk balance in the middle.

2.5 The yolk attachment pole (chalazae) is a twisted,

glial cord protruding from the yolk membrane on both the oblique and acute sides of the egg, helping to hold it from moving back and forth, but if the egg is kept for a long time, this part will weaken, making it more mobile.

3. Property of hen eggs and duck eggs in Thai cuisine .

Hen eggs and duck eggs can be used differently. In other words, duck eggs are often used for making Thai food and Thai desserts, depending on the purpose of the use. Duck egg yolk is often used for Thong Yip, Thong Yod, Foi Thong., On the other hand, whole duck eggs are used for cooking Thai custard, egg custard pudding., The yolk of a hen egg is used for bakery, and salad dressing, while whole hen eggs are for common foods and sweets. The difference in the usage of egg components is a result of different types and amounts of protein in each egg, which affect food characteristics differently. The components of duck eggs and hen eggs are shown in Table 3.

3.1 Protein-Most egg proteins are water-soluble. Whole eggs contain about 10.7-12.9 g of protein per 100 g. The amount of protein in the yolk and egg white is different because the yolk protein is lipoproteins while the egg white protein is a glycoprotein, each containing the following components:

3.1.1 The protein in the yolk, vitellin, is composed of large molecules attached to lipoprotein, so it is named "lipovitellin" and "ovolivettin".

3.1.2 Proteins in egg white are mostly water-soluble, second-most to water, and proteins in egg whites have various properties. Each has different properties, as the author will soon explain in paragraphs under Table 3.

Table 3 Food Composition of Thai Foods

Nutrient	Components of duck egg			Components of hen Egg		
	a whole egg	White eggs	Egg yolk	a whole egg	White eggs	Egg yolk
Moisture (g)	70.6	87.8	47.5	73.7	87.4	52.1
Energy (Calorie)	188	50	368	163	52	336
Fat (g)	14.2	0.1	32.3	11.5	0.2	29.0
Carbohydrate (g)	0.7	0.8	4.8	0.8	1.1	0.9
Fiber (g)	0	0	0	0	0	0
Protein (g)	13.2	10.7	13.6	12.9	10.7	16.3
Calcium (mg)	64	6	146	61	10	154
Phosphorus (mg)	220	8	328	222	13	479
Iron (mg)	3.6	Tr.	5.6	3.2	0.4	6.3
Vitamin A (I.U.)	1,541	0	6,575	1,950	0	4,025
vitamin B1 (mg)	0.16	Tr.	0.94	0.10	0.01	0.24
Vitamin B2 (mg)	0.40	0	0.94	0.40	0.32	0.47
Niacin (mg)	0.2	0.2	0.2	0.1	0.1	Tr.

Source: Origin, Bureau of Nutrition, Department of Health, Ministry of Public Health (2018)

1) Ovalbumin is the most common protein in egg whites, accounting for 75% of the total protein content in egg whites. Ovalbumin is composed of carbohydrates and phosphates. It has properties that help gel formation and foam formation; this protein changes properties when heated.

2) Ovomucoid is a glycoprotein containing 14% glucosamine and 7% mannose, which will change in properties upon exposure to heat, but is more heat-resistant than ovalbumin and Ovotransferrin (conalbumin).

3) Ovomucin is a protein that forms the gel form of the condensed egg white by forming a mesh structure, and the amount of ovomucin in the clear egg white is differentiated by 5.11% ovomucin. The inner part of the clear egg contains 1.91%, about four times more ovomucin in the thick egg white than in the clear egg white. Ovomucin has water-insoluble properties, each soluble in a saline solution at pH 7.2-10.4. And when stored for a long time, the pH of the egg increases, causing ovomucin to secrete, the condensed egg white becomes liquid egg white and another important property of ovomucin is that it is a substance that helps stabilize the foam generated by beating.

4) Ovoclonalbumin or "ovotransferrin" has the property of inhibiting bacterial growth and when heated, it is easier to change its properties than ovalbumin and coagulation occur at 63°C, which is the same temperature at which the egg whites coagulate.

5) Ovoglobulin is a protein with a property that helps cause foam in the egg white.

3.2 Fat is abundant in about 29-32.3 g in 100 g of yolk, while very little fat is found in egg white, around 0.1-0.2 g in 100 g of egg white. Most of the fat in the yolk is a saturated fatty acid and partly cholesterol. One large egg consists of 5 g of fat, 2 g is saturated fat and 213 mg of cholesterol. The type and number of fatty acids will change according to the food used to feed animals.

3.3 Carbohydrates are rarely found in eggs. They are often found in free forms, such as glucose, with proteins in the form of glycoproteins found in egg whites.

3.4 Water is present in all parts of the egg. An egg has a 65.5% water content of the whole egg weight. The amount of water varies according to each part of the egg. The egg white has the largest amount of water, causing osmosis pressure, which creates movement from the egg white into the egg yolk. When stored for a long time, egg yolk size will increase. The yolk will not be in the

middle, and when you poke it out, the yolk becomes flat and breaks easily.

3.5 Minerals, essential minerals in eggs, consist of sulfur, potassium, sodium, calcium, magnesium, and iron. The number of minerals will also change according to the animal environment, the food used, the season, and the age.

3.6 Vitamins in eggs, vitamins both dissolve in fat and water. Most vitamins are dissolved in large amounts of fat found in the yolk. Vitamin B2 and niacin are also found in the yolk. All water-soluble vitamins were found within egg whites except vitamin C.

Dessert type boiled in syrups using egg as a component

1. Foi Thong

Foi Thong is a dessert made of yolk and an outer liquid layer (Thin egg white). In the past, only duck eggs were used. Hen eggs were rarer because Thai people did not usually raise a chicken to get eggs. Duck eggs had a stronger smell than hen eggs, so when baking using duck eggs, jasmine flowers are put into the syrup, or pandan leaves are boiled to make the syrup to cover the smell of the eggs., When using the eggs, they need to be washed and dry. The egg then is cracked into a bowl and the eggshell is kept because the eggshell can be used to purify the syrup. When cracking an egg, separate thin egg white from every egg, and the storing of thin egg white is done by quickly turning the eggshell upside down when the clumpy egg white and yolk go down, you will see a little bit of clear water left over, pour all thin egg white into a cup together. However, the author has an easier way of storing thin egg white: First, cracking the egg into a container. Second, separate the yolk using both hands. Third, separate the yolk, and gently remove the stuck egg white on the yolk. Fourth, remove the yolk. Fifth, pour the egg white through a sieve (no need for a spoon or stirring with a ladle). The eggs that can flow through the sieve are thin egg white and the remaining eggs on the sieve are thick eggs white. Sixth, separate the thin egg white and keep them for making Foi Thong. Then put the yolk ironed through a thin white cloth to separate the yolk membrane. Lastly, mix the yolk and the thin egg white lightly. Do not beat until foam, or else the Foi Thong will not form long lines.

In the old days, syrups were prepared by putting small pieces of eggshells together with sugar, jasmine float and pandan leaves then put on the stove. Eggshells help the syrup to have a clear color. The reason why it helps the syrup to obtain a clear color can be scientifically explained. That is the egg white attached to eggshells catches sediment and dust particles attached to sugar. The syrup at the end of the process will need to be filtered through a cloth to get a clear color syrup as the final product. After the syrup has been made, it needs to be put on a heated stove and sprinkled in the Foi Thong, which requires banana leaves to be made into cones for sprinkling. Few cones may be made and then sewn together with a brooch and then lap the prepared egg into a cone, allowing the egg to flow out in a continuous, uninterrupted line. Sprinkle over boiling syrup into a circle, and when you have enough Foi Thong, stop sprinkling, and when the eggs are cooked, use sharp sticks, chopsticks, or long small bamboo to lift them from the syrup and fold it.

The early versions of Foi Thong are made by using the banana leaves cone to have a large plate shape. These versions of Foi Thong are not as finely shaped as they are today because the latter developed tools, stainless steel cones, or brass cones that make the Foi Thong simple, easy, more beautiful, and more delicate. The complicated methods of making Foi Thong make it unpopular for home cooking and are often made for various merit works.

When the author makes syrup for making Foi Thong, the ratio of white sugar to water is 1: 1.5, boiling at the highest boiling point. Temperature and sweetness measures are 103°C and 55-57°Brix, respectively and the viscosity of the syrup is only slightly thickened. When scooped up, it forms a thin, straight, and soft line (thread/gloss), consistent with Dholvitayakhun (2013). The Foi Thong made by the author, on the other hand, forms into thin smooth circular thin straight lines, sticky, soft, silky, glossy, nicely coated in syrup, and not very sweet. However, Foi Thong made without adding any water, Foi Thong will still be round and sticky, as the sugar coat and grips on to the Foi Thong more than usual, but the glossiness will decrease due to the high concentration of sugar, which from crystallizing sugar, the softness will decrease and turn crispy instead, and has a sweeter taste. Therefore, syrups at high concentrations are used to make Foi Thong Krob (crispy Foi Thong), so they are not suitable for soft Foi Thong. The sweetness of such syrup is not consistent with the data of the study, Mongkonworawan et al. (2002) found that Foi Thong made in 68±1°Brix concentration syrup is rich and the Foi Thong is formed into soft round lines, but Foi Thong production method of Mongkonworawan et al. (2002) was stirred in clear syrup to reduce sugar concentration,

but Foi Thong line is still round and soft.

2. Med Kanoon

Syrups for making Med Kanoon has white sugar to water ratio at 1: 1, boiling for about 5-7 minutes until measured temperature and sweetness are at 104°C and 65-66°Brix, respectively. The characteristics of syrup are thicker and harder (Large thread) consistent with Dholvitayakhun (2013). The reason why the syrup of Med Kanoon is more saturated than Foi Thong is that the high-concentration syrup increases osmotic pressure to cook the yolk until pea conserved coat is tightened to maintain the shape of a coating film, this will not break the pea conserved, creating beautiful pellets, and a smooth yolk coating surface. But if the concentration of syrup is greater than this, Med Kanoon from the syrup will not be as glossy when the temperature drops, because the sugar will crystallize.

3. Thong Yip

Egg preparation is done just like Foi Thong, and the only difference is that yolk does not use thin egg white, do not have to filter through a thin white cloth (there are two types of syrup used: a very strong syrup for the egg to be cooked, and the other is a very low concentration syrup with a normal temperature for soaking the cooked egg, used to lower the temperature of the egg so that it can be picked without burning the hands.) and the yolk needs to be beaten until foaming. In the past, brass with a spring-like appearance was used and there were three lines with a long handle for holding. The eggs must be beaten until the foaming is smooth. Drip the egg in syrup into a small circular sheet and when the egg is cooked, the edges are inflated, not flattened. After a minute or two, use a skimming ladle to flip the other side of the egg into the syrup and when the egg meat is cooked, lift it from the syrup and soak it in the cooled clear syrup. When the temperature of the egg drops enough for the hand to endure, quickly grasp the petals, the grip of the petals ranges from 3, 5, 6, 7, 8, 12 and 16 and the more the petals, the more skilled the maker is.

In the early days when Thong Yip was made, only three petals were caught. Later, the petals were added, because the more the petals, the more they showed effort, intention and creativity, which was a common practice to elevate Thai desserts in that period. In general, only five Thong Yip pedals are made for each Thong Yip because it is not too difficult to make and gives the Thong Yip a beautiful petal shape. When you get the petals to be as pretty as you prefer, put them in a lattice or bottle cap to achieve the shape of a cup, let it cool and when you serve it, use a stick to pull out it from a bowl, put it in a plate, cup or another container.

Condensed syrup to make Thong Yip has a white sugar to water ratio of 1.5: 1, boils, and continues to boil for about 10-15 min, temperature and sweetness are measured at 110°C and 70-72°Brix, respectively. Syrup, while boiling, will foam into small bubbles throughout the pan. The syrup when flowing down from a scoop will form a thread-like line about 1-2 inches long (Thread) following Sinthawalai (1982). The concentration of syrup at different boiling points was said to be at 110-112 °C, the syrup boils up to medium-sized bubbles and the color is clear, the syrup is in the form of a thread, a line or spout about 2 inches long. It was used for making Thong Yip, Thong Yod, Foi Thong, and Krobkam. Meanwhile, A Cuisine (2022), Mongkonworawan et al. (2002) and chemistry of textiles, food, and surroundings (2016) provided information on the syrup concentration of 74±1°Brix stating that it gives softer Thong Yip and makes it so that yolks in the syrup will not spread as much, making it easier to be shaped into petals. However, this information did not match the concentration the author uses. The author's syrup concentration to make Thong Yib is at 71-72 °Brix, which gives Thong Yip a fluffy, dripping syrup, and soft characteristics, and the shape stays when formed into petals.

4. Thong Yod

Preparation of eggs and syrup are like those of Thong Yip. The syrup of Thong Yod when boiling is a pan full of fine bubbles (like boiling coconut milk or boiling milk). Boiling syrup helps to hold the Thong Yod into round and fine grains. The eggs are beaten slightly more than Thong Yip. Mix the rice flour baked in candle smoke until fragrant (now there are Thong Yod prebaked in candle smoke. The aroma of the flour depends on the brand of the flour.) then slowly knead lightly with a spoon to mix until the whole mixture is uniform, but do not stir for long or the ingredients will be sticky. If a person beats the egg too fast until the foam is dissolved, the dessert will not get a pretty drop shape., The shape of the drop of Thong Yod must be like a drop of water. The drop is made using the index finger, the middle finger and the thumb to scoop the dessert out of the index finger to drop into the boiling syrup. Some people may use the tip of a short spoon to spread the ingredients from the edge of the cup and then push the mixture into the syrup. When Thong Yod is ripe, there is no white part seen in the center, soak up the clear syrup to reduce the sweetness.

As for the concentrated syrup used to make Thong Yod, the ratio of white sugar to water is 1.5: 1, boil and continue to boil for about 15-17 minutes, temperature and sweetness are measured at 112°C and 72-74 °Brix, respectively. Syrup, while boiling, will foam into small bubbles throughout the pan. The flow characteristics of syrup are thread-like, about 1 to 2 inches long (Thread) same as the one for Thong Yip, consistent with Ketthongkam (2014). The concentration of syrup used for making Thong Yod is 72 °Brix, and the Thong Yod will be syrupy, soft and has a droplet-shaped. If less concentration of syrup was used, the Thong Yod will not have a rounded shape, it will be flat, and roughened. While the excessively high concentration will result in a Thong Yod that does not carry syrup and the gel of protein will be formed before the syrup is penetrated to the center of Thong Yod. On the other hand, the data of A Cuisine (2020), Mongkonworawan et al. (2002), and "The chemistry of textiles, food and surroundings" (2016) provided information on the concentration of syrup in making Thong Yod equivalent to 71±1°Brix. Mongkonworawan et al., (2002) found that Thong Yod using such syrup concentration has a droplet-like shape, syrupy and had less hardness than at concentrations of 68±1 and 74±1°Brix.

5. Clear syrup - There are 2 parts of syrup for making Thong Yip and Thong Yod: clear syrup and concentrated syrup. The clear syrup is used to soak the dessert after they are cooked in the concentrated syrup. The purpose of soaking dessert in it is to reduce the sweetness of the syrup within the dessert using the osmosis principle and to reduce the temperature of the dessert so that it can be picked by hand, in the case of Thong Yip. The ratio of white sugar to water in the clear syrup is 1: 2. Boil until peak boiling point and temperature and sweetness measure reached 100°C and 36-38°Brix, respectively. The sweetness of this clear syrup will dictate how much sweetness is given to the dessert. It is found in Ketthongkam (2014), A Cuisine (2020), Mongkonworawan et al. (2002) and "The chemical physics of textiles, food and surroundings" (2016) mentioned the concentration of clear syrup at the sweetness of 48±1°Brix. At the present, there is a campaign to reduce sweetness and reduce salt. As a result, the author was frequently asked when teaching how to make Thong Yip and Thong Yod whether it would be possible to lower the sweetness of this kind of dessert. The answer is that we can't lower the sweetness of the concentrated syrup, but we can lower the sweetness of the clear syrup. Therefore, it is the origin of the clear syrup concentration of 36-38°Brix.

Conclusion

In conclusion, the concentration of syrup has an effect to give desirable attributes to each dessert. Foi Thong should have the concentration of syrup at 55-57 °Brix, the dessert is a round thread, sticky and glazed. Med Kanoon should be at 65-66°Brix, it is glazed, sugar non-crystalline sugar. Thong Yip should be at 70-72°Brix, it is fluffy, sticky, and juicy with syrup. Thong Yod should be at 72-74°Brix, it has a droplet-shape and is non-flat, fluffy and juicy with syrup. The concentrated clear syrup at 36-38°Brix which is used to soak Thong Yip and Thong Yod may be reused to dilute Foi Thong's sweetness.

However, to make these kinds of desserts, the maker must be relatively skilled, especially to produce different kinds of syrup needed for different types of desserts. The author, practiced making Thong Yod and Thong Yip many times before achieving the intended shape, flavor, uniformity, and other desired characteristics. These experiences led the author to conclude that the sweetness of the clear syrup determines the sweetness of Thong Yip and Thong Yod. When the strong syrup is used to make Foi Thong, one should stir it in clear syrup first to reduce sweetness because consumers have become more health-conscious.

References

A Cuisine. (2019). *"Gold dessert" Many stories in the Thai dessert stand.* Retrieved April 21, 202 2 from https://acuisineth.com/food-story

Anonymous. (2020). *What is a Brix refractometer, and how do they work?* Retrieved April 21, 2022 from https://www.instrumentchoice.com.au/news/what-is-a-brix-refractometer-and-how-do-they-work

Bureau of Nutrition, Department of Health, Ministry of Public Health. (2018). *Food composition table of Thai foods.* Retrieved April 22, 2022 from https://nutrition2.anamai.moph.go.th/th/thai-food-composition-table/download

Bureau of Nutrition, Department of Health, Ministry of Public Health. (2002). *Fatty acids composition and cholesterol in Thai foods.* Retrieved April 22, 2022 from https://nutrition2.anamai.moph.go.th/th/thai-food-compositiontable/download

Dholvitayakhun, A. (2013). Food experimental (2th ed.). Bangkok: Odeans store.

Ketthongkam, P. (2014). *The production of simulated egg yolk for Thong Yoad (Thai dessert)* (Master's thesis). Bangkok: Kasetsart University.

Kyzlink, V. (1990). *Principle of preservation*. Amsterdam: Elsevier.

Mongkonworawan, K., Jangchud, K., & Wongtong, O. (2002). The effects of syrup concentertion on the qualities of Kha-nom Tongyod, Tongyib and Foytong. In *The 40th Academic Conference of Kasetsart University* (pp. 454-461). Bangkok: Kasetsart University.

Simons, C. (2022). *Properties of water*. Retrieved May 1, 2022 from https://cwsimons.com/properties-of-water

Sinthawalai, S. (1982). *Sugar*. Retrieved May 1, 2022 from https://sites.google.com/sitexxyphuchsersthkic/khunsmbati-kayphaph-khxng-natal-thray

Suan Dusit Poll. (2021, 7 November). *Thai dessert and Thaipeople, Suan Dusit University*. Retrieved May 1, 2022 from https://suandusitpoll.dusit.ac.th

The chemistry of textiles, food and surroundings. (2016). *The concentration level of syrup in making egg desserts or "Golden Desserts"*. Retrieved May 1, 2022 from https://www.facebook.com/textile.phys.and.chem/photos/

Guidelines for Writing and Submitting Original Manuscripts for Publication in Journal of Food Health and Bioenvironmental Science

Journal of Food Health and Bioenvironmental Science is an academic publication that aims to publish manuscripts such as original articles, review article, and book review in the fields of food, health, biological and environmental disciplines and other related fields.

The journal is published thrice annually. All manuscripts accepted for publication are copyrighted by Suan Dusit University; reproduction, in whole or in part, requires written approval from Suan Dusit Unversity. Excluding errors incurred during the printing process, all content contained within articles is the author's responsibility.

To encourage and support researchers, all the publication process is free of charge.

Publication Process

1. The journal accepts original manuscripts for consideration, from January to December. Due to self-supporting of Journal of Food Health and Bioenvironmental Science, authors are required to pay 500 Bath as a processing fee and 3,000 Bath for peer review process after the submission
2. The editorial board adjourns to consider the merits or submitted manuscripts and the scope of the journal. During this phase the integrity and accuracy of the manuscripts content is assessed.
3. An editorial letter is issued to the author for manuscripts that the editorial board deems inappropriate for publication. If the editorial board approves the manuscripts, an editorial letter will be sent to the author and the article will be subjected to peer review.
4. Articles that are deemed appropriate for publication are subjected to peer review by a panel of three experts in the appropriate field. In order to be deemed appropriate for publication, an article must be by recommended two of the three experts via the double-blinded review system.
5. The qualitative assessments of the expert panel returned by the manuscript’s author. The author is expected to make the appropriate alterations indicated by the experts’ feedback.
6. The author returns the edited document; the editorial staff examines the changes to make sure they are congruent with the experts' recommendations as well as the journal format.
7. The revised version is granted the University's recognition of “Accepted” for publication status with the Journal of Food Health and Bioenvironmental Science Stamp on every page. Information regarding publication status (Accepted) is located on the journal's website (http/ research dusit ac.th/new/e-Journal)
8. The editorial tearm conducts an accuracy check for all articles before sending the manuscripts to the printer to create a draft journal issue.
9. The editorial board conducts a review of the draft journal issue before publication on the journal's website (http://research.dusit.ac.th/new/e-Journal). Suan Dusit University will place their official seal of approval on each page of the manuscript and to verify before formal publication.
10. Upon approval by each author, the final version of the journal will be published as a physical journal and online publication, accessible on website (http://research.dusit.ac.th/new/e-Journal). Together with sending a physical journal to peer reviews, authors and involved sectors.

Publication Criteria

1. The original manuscript is concise and interesting to the academic community.
2. The content of the manuscript represents quality and theory of the discipline and also possesses knowledge with practical applications.
3. The manuscript's content is consistent with the aim and scope of the journal.

4. Manuscripts submitted to Journal of Food Health and Bioenvironmental Science must not have been published previously in or actively involved in the publication process of another journal.

5. All content within the manuscript must be the product of the author himself. Any use of intellectual property within must be appropriately credited to its original authors.

6. The author must comply with the writing style established by Journal of Food Health and Bioenvironmental Science.

7. There are four levels of assessments given to reviewed manuscripts:

7.1 Requires minor or no revisions prior to publication.

7.2 Requires moderate revisions prior to publication.

7.3 Requires intensive editing and revisions followed by a future evaluation. 7.4 Unsuitable for publication

In order to be assigned the "Accepted" status, an article must be assessed as "Requires minor or no modification prior to publication" by two of the three experts from the peer review process.

Formatting Guidelines

It is the author's responsibility to format manuscripts to the standards of Journal of Food Health and Bioenvironmental Science. The details of format style are contained herein,

1. Format

1.1 Single page printing on A4 paper with a width of 19 cm and height of 26.5 cm. The vertical and horizontal spacing from the margins must be 3.5 cm and 2.5 cm, respectively.

1.2 Typefaces and layout: English must be typed using Time New Roman using Microsoft word. Specific font format guidelines are as follows.

1.2.1 The header contains the page number, aligned on the right side, in 12 pt. font.

1.2.2 The title in English languages must be 12 pt. font, bolded, and center aligned. The title should not exceed two lines of text.

1.2.3 The author's name in English language must be typed 9.5 pt. font and centered below the title. Asterisks (*) should proceed the authors' names which is correspond to the appropriate author.

1.2.4 Affiliations should match each author with their appropriate affiliated institutions and organizations. In case of different affiliations, superscript numbers should follow the surname a and affiliation a.

1.2.5 A footnote must be placed on the first page of the article with the text "*Corresponding Author", and the next line of text should contain "e-mail".

1.2.6 "Abstract" in English must be 9.5 pt. font, bolded, left aligned, and placed below the Thai keywords section. Abstract text must be 9 pt. font, with 1 tab indentation from left and right margins.

1.2.7 "Keywords:" should appear in English language in 9.5 pt. font, placed beneath the English abstract text and be aligned with the left margin. English keywords must be 9 pt. font, and should not exceed four words. Each keyword should be separated by a comma (,) and space.

1.2.8 Regardless of language choice, the main text headings used throughout the paper must be 9.5 pt. font, bolded, and aligned with the left margin.

1.2.9 Bulleted items must appear as 9 pt. font, bolded, and be indented 1.5 tabs from the left margin.

1.2.10 Body text must appear as 9 pt. normal font, and be indented 1 tab from the left and right margins.

1.2.11 "References" must be 9.5 pt. font, bolded, and be aligned with the left margin. Individual entries must be 9 pt. font and should follow American Psychological Association (APA) formatting guidelines. Any lines of text for a single entry that exceed the first line should use a "hanging indent" of 1.5 tabs from the left margin.

1.3 An appropriate page length for publication in the Journal is approximately 15 pages.

2. Citing

Should follow American Psychological Association (APA) formatting guidelines. Click http://jfhb.dusit.ac.th/flie/Ref%20Guidelines. pdf to see the example.

3. Ordering of Titles in Journal of Food Health and Bioenvironmental Science

The written manuscript may contain only English. The content should be easy to understand and clear. If the author uses abbreviation, full word must appear before any abbreviation.

3.1 The title should be brief, the length should not exceed 100 characters.

3.2 The authors if there are more than six authors only the first author is listed, followed by "et al."

3.3 Affiliated entities associated with the author should appear in English languages.

3.4 The abstract must be written in English language. The abstract should briefly summarize the research and not exceed 250 words or 15 lines of text.

3.5 The "Keywords" section must contain no more than four keywords that allow for appropriate searching and selection based upon the article's topic.

3.6 The "Introduction" section should provide background information relevant to the research, provide information regarding the manuscript's content and state the objectives of the work.

3.7 The "Materials and methods" section delineates the procedures, how the research was conducted, sampling method (i.e. simple random samples) and population, and the creation and development of research tools used for data collection and analysis.

3.8 The "Results" section or "Results and Discussion" presents data obtained during the research and may be displayed as tables, graphs, illustrations, and accompanying explanations. Tables should be not have left and right borders and are normally black and white printed. No more than five tables should be present in the "Results" section. Pictures within the section should be clear and use simple black and white coloring with an accompanying caption, the author wishes to use colors for any item they may do so; however, the author will be responsible for the additional costs of color printing.

3.9 The "Discussion" section or "Result and Discussion" should explore the significance of the results of the work and address whether or not the data support the research hypothesis and compare research findings to other similar research works.

3.10 The "Conclusions" section should summary of the main topic covered or a re – statement of the research problem.

3.11 The "Acknowledgements" (if any) section should provide help during the research (e.g., providing materials, laboratory, equipment, etc.) and funding.

Sending Original manuscript

1. Compose the manuscript using the format of the Journal of Food Health and Bioenvironmental Science.

2. Send the manuscript via ScholarOne website https://mc03.manuscriptcentral.com/jfhb

Journal of Food Health and Bioenvironmental Science Publication Ethics

Editorial Regulations

- It is the duty of the Editors to consider the submitted manuscripts related to field of food, health, biological and environmental disciplines and other related fields. The consideration will be based solely on the content. The ethnicity, country of origin, gender, sexual orientation, political affiliation, or religious belief of authors does not influence the editor's decision.
- Throughout the submission, the editors must not share the information about the submissions to anyone except the authors, reviewers and JFHB staffs.
- Editors must make sure the manuscript has no substantial vested interests authors or affiliated organizations.
- The editorial staff have to assure that the manuscript has been peer-reviewed by at least two reviewers in the field of Food, Health, biological and environmental disciplines or other related field appropriate for each manuscript. The editorial staffs also have to be careful about the copyright Infringement, falsification of data, and plagiarisms. If there is an offense according to the said regulations, the editor must investigate and seek for evidence before consider reject the manuscript.
- In case that the Editors suspect that the manuscript might have been published elsewhere, the editor should investigate and consider reject.
- In case of unethical publishing practices that are later uncovered, the action will still be taken seriously.

Reviewer Regulations

- Reviewers are expect to give constructive and professional comments. Improper criticism must be avoided.
- If the manuscript given is lies beyond area of expertise, the reviewers should inform the staff immediately.
- Reviewers must keep the manuscript confidential. Do not share any information of the manuscript to anyone other than the editorial staff.
- In case that the reviewers find that the other works contained in the manuscript are not well credited, reviewers are required to inform the editorial staff.
- If there are conflicts of interests, reviewers should inform the editorial staff. Editors will decide whether the reviewer is appropriate for the manuscript or not.

Author Regulations

- The authors should write the manuscript related to the theme of Food, Health, biological and environmental disciplines. The research manuscript should contained relevant background information, efficient methodology, APA style citation, accurate results, and reasonable discussion.
- The authors should follow the journal guidelines strictly.
- Any opinion or perspective made in the manuscript must be explicitly highlighted as "opinion" or "perspective"
- The authors must be careful and aware that fraudulent information and omission of important information are unethical author behaviors.
- The authors must be able to provide research data if the Editor see needed.
- Authors must reference other works properly. Any work involved in the manuscript also must be well credited.
- The authors must make sure that the manuscript has not been published elsewhere before and is not currently in the publication process in other journals.

- The person must have made significant contributions to the manuscript, participate and give important efficient content during revisions and provide approval for publication in order to be listed as an author. Researchers who do not meet the above criteria should be listed in the Acknowledgements section.
- Author should identify any conflicts of interest that might have influenced the data and/or interpretations of data.
- To make the efficient revision, the authors should respond to all the given critiques and suggestions during the revision.
- If the authors find errors in their works that need to be correct, the author should inform the editors immediately.

Journal of Food Health and Bioenvironmental Science

Vol. 15 No. 3 September – December 2022

Original Articles

Review article

RDI
Research and Development Institute
Tel: 0-2244-5280-2 Fax: 0-2668-7460
Website: http://research.dusit.ac.th