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Characterization and Properties of Chitosan/PVA Bio-based Film Incorporated with *Clitoria ternatea* L. (Butterfly pea) Extract and Its Application in Foods

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Abstract

Natural anthocyanin pigments gained awareness to use to improve the function of food packaging based on dye sensitivities when chemical composition changes. In this study *Clitoria ternatea* L. (Butterfly pea) extract (10-30%) was incorporated into chitosan/poly-vinyl alcohol (PVA) based film to produce pH-sensing elements. Physical, mechanical and barrier properties of film, e.g., swelling index, thickness, water vapor permeability coefficient (WVPC) and tensile strength were evaluated. Film incorporated with 30% *Clitoria ternatea* L. extract showed an increase in film thickness and decreased in tensile strength and swelling index. No significant difference ($p>0.05$) was observed in WVPC between treatments. Films were immersed in six levels of pH buffer (1, 3, 5, 7, 9 and 12) and exhibited color response ranging from brownish-red (acidic), bluish-green (neutral) and yellow (basic), respectively, within 15 min which was confirmed by color parameters (L*, a*, b*, chroma and hue angle). Total color different (ΔE) greatly rose under extreme pH conditions (pH 1 and 12). Films were also performed on a model of low (pasteurized milk and chicken meat) and high (fresh-cut pineapple) acid foods. The visual color of film responded when pH shifted, which changed into pale pink (pasteurized milk), green (chicken meat) and red (fresh-cut pineapple) compared to an initial color (bluish-green). This study revealed that an embedding of *Clitoria ternatea* L. extract into chitosan/PVA based films had a pH-sensing potential material for application in smart packaging.

Introduction

In the current consumer environment, quality and safety of food are important factors that consumers are concerned about. The modern consumer demand is for convenience products due to changes in modern lifestyles, however, food safety must go hand in hand with good nutrition. Therefore, fresh food and minimally processed products are now gaining greater attention, especially among the healthy-lifestyle consumer because of the high nutritional content. However, shelf-life of fresh food is short and must be stored in a temperature controlled condition throughout the storage periods. Likewise, foods that have been on the shelf for a long

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period result in a reduction of food quality which increases with storage times. Intelligent or smart packaging has been developed for solving this problem and guaranteeing quality, food safety, and traceability (Vanderroost et al., 2014). The system comprises a smart function such as sensing, detecting, tracing, recording, communicating and sending information to the external environment (Schaefer & Cheung, 2018). Examples of intelligent packaging are radio frequency identification (RFID) sensors (Vanderroost et al., 2014), time-temperature indicators (Lu et al., 2013b; Pereira et al., 2015; Wu et al., 2015), gas sensing devices (Vu & Won, 2013), freshness and ripeness indicators (Kuswandi et al., 2013), and a microbial indicator (Yousefi et al., 2018).

Freshness sensor offers quality data resulting from microbial metabolism or chemical change of the food product, based on a color shift (Vanderroost et al., 2014) which is made by embedding a pigment in various kinds of material such as natural polymers and paper. Currently, natural pigments are noticeable used instead of artificial dyes such as phenol red and bromothymol blue (Hidayat et al., 2019), since its plays an important role in non-poisoning and widely response to pH ranges (Kungsuwan et al., 2014). Anthocyanin, a water-soluble natural pigment, has a great potential as a pH sensor which is exhibited in red, purple, or blue based on the pH of the environment. Moreover, the expression of anthocyanin color is markedly influenced by its structure, temperature, pH, ultraviolet light, and oxygen (Kungsuwan et al., 2014). Extraction of anthocyanin from red cabbage (Pereira et al., 2015), grape peel (Golasz et al., 2013), blakeana flower (Zhang et al., 2014), rose flower (Shukla et al., 2016) and purple sweet potato (Liu et al., 2017) have been noted for utilizing in a smart packaging system.

Clitoria ternatea L. flowers (Butterfly pea) are a perennial herbaceous plant with a distinctive deep blue color that grows in Tropical Asia, and are found in Africa, Australia and America (Sinha et al., 2012). In Asia, a blue color of *Clitoria ternatea* L. is widely used as a natural coloring for foods (Kungsuwan et al., 2014), textiles and other industries (Sinha et al., 2012). The color of anthocyanin extracted from *Clitoria ternatea* L. can shift to various shades which show a red color in acid, blue in neutral and green in base condition (Kungsuwan et al., 2014). The embedding *Clitoria ternatea* L. extracted in polyvinyl alcohol (PVA) film showed positive response to NH_3 vapor as a model volatile amine. However, the limitation of PVA based film has shown poor performance and appearance because of a swelling problem in the excess moisture environment which should be modified by blending with other polymers (Sukprasong, 2013). Based on the results of the above study, there is a possibility to use anthocyanin from *Clitoria ternatea* L. in an intelligent packaging system. However, studies on the blend of PVA with other polymers, a composite film, are needed to reduce their limitations accordingly. The ratio between chitosan and PVA embedded with fresh Butterfly pea extract was noted by Hidayati et al. (2021). However, the optimum anthocyanin concentration to produce the colorimetric pH indicator and its application in various kinds of food have not been reported.

Currently, the demand for modern packaging in the global food industry is growing continuously in order to provide food safety and shelf stability. Unfortunately, it also brings a pollution crisis on the resources and the environment. From this perspective, increase interest is occurring in environment-friendly packaging materials, a natural source, such as polysaccharide (gum, starch, chitosan, cellulose, and their derivatives), protein (whey, soy, corn, zein, gelatin and wheat gluten, etc.) and lipid (Mangaraj et al., 2019). Chitosan is a cationic amino polysaccharide derived from chitin (N-deacetylated derivative) which has been widely used as edible coating and film for preserving food qualities. The properties of chitosan film depends on the source (molecular weight and degree of methylation), solvent, drying condition, temperature and storage time (Park et al., 2002). However, low mechanical strength is a limit of chitosan film, so other polymers are usually incorporated, e.g., PVA (Pereira et al., 2015) which is non-toxic, biodegradable and a water-soluble. PVA is often used by mixing with other types of polymers for reducing the water solubility and improving mechanical properties (Jayasekara et al., 2004). Higher molecular weight of chitosan improved the strength of the film but did not affect water vapor permeability (Park et al., 2002). Moreover, the antimicrobial effect of chitosan has been noted (Zivanovic et al., 2005). There have been reports that a blend of chitosan and PVA cross-linked with formaldehyde using glycerol as a plasticizer enhanced thermal stability by reducing the percentage of weight loss (Abraham et al., 2016). Liu et al. (2018) found that chitosan/PVA blended film (75:25) prepared by electrospray technique improved the permeability of water vapor and showed a higher antimicrobial effect. Thus, the physical, mechanical, and barrier properties of film are an important factor for food packaging in order to be durable to use in various storage

conditions. These factors also has an effect on the quality and shelf life of food. The potential of chitosan/PVA solution or film for application in contact food has been reported in minimally processed tomato (Tripathi et al., 2009), sliced fresh *Channa argus* (Lu et al., 2013a) and chicken sausage (Nwabor et al., 2020).

The objective of this study was to evaluate the performance of a chitosan/PVA based film incorporated with an anthocyanin extract from *Clitoria ternatea* L. as pH-sensing elements. The efficacies of the sensing film were tested in various pH-solutions and both low (pH > 4.6) and high acid food (pH < 4.6) samples. Mechanical, physical and barrier properties of films were also investigated.

Materials and methods

1. Raw material and reagents

Dried *Clitoria ternatea* L. was bought from domestic markets in Sathorn District, Bangkok, Thailand. Chitosan medium molecular weight with 75-85% deacetylate was obtained from Sigma-Aldrich, Germany. Sodium tripolyphosphate and polyvinyl alcohol were used as food grade, which was obtained from Chemipan, Thailand. Sodium dihydrogen phosphate (Ajex Finechem, Australia), disodium hydrogen phosphate (Loba Chemie Pvt., Ltd, India), lactic acid (Loba Chemie Pvt., Ltd, India), acetic acid (food grade; QP, Thailand) and sodium hydroxide (NaOH; Sigma-Aldrich, Germany).

2. *Clitoria ternatea* L. extraction

Clitoria ternatea L. was extracted according to the method of Pereira et al. (2015) with minor modifications. Fifty grams of dried *Clitoria ternatea* L. was ground in 150 mL of the mix solutions of water and ethanol (7:3) followed by filtering using cotton cheesecloth. The extract was adjusted to pH 2.0 using 1 mol/L HCl and kept at 5°C in the refrigerator for 24 h. After that, the extract solution was filtered again through Whatman filter paper No. 1. The pH of supernatant was adjusted to 7.0 using 2.5 mol/L NaOH and kept in an amber bottle at 5°C until the experiment. Total anthocyanin content was analyzed by spectroscopic method at 535 nm and calculated using extinction coefficient (ε) of 98.2 given by Ranganna (1986) as shown in equation (1) and (2).

$$\text{Total optical density} = \frac{\text{OD at 535 nm} \times \text{Volumn made}}{\text{Weight of sample}} \times 100 \quad (1)$$

$$\text{Total anthocyanin content} = \frac{\text{Total optical density}}{98.2} \quad (2)$$

3. pH-sensing film preparation

The biodegradable base film was prepared by two types of polymer, chitosan and PVA, as a composite film. Chitosan/PVA film preparation was described by Pereira et al. (2015) using a casting technique with some adjustments. One percent of PVA was prepared in distilled water at 70±2°C. One percent of chitosan solution was prepared in 1% (w/v) acetic acid and heated up to 70±2°C while stirring continuously using a magnetic stirrer for 24 h. The composite film was done by mixed PVA and chitosan in the ratio of 3:7 (v/v). Then, *Clitoria ternatea* L. extract (10, 20 and 30%) was added in the chitosan/PVA solution. Sodium tripolyphosphate (1.5%) was used as a plasticizer by adding the volume of 0.1% (w/v) into the mixed solution. The final pH of the mixed solution was adjusted to 6.1 using 0.1 mol/L NaOH (shade changed from purple to blue) and then poured onto a petri dish (90 mm of diameter) with 42 mL per dish. To eliminate the solvent in the composite film, the petri dishes were placed in a hot air oven at 35°C for 5 h followed by placing at room temperature (25°C) for 48 h. Dried film was kept in an aluminum foil bag and placed in a desiccator filled inside with silica gel until experiment.

4. Swelling index (%Si)

Dried film was tested for swelling index which was described by Cavalcanti et al. (2002). The method was done by cutting the film into a size of 2 cm^2. Weight of initial film was measured before immersion in a plastic cup containing 200 mL of distilled water. Then, the film was weighed after immersion for 0.5, 1, 3, 5, 7, 10, 15 and 20 min, respectively, using two decimals digital scale. The experiment was done at 25°C using three samples per treatment. Swelling index was calculated according to the following equation (3).

$$\text{Si (\%)} = \frac{\text{(Final weight – Initial weight)}}{\text{Initial weight}} \times 100 \quad (3)$$

5. Film thickness

Film thickness was measured using a manual micrometer (Mitutoyo, Japan) at room temperature (25°C). Film was cut into a size of 2 cm^2 and then randomly measured in 3 points with three replications of each treatment.

6. Tensile strength

Mechanical property of film was reported as tensile strength using Texture analyzer (Ta.xt.plus, Stable Micro Systems, Surrey, UK) according to Zhang et al. (2019) with some modifications. Film was cut into a size of 20

mm x 75 mm. Tensile strength was measured using A/SPR probe at 25°C. Texture analyzer was set to a test speed of 1 mm/sec, pre-test speed of 1mm/sec, post-test speed of 10 mm/sec and distance of 10 mm. Three samples were analyzed in each test and tensile strength was calculated by the following equation (4)

$$\text{Tensile strength (MPa)} = \frac{\text{Maximum force (N)}}{\text{Film width (mm)} \times \text{Flim thickness (mm)}} \quad (4)$$

7. Water vapor permeability

Water vapor transmission (WVTR) of film was measured by the cup method using ASTM E96-87 (1989), which was utilized in accordance with the recommendation in Caner et al. (1998) with some modifications. This method was based on water vapor transmitted out of the cup. The non corroding aluminum cup was filled with distilled water. The film without visible scratches or leaks was mounted on the cup and tightly fixed with paraffin wax and then placed in a desiccator with 11% RH monitored at room temperature (26±2°C). The weight loss of the test cup was recorded using a four-digit digital scale (ATX224; Shimadzu Corporation, Japan) as a function of time for 48 h. WVTR ($g/h.m^2$) was calculated using equation (5) given by ASTM E97-87 (1989). To calculate the water vapor permeability coefficient (WVPC), film thickness was used to multiply with WVTR and the obtained value was then divided by the pressure difference as expressed in equation (6).

$$\text{WVTR} = \text{Slope/ Film Area} \quad (5)$$

$$\text{WVPC} = \text{WVTR} \times \text{thickness}/\Delta\text{Vapor pressure} \quad (6)$$

8. Film sensitivity in standard pH solutions

The color response of pH-indicator film was studied in six levels of standard pH solution (pH 1.0, 3.0, 5.0, 7.0, 9.0 and 12.0). Phosphate buffer was prepared by mixing of 0.1% sodium dihydrogen phosphate (acid) and 0.1% disodium hydrogen phosphate (base) into pH 7.0 and 9.0. Lactic acid (0.2 M) was used to adjust the pH of the phosphate buffer into an acidic condition (pH 1.0, 3.0 and 5.0). For basic conditions, NaOH (0.1 M) was used to adjust the pH of phosphate buffer to 12.0. Films were cut into a size of 2 cm^2 and then immersed into pH solution for 15 min at 25°C to allow a development of color. The changes in color of indicator films were measured by colorimeter (MiniScan XE Plus, USA) and reported in both of the CIE L* C* h and CIE L*, a*, b* scale. L* value indicated lightness which ranged from black (0) to white (100). Chroma (C*) represented the distance from the lightness axis (L*) and ranged from 0-100. Hue angle (h) expressed in degrees, which ranged from 0° (red) to 270° (blue). The values of a* represented red-green and b* was yellow-blue. Total color difference (ΔE) between each pH level (L, a and b) and the film element before experiment (L_0, a_0 and b_0) was calculated using equation (7). Tests were performed in triplicate at 25°C.

$$\Delta E = \sqrt{(L - L_0)^2 (a - a_0)^2 (b - b_0)^2} \quad (7)$$

9. Film sensitivity in food samples

The efficacy of pH-sensing film was tested on low (pH > 4.6, a_w > 0.85) and high acid food (pH <4.6, a_w > 0.85). Low acid foods were studied in pasteurized milk (pH 5.50) and chicken meat (pH 6.80), while pineapple cv. Pattawia was considered as a high acid food sample (pH 3.36). Cow pasteurized milk was purchased from a convenience store and transferred to the laboratory within 15 min. Skinless chicken breast and pineapple were bought from a wholesale hypermarket store in Sathorn District, Bangkok, Thailand, and then brought for preparation within 1 hour.

Pineapple was washed with tap water and then drained to remove excess water. Fruit was peeled and prepared into a cube of 3x4 cm (6 g per piece) using a sharp stainless knife. Skinless chicken breast was cut into a size of 3x3 cm with weights of 6 g per piece. Each piece of both food samples were placed in a plastic cup and had a pH-sensing element on the top followed by a covering with PVC film (Aro, Thailand) and then stored at 5±1°C for 4 days. The study in pasteurized milk was conducted by the procedures according to Pereira et al. (2015) which a film was maintained in contact with food (40 mL) in a sterile petri dish (diameter 9 cm) covered with a glass lid and then stored at a controlling temperature of 25±2°C for 4 days. Changes in pH of food samples and visual color of films were observed during storage periods. pH was measured using pH meter (Model 7011; Ezdo, Taiwan) which was calibrated with standard buffer 4.0, 7.0 and 10.0 before each measurement.

10. Statistical analysis

All measurements were done with three replications and represent all data as mean ± standard deviation. Statistical program was used (SPSS V. 26; An IBM Company, Ontario, Canada) to analyze data. Mean values were compared by Duncan's multiple range test to determine the difference between treatments.

Results and discussion

1. Physical, mechanical and barrier properties of pH-sensing film

Physical, mechanical and barrier properties of pH-indicator film were reported by%Si, WVPC, film tensile strength and thickness. Percent swelling index of pH-indicator film incorporated in 10-30% *Clitoria ternatea* L. extract is shown in Table 1. Overall, it can be seen that that higher percentage of butterfly pea extract (30%) results in a lower%Si compared to other treatments. Percent swelling index of all treatments increased gradually from an initial at 0.5 min (36-46%) reaching the peak at around 5 to 7 min and then falling, with table 1 showing 59.22 and 46.03% for 10 and 20% film, and 44.99% for 30% film, respectively. The results showed that the composite film between chitosan and PVA demonstrated swelling ability in the water as a hydrogel due to the high hydrophilic properties of PVA. It was noticeable that, a higher content of *Clitoria ternatea* L.extract, a phenolic compound, retarded a rise of swelling rate which was observed in 30% treatment at 7 min. Liu et al. (2019) reported the%Si of PVA film decreased by adding the tea polyphenol extract because of the hydrophobic properties of phenyl rings resulting in an increase of the hydrophobicity of the matrix systems leading to reduce swelling capacity. Similar observation was noted in the incorporation of lignin nanoparticles in PVA/chitosan hydrogel film (Yang et al., 2018).

Table 1 Swelling index (%Si) of pH-indicator film incorporated with 3 levels of *Clitoria ternatea* L. extract

Time (min)	*Clitoria ternatea* L. extract (%)		
	10	20	30
0.5	46.37 ± 2.84^{bA}	41.92 ± 2.69^{abcAB}	36.17 ± 0.21^{bB}
1	50.99 ± 2.24^{abA}	38.75 ± 1.91^{cB}	37.93 ± 1.24^{bB}
3	51.20 ± 3.14^{abA}	37.41 ± 0.34^{cB}	39.84 ± 1.45^{bB}
5	59.22 ± 6.59^{aA}	46.03 ± 0.67^{aB}	38.98 ± 1.71^{bB}
7	54.10 ± 1.08^{abA}	44.51 ± 1.09^{abB}	44.99 ± 0.91^{aB}
10	45.81 ± 0.30^{bA}	39.96 ± 5.02^{abA}	36.81 ± 4.26^{bA}
15	52.90 ± 2.12^{abA}	40.36 ± 1.73^{bcB}	36.32 ± 0.10^{bB}
20	49.69 ± 4.68^{bA}	42.61 ± 0.54^{abcA}	25.57 ± 2.08^{cB}

Remark: Data represented mean ± standard deviation
Mean value in each column of each immersion time followed by distinct lower letter cases indicates significant differences ($p \leq 0.05$).
Mean value in each row of each film treatment followed by distinct upper letter cases indicates significant differences ($p \leq 0.05$)

Film thickness, tensile strength and WVPC properties of pH-sensing film incorporated with 10-30% *Clitoria ternatea* L. extract is shown in Table 2. Addition of the highest percentage of *Clitoria ternatea* L. extract (30%) into composite resulted in the elevated thickness of the film which was 0.094 mm. Tensile strength was considered to reduce as the amount of *Clitoria ternatea* L. extract increased from 10 to 30%, with Table 2 showing 1.310, 1.112 and 0.874 MPa, respectively. This could be due to the supplementation of natural extract causing a change in proportion and density between base polymers chitosan/PVA and sodium tripolyphosphate as a plasticizer that resulted in a weakening of internal interaction bonding. This result was in accordance with Pereira et al. (2015) who revealed that the chitosan/PVA blend film embedded with red cabbage extract presented a lower tensile strength compared to pure chitosan or PVA film. However, the film form from pure chitosan with glycerol as a plasticizers incorporated with green tea extract showed an increase in tensile strength (Siripatrawan & Harte, 2010) which was the effect of hydroxyl groups (-OH) of polyphenols and NH_3^+ reactive groups of the chitosan backbone.

Table 2 Thickness, tensile strength and water vapor permeability coefficient (WVPC) of pH-sensing film incorporated with 3 levels of *Clitoria ternatea* L. extract

Clitoria ternatea L. extract (%)	Thickness (mm.)	Tensile strength (MPa)	WVPC^{ns} (10^{-8} g/ m.h.atm)
10	0.062 ± 0.006^{c}	1.310 ± 0.117^{a}	2.81 ± 0.10
20	0.075 ± 0.012^{b}	1.112 ± 0.220^{ab}	2.63 ± 0.10
30	0.094 ± 0.007^{a}	0.874 ± 0.112^{b}	2.46 ± 0.22

Remark: Data represents mean ± standard deviation. Mean value in each column of each film property followed by distinct lower letter cases indicates significant differences ($p<0.05$). ns = not significant

The capabilities of water vapor molecules moved through the film was slightly limited when the amount of *Clitoria ternatea* L. extract increased. However, no significant difference ($p>0.05$) was observed as compared to other treatments. From this result, the thickness of the film may be an important factor that affected WVPC apart from the nature of molecules incorporated (Miranda et al., 2004). WVPC is an important factor for high moisture food, e.g., meat and poultry when kept in a low WVPC packaging generally presents a fog inside which could enhance microbial growth and spoilage. Fruit and vegetables still remain respiration and transpiration which enhanced a deterioration rapidly because of a condensation problem (Turan, 2021).

2. Film sensitivity in standard pH solutions

Efficacy of pH-sensing films incorporated with 10-30% *Clitoria ternatea* L. extract were tested in a standard pH solution ranging from 1.0-12.0. The visible shade and color parameters are shown in Fig. 1 and Table 3. The visual color in all film treatments ranged from brownish-red (pH 1.0) to yellow (pH 12.0), which had

stronger color intensity when anthocyanin content increased. An indicator film observed at pH 1.0 and 3.0 represented in a brownish-red color which was evidenced by hue angle, with table 3 showing 31.96° and 69.46°, respectively. Increasing *Clitoria ternatea* L. extract (30%) was noted in a more vivid shade of the same color which was confirmed by positive a* value approximately 13, while the 10 and 20% treatments were 2 and 6, respectively (Table 3). The pH-sensing film showed a dark blue color at pH 5.0 and the shade was changed into green when the pH increased to 7.0 and 9.0, respectively, which was approved by negative a* value when pH increased (Table 3). At pH 12.0, a yellowish-green color was observed and showed the highest b* value (40.94-59.90) compared to pH 7.0 (2.30-25.18) and 9.0 (12.86-35.63) (Table 3). The 30% film showed a more intense yellowish-green sight by eye while the vivid color was faded in 10% treatment (Fig. 1). The hue angle of film incorporated with 10, 20 and 30% *Clitoria ternatea* L. extract were 102.82°, 95.31° and 79.65°, respectively, which indicated that the color appearance ranged from yellow (60°) to green (120°). The degree of color brilliance was explained by C* value which was greater in treatment with 30% *Clitoria ternatea* L. extract leads to a decline in lightness (L* value) (Table 3). Total color change (ΔE) approved that all film treatments had responsibility for pH shift (Table 3). A little change of ΔE for all film samples investigated at pH between 5 to 7 due to its revert to a color close to the beginning (pH 6.1). The color of the film from this result was more intense than that of the Hidayati et al. (2021) which was probably due to the higher amount of anthocyanin content incorporated. However, the color shade of the film at different pH levels were similar.

Similar observation of the color pattern of anthocyanin extract from *Clitoria ternatea* L. was reported by Kungsuwan et al. (2014) and Ahmad et al. (2020) which exhibited a red in acidic and green in basic condition. Total anthocyanin content in *Clitoria ternatea* L. extract in this study was 12.22 mg/100 g, demonstrating that there is a potential for utilization as a source of anthocyanin for an indicator in pH measurement. The results were roughly similar to the experiment made by Pham et al. (2019), the maximum amount of anthocyanin content in *Clitoria ternatea* L. was 13.28 mg/100g. For color shift sensitivity, anthocyanin structure can form as the predominant flavylium cation under acidic conditions which could absorb photons in higher wavelengths of visible spectra such as red and magenta. In basic conditions, the degree of disruption in the conjugated double bond in the central ring controlled an absorption of photons in a higher wavelength such as yellow, blue and purple (Chen & Gu, 2013; Kungsuwan et al., 2014). However, numerous factors are affected on anthocyanin color which depend on the main type of anthocyanin raw materials, structure and the test conditions, such as pH ranges, type of solutions and temperatures. The performance of anthocyanin pigment on pH sensitivities were recognized in red cabbage (Pereira et al., 2015; Silva-Pereira et al., 2015), purple sweet potato (Choi et al., 2017; Liu et al., 2017) and black bean seed coat mixed with red cabbage (Prietto et al., 2017). The embedding

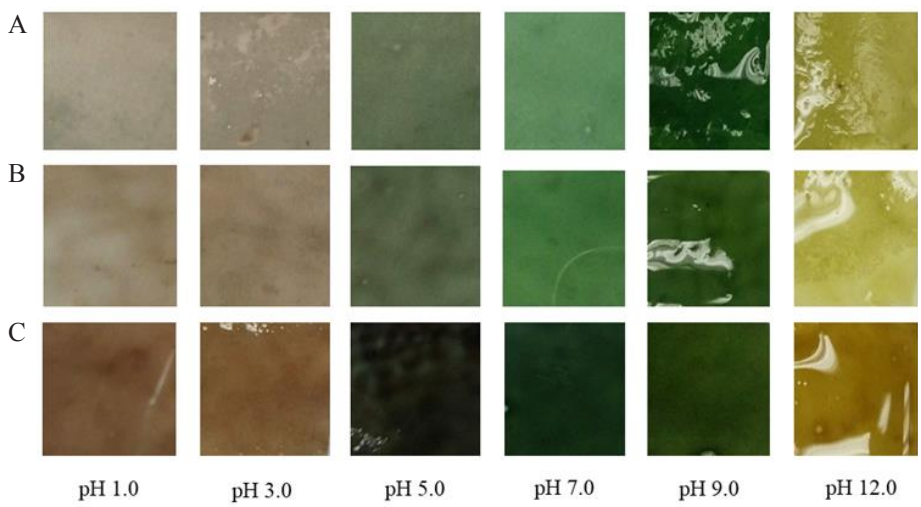

Fig. 1 Changes in color of pH-sensing film incorporated with 10 (A), 20 (B) and 30% (C) *Clitoria ternatea* L. extract in a variety of pH levels

red cabbage extract in chitosan/PVA composite film showed a wide color spectrum between pH 1-12, shifted from red to pink, purple, light blue, blue and green, respectively (Pereira et al., 2015).

3. Film sensitivity in food samples

The efficacies of pH-sensing films were tested in chicken meat, pasteurized milk and pineapple, as the model for low and high acid food. Changes in pH samples and visual color of film incorporated with 10, 20 and 30% *Clitoria ternatea* L. extract are shown in Fig. 2-4. Tested in pasteurized milk, the color of film changed from an initial of blue-green to light red color at the end of the storage. An initial pH of pasteurized milk was 6.78-6.80 (Fig. 2 A-C) which showed a similar shade of color as observed in standard pH solution at pH 7.0 (Fig. 1). During storage at 25°C (accelerated condition), pH of pasteurized milk continuously decreased to 3.48-3.51 on day 4 appearing as a light red color, corresponding to the color observed in standard pH solution at pH 3.0 (Fig. 1). However, no visual difference between all treatments after pH dropped below 4.2 on day 2 (Fig. 2 A-C) clearly indicating milk spoilage due to an accumulation of lactic acid by microbial metabolism can cause the declining pH value (Pereira et al., 2015).

The test of pH-sensing film in chicken meat is shown in Fig. 3. During storage at 5°C for 4 days, pH of chicken meat rose from an initial of 5.42-5.61 to 6.05-6.49 at the end of the storage, which was detected as off-flavor (Fig. 3 A-C). The blue color of pH-sensing film on an initial storage was shifted to green after storage for 4 days as clearly observed in 10% treatment which was expressed in a similar pattern with 7.0 pH solution. On the contrary, the change in color of 20-30% treatments were not noticeable when viewed with eyes. A change in film color studied in chicken meat was in accordance to Ahmad et al. (2020) in which the sago film incorporated with Butterfly pea extract turned to a green color after 48 h storage at exposed condition. The deterioration of chicken meat is caused by microorganisms and activity of protease enzymes. Proteolytic bacteria is able to hydrolyze protein into short chain peptide or amino acid and also produce ammonia as a volatile compound resulting in an increase of pH value (Zhang et al., 2016). The response of film embedded with anthocyanin extract from butterfly pea on volatile amine (NH_3 vapor) was reported by Sukprasong (2013).

An initial pH of fresh-cut pineapple was acidic ranging between 3.34 to 3.39 (Fig. 4 A-C). The remarkable changes in color was obviously found on day 1 while the pH values slightly dropped to around 3.17-3.18. After 2 days of storage, the color of 10-30% treatments shifted from red to more vividness when the pH dramatically fell to 2.45-2.60 on day 4. It is noticeable that a red color of *Clitoria ternatea* L. extract

Table 3 L value (L*), a*, b*, Chroma (C*), Hue angle (h) and ΔE of indicator film incorporated with 10-30% *Clitoria ternatea* L. extract immersed in various pH solutions

Treatments	pH	L*	a*	b*	C*	h	ΔE
10%	control*	39.55±0.67^{dA}	-28.48±0.57^{eB}	7.66±0.27^{cB}	29.76±0.71^{bB}	164.55±0.78^{cA}	-
	1	57.83±0.40b^{cA}	1.80±0.19^{aC}	1.20±0.52^{eC}	2.20±0.31^{fC}	31.96±1.22^{gC}	35.96
	3	76.73±0.40^{aB}	1.90±0.13^{aB}	5.27±0.22^{dB}	5.47±0.11^{eB}	69.46±0.75^{fB}	48.07
	5	39.77±0.20^{dA}	-15.80±0.20^{eA}	-2.51±0.16^{fC}	15.95±0.10^{dA}	188.81±0.45^{aA}	16.26
	7	56.42±01.21^{cA}	-14.97±0.37^{cA}	2.30±0.30^{eC}	15.35±0.31^{dB}	171.07±2.17^{bA}	22.27
	9	27.43±0.91^{eA}	-19.90±0.39^{dB}	12.86±0.41^{bC}	21.85±0.77^{cB}	149.42±0.44^{dA}	15.73
	12	60.70±0.23^{bA}	-9.29±0.06^{bC}	40.94±0.26^{aC}	40.98±0.24^{aC}	102.82±0.59^{eA}	43.85
20%	control	40.75±0.67^{eA}	-30.29±0.57^{fB}	13.99±0.27^{dA}	32.94±0.71^{cA}	155.72±0.78^{aB}	-
	1	58.84±0.40^{bA}	6.07±0.19^{aB}	10.39±0.52^{eB}	12.02±0.31^{eB}	57.83±1.22^{dA}	40.77
	3	87.22±0.40^{aA}	-0.31±0.13^{bC}	2.82±0.22^{gC}	2.54±0.11^{gC}	96.50±0.75^{cA}	56.42
	5	37.05±0.21^{fB}	-7.28±0.20^{cB}	7.11±0.16^{fB}	9.69±0.10^{fC}	137.06±0.45^{bB}	24.30
	7	49.29±1.21^{dB}	-23.60±0.37^{dC}	17.46±0.30^{cB}	28.79±0.31^{dA}	140.64±2.18^{bB}	11.39
	9	39.94±0.91^{eB}	-26.79±0.39^{eC}	25.14±0.41^{bB}	36.44±0.77^{bA}	137.21±0.44^{bB}	11.71
	12	57.32±0.23^{cB}	-6.94±0.06^{cB}	54.47±0.26^{aB}	54.60±0.24^{aB}	95.31±0.58^{cB}	49.58
30%	control	19.22±0.67^{fB}	-10.09±0.57^{eA}	4.98±0.27^{gC}	10.27±0.71^{gC}	155.97±0.78^{aB}	-
	1	47.13±0.40^{aB}	13.25±0.19^{bA}	18.20±0.52^{eA}	22.02±0.31^{eA}	53.47±1.22^{gB}	38.71
	3	42.63±0.40^{cC}	14.56±0.13^{aA}	22.34±0.22^{dA}	26.23±0.11^{dA}	56.50±0.75^{fC}	38.17
	5	26.49±0.20^{eC}	3.10±0.20^{dC}	14.14±0.16^{fA}	14.24±0.10^{fB}	78.30±0.45^{dC}	17.73
	7	26.33±1.20^{eC}	-17.26±0.37^{gB}	25.18±0.30^{cA}	29.75±0.31^{cA}	124.85±2.17^{bC}	22.58
	9	29.26±0.91^{dA}	-10.80±0.39^{fA}	35.63±0.41^{bA}	36.29±0.77^{bA}	107.00±0.44^{cC}	32.26
	12	44.13±0.23^{bC}	10.96±0.06^{cA}	59.90±0.26^{aA}	60.85±0.24^{aA}	79.65±0.58^{dC}	63.87

Remark: * Control represents an indicator film before examination. Data represents mean ± standard deviation
Mean followed by the distinct lower letter case represents the significantly different results between pH levels ($p \leq 0.05$)
Mean followed by the distinct upper letter case represents the significantly different results between film treatments ($p \leq 0.05$)

film at pH ranging from 2-3 was different which showed a brownish-red color observed in standard pH solution (pH 2.0-3.0) since it used lactic acid for adjusting pH. These results indicated that the type of acid in raw material along with other components may be affected by color expression. In this case, citric acid was the main feature acid found in pineapple. However, yeasts and lactic acid bacteria can metabolize sugar (glucose, sucrose and fructose) as a nutrient resulting in a production of lactic acid that leads to deterioration in fresh-cut pineapple (Zhang et al., 2013). The decline in pH was shown in the same pattern which was reported by Antoniolli et al. (2012) in minimally-processed pineapple dipped in ascorbic and citric acid as a browning agent. Therefore, changes in flavor quality or spoilage of fresh-cut pineapple can be detected and monitored through a pH-sensing film in this work.

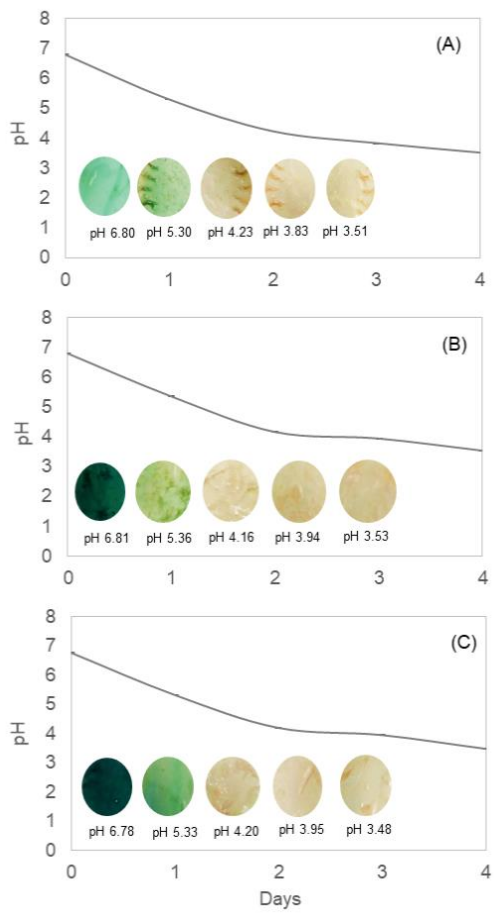

Fig. 2 Changes in pH and visual color of pH-sensing film incorporated with 10 (A), 20 (B) and 30% (C) *Clitoria ternatea* L. extract observed in pasteurized milk during storage at 25°C for 4 days

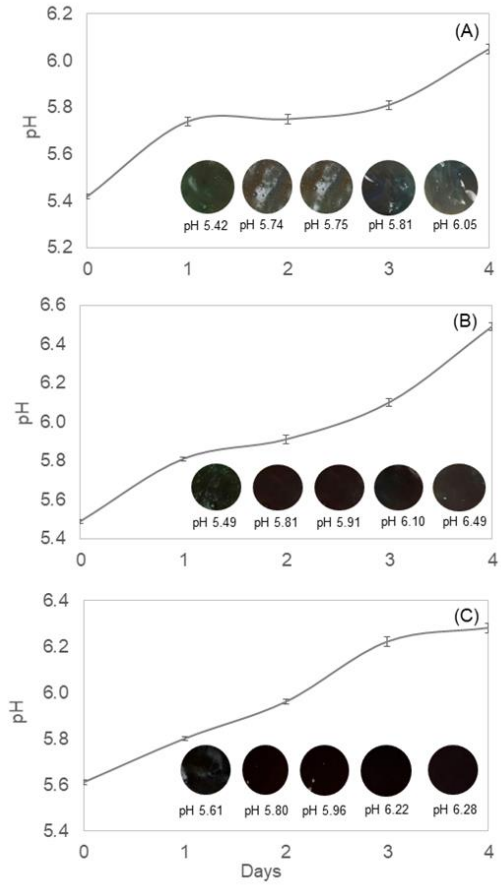

Fig. 3 Changes in pH and visual color of pH-sensing film incorporated with 10 (A), 20 (B) and 30% (C) *Clitoria ternatea* L. extract observed on chicken meat during storage at 5±1°C for 4 days

Conclusion

Ten and/or twenty percent of *Clitoria ternatea* L. extract showed the optimum concentration for producing pH-sensing elements depending on the type of food applied. Both treatments showed no difference of visual color shift (pH buffers and food samples) when compared to 30% corresponding to the total color difference represented by ΔE value. The 20% films contained good physical properties with neither too much nor too little in terms of swelling index, tensile strength and WVPC. For description, the swelling was minimal and had the ablity to maintain its shape when exposed to water or humidity. Film physical properties were flexible, not hard and brittle, and water vapor was allowed to pass more than 30% treatment. Shelf-life and film performance in food samples by noncontact method should be studied in the future for the detection of volatile chemical changes upon food spoilage.

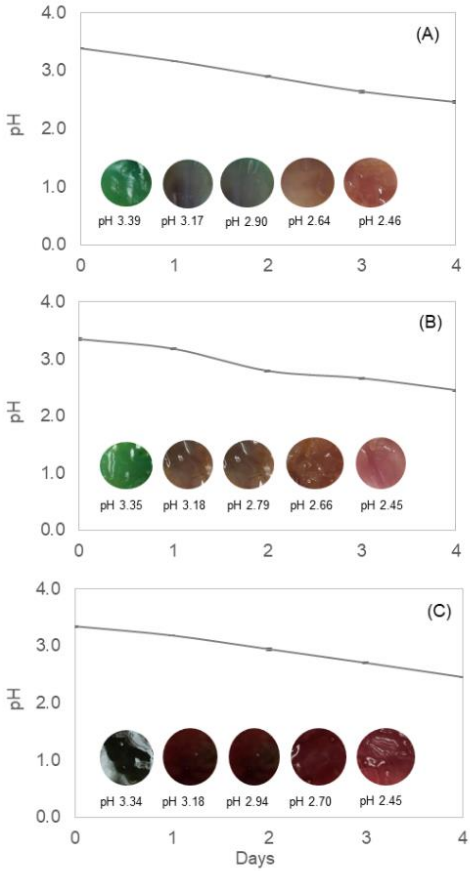

Fig. 4 Changes in pH and visual color of pH-sensing film incorporated with 10 (A), 20 (B) and 30% (C) *Clitoria ternatea* L. extracted observed on pineapple cv. Pattavia during storage at 5±1°C for 4 days.

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Degradation of Poly (lactide), Poly (butylene succinate) and Poly (butylene Succinate/poly (lactide) by UV Irradiation in Combination with Enzymatic Hydrolysis Method

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Abstract

This study elucidated an approach for hydrolysis of bioplastics by UV irradiation in combination with enzymatic hydrolysis. The hydrolytic enzymes, including commercial lipase and alkaline protease, were used for hydrolysis bioplastics of poly (lactide) (PLA), poly (butylene succinate) (PBS), and poly (butylene succinate)/poly (lactide) blend (PBS/PLA) at pH 9.0, 50°C for 72 h. The results showed that each enzyme could hydrolyze all kinds of bioplastics. The combination of commercial enzymes improved the degradation of PLA, PBS, and PBS/PLA blend, which showed the highest weight loss of bioplastic concentration (100 g/L) yielded, 37.70±1.23, 32.60±1.15 and 34.87±3.44%, respectively. The optimum temperature and pH of the hydrolysis reaction were found at 50°C and 9.5, respectively, which gave the highest percent degradation at 39.13±0.71, 35.77±1.94 and 37.90±1.99%, respectively when using each bioplastic at 100 g/L. The exposure of UV irradiation at 254 nm for 36 and subsequent hydrolysis with mixed enzymes at pH 9.5, 50°C for 36 h increased the percent degradation up to 48.45±2.85, 42.8±2.56, and 44.1±1.75%, respectively. The hydrolysis of PLA, PBS, and PBS/PLA blend in a 2.0 L stirrer fermenter led to a percent degradation at 49.50±2.29, 44.33±1.52, and 48.17±3.01%, respectively. Scanning electron microscope (SEM) confirmed the change of the physical structures of the degradation products. These results showed the alternative approach to reduce the bioplastic wastes by applying the UV irradiation with hydrolytic enzymes that could develop at an industrial level in the future.

Introduction

Biodegradable aliphatic polyesters such as poly (lactide) (PLA), poly (butylene succinate) (PBS) and poly (butylene succinate)/poly (lactide) blend (PBS/PLA) have been interesting as a solution for reducing the use of petroleum-based plastic and reducing the global environmental problem (Hu et al., 2018; Su et al., 2019; Accinelli et al., 2020). Biodegradable polyesters could be produced from the fermentation of various renewable

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materials and polymerized to a polymer by condensation (Rajeshkumar et al., 2021). PLA is a polymer of lactic acid (Singhvi et al., 2019), while PBS is an aliphatic polyester polymer, produced from the condensation of succinic acid (SA) and 1,4-butanediol (BD) (Su et al., 2019). PBS and PLA (PBS/PLA) blends were also currently applied to produce alternative biodegradable plastic, which improved physical and chemical properties such as tensile properties, crystallization, and thermal stability (Hu et al., 2018; Su et al., 2019). Although these plastics could degrade in a natural environment, however long time and optimum conditions were required, which also caused the environmental problem from the accumulation of these plastics in nature (Lomthong et al., 2020). Recently, Lomthong et al. (2020) reported that PLA film could degrade about 20% when incubated the reaction without enzyme at 50°C for 24 hours. While the reaction contained serine protease produced from the *L. sacchari* LP175 strain could degrade up to 90% at 24 hours of incubation.

Biodegradable aliphatic polyesters could degrade by various hydrolytic enzymes such as serine protease produced from *L. sacchari* LP175 (Lomthong et al., 2021), lipase from *Cryptococcus* sp. MTCC 5455 (Thirunavukarasu et al., 2016) and cutinase from *Fusarium* sp. (Shi et al., 2019) which are interesting to apply for degradation of bioplastics at high concentrations. Various commercial hydrolytic enzymes were also reported for degradation of aliphatic polyester polymers such as commercial lipase (Hoshino et al., 2002) and protease (Kawai et al., 2011; Luzi et al., 2015), which are more suitable for use at an industrial level due to high stability, high activity and commercially available for large scale application. However, the optimal conditions still need to be researched in more detail

Ultraviolet (UV) radiation has been reported to break the biodegradable aliphatic polyesters's chemical bonds, which enhances the degradation of bioplastic samples by causing the sample cracking and decreasing the melting temperature (Podzorova et al., 2017). From our knowledge, the application of UV irradiation with the synergistic hydrolysis of hydrolytic enzymes has not yet been reported for the degradation of bioplastics.

Therefore, this study's objective was to investigate the hydrolytic efficiency of PLA, PBS, and PBS/PLA blends using commercial lipase and alkaline protease at different substrate concentrations. The synergistic hydrolysis of commercial hydrolytic enzymes at optimum conditions together with UV irradiation on the degradation of bioplastics at a high concentration were also evaluated.

Materials and methods

1. Enzymes and bioplastics

Lipase produced from *Camlicla lipolytica* and alkaline protease produced from *Bacillus licheniformis* 2709 were obtained from Reach Biotechnology Co., Ltd., Thailand and stored at -20°C until required for use.

PLA film was prepared following the procedure described in Lomthong et al. (2021), when dissolved 2.0 g of PLA pellets (Terramac TP-4000, Unitika. Co., Ltd., Japan) in 200 mL of dichloromethane (Merck, Germany). The clear solution was poured into a stainless-steel tray, covered with aluminium foil and then dried overnight at room temperature. PBS powder was prepared according to the method of Hu et al. (2018) with some modifications by dissolving 2.0 g of PBS pellets (FZ91PD, Mitsubishi Chemical Corporation, Japan) in 100 mL of chloroform (Merck, Germany). The dissolved solution was poured into a stainless-steel tray which contained aluminium foil covered on the surface. PBS/PLA blends were prepared by the modified method of Hu et al. (2018). Dissolved 1.0 g of PBS and 1.0 g of PLA pellets in 100 mL of chloroform (Merck, Germany). Then, the dissolved solution was poured into a stainless-steel tray which contained aluminium foil covered on the surface.

2. Degradation of PLA, PBS and PBS/PLA by commercial lipase and alkaline protease

The degradation of bioplastic polyester including PLA, PBS and PBS/PLA blends were performed in a 250 mL Erlenmeyer flask using 50 mL of enzyme solution. Each 10 mL of the commercial lipase (30,000 U/mL) or alkaline protease (20,000 U/mL) was mixed with 40 mL of 0.2 M Tris-HCl buffer, pH 9.0 (Lomthong et al., 2017). Each bioplastic at different concentrations (10, 20, 50 and 100 g/L) was added to the flask and incubated in a shaking incubator at 150 rpm and 50°C for 72 h (Lomthong et al., 2021). The dry weight of the obtained powder after filtration through Whatman® No. 1 filter paper and drying at 50°C for 12 h was used to calculate the percentage degradation according to the equation below:

$$\text{Percentage Degradation} = \frac{(\text{Initial film weight} - \text{Retained film weight}) \times 100}{\text{Initial film weight}}$$

3. Synergistically hydrolysis of bioplastics using a combination of commercial lipase and alkaline protease

Bioplastics of PLA, PBS, and PBS/PLA blends at 10, 20, 50 and 100 g/L were hydrolyzed in a 250 mL Erlenmeyer flask using 50 mL of enzyme solution as described above. The enzyme solution was prepared by added 5.0 mL of each commercial lipase (30,000 U/mL) or alkaline protease (20,000 U/mL) to the 40 mL of 0.2 M Tris-HCl buffer, pH 9.0. All flasks were incubated at 150 rpm and 50°C for 72 h and then the percentage of degradation was determined as described above.

4. Optimum temperature and pH for degradation

The experiments regarding the degradation of PLA, PBS, and PBS/PLA blends were performed at 100 g/L of each bioplastic using the mixed enzyme of commercial lipase and alkaline protease at different temperatures (30, 40, 50, 60 and 70°C) for 72 h and then the percentage of degradation was determined as described above.

The effects of pH at 8.0-10.0 were investigated by dissolving the enzymes in different buffers, including Tris-HCl buffer (pH 8.0–9.0) and glycine-NaOH buffer (pH 9.5-10.0). The reactions were incubated at optimum temperature for 72 h and and then the percentage of degradation was determined as described above.

5. Effect of UV irradiation on the degradation of bioplastic polymers

All bioplastic samples at 100 g/L were exposed to UV irradiation (254 nm) for 36 h at room temperature and air atmosphere using a mercury vapor lamp (TUV G8 T5, Philips, Poland) at a distance of 10 cm, which modified the method from Olewnik-Kruszkowska et al. (2015). The exposed bioplastic samples were subsequently subjected to hydrolysis with a mixed of commercial lipase and alkaline protease enzymes as described above for 36 h at 50°C. The experiment without the UV irradiation was used as a control for each bioplastic sample. At each 12 h of incubation, the flasks of each bioplastic were taken to determine the dry weight and calculate the percentage degradation as described above.

6. Degradation of PLA, PBS, and PBS/PLA blends polymers in a 2.0 L stirrer fermenter

The degradation of PLA, PBS, and PBS/PLA blends at 100 g/L were up-scaled degradations in a 2.0 L stirrer fermenter using 1.0 L of total suspension. The bioplastic samples were exposed to UV radiation for 36 h and subsequent hydrolysis with mixed commercial lipase and alkaline protease enzymes at the ratio described above. The hydrolysis conditions were operated at the optimum pH and temperature from the result above, 200 rpm for 36 h (Lomthong et al., 2021). At the end of hydrolysis, the dry weight of each bioplastic was used to calculate the percentage degradation as described above.

7. Scanning electron microscope

A scanning electron microscope (SEM) was used to evaluate the physical structure change of the degraded bioplastics compared to the native structure. At the end of the reaction, the degraded bioplastic was washed with distilled water, dried at 50°C for 24 h, and examined under SEM at 10.0 kV (Sriyapai et al., 2019).

8. Statistical analysis

Results were reported as means of three replicates (n = 3) with SD, and the significance of the data was analyzed by one-way analysis of variance (ANOVA) (SPSS 21.0, USA). Values were considered significant at $p < 0.05$ using Duncan's multiple range tests.

9. Enzyme activity

Alkaline protease activity was determined using the standard assay method reported by Zhou et al. (2020). One unit of alkaline protease equals the enzyme amount, which hydrolyzes casein to get 1 µg of tyrosine in 1 min under standard conditions. The lipase activity was determined using the p-nitrophenyl laurate (p-NPL) as substrate, as Isobe et al. (1988) described. One unit of lipase enzyme was defined as the amount of enzyme that liberates 1.0 mmol of p-nitrophenol per minute under the standard assay conditions.

Results and discussion

1. Degradation of PLA, PBS, and PBS/PLA by commercial lipase and alkaline protease

Commercial hydrolytic enzymes have been used at an industrial level for a long time due to highly stable, high concentration and are commercially available for large-scale requirements compared to the non-commercial hydrolytic enzymes in the lab scale. In this study, the commercial lipase and protease were used to investigate the degradation of bioplastics as described above, aiming to provide a choice for up-scaled degradation at an industrial process in the future. The results of bioplastics degradation by commercial lipase and alkaline protease are shown in Tables 1 and 2. For commercial lipase, the maximum% degradation was found in 10 g/L of PLA, PBS, and PBS/PLA blends, which yielded 56.36±1.87, 65.37±4.03 and 60.43±0.55%, respectively. While at 100

g/L, the percent degradation was decreased to 27.46±2.20, 32.70±0.25 and 29.80±2.75%, respectively (Table 1).

In commercial alkaline protease, the maximum percentage degradation of PLA, PBS, and PBS/PLA was found at 10 g/L (68.60±3.65, 48.30±2.15 and 60.90±1.85%, respectively), corresponding to the results of Lomthong et al. (2021). While at 100 g/L, it yielded 29.15±2.45, 18.70±0.61 and 23.50±2.29%, respectively (Table 2).

Table 1 Degradation of PLA, PBS, and PBS/PLA blend by commercial lipase at pH 9.0, 50°C for 72 h

Substrate concentration (g/L)	% Degradation		
	PLA	PBS	PBS/PLA
10	56.36 ± 1.87^{d}	65.37 ± 4.03^{d}	60.43 ± 0.55^{d}
20	40.97 ± 1.00^{c}	54.30 ± 1.30^{c}	44.70 ± 2.70^{c}
50	33.95 ± 1.03^{b}	41.17 ± 2.78^{b}	35.10 ± 0.90^{b}
100	27.46 ± 2.20^{a}	32.70 ± 0.25^{a}	29.80 ± 2.75^{a}

Remark: Different letters within the same column are statistically different at $p < 0.05$

Tan et al. (2021) and Satti et al. (2019) reported that microbial lipase could degrade bioplastics such as PLA and PBS, while Oda et al. (2000) reported of the degradation ability regarding PLA polymer of commercial protease produced from *Bacillus* sp. From the results of this study, commercial lipase and alkaline protease could degrade PLA, PBS, and PBS/PLA blends polymer, which revealed the possibility for an application in the bioplastics recycling process (Panyachanakul et al., 2020). However, further development to increase the percentage of degradation of these bioplastics at high substrate concentration was required to investigate.

Table 2 Degradation of PLA, PBS, and PBS/PLA blend by alkaline protease at pH 9.0, 50°C for 72 h

Substrate concentration (g/L)	% Degradation		
	PLA	PBS	PBS/PLA
10	68.60 ± 3.65^{d}	48.30 ± 2.15^{d}	60.90 ± 1.85^{d}
20	51.71 ± 2.06^{c}	37.16 ± 1.26^{c}	49.00 ± 2.00^{c}
50	39.40 ± 0.50^{b}	26.00 ± 2.00^{b}	37.60 ± 1.35^{b}
100	29.15 ± 2.45^{a}	18.70 ± 0.61^{a}	23.50 ± 2.29^{a}

Remark: Different letters within the same column are statistically different at $p < 0.05$

2. Synergistically hydrolysis of bioplastics using a combination of commercial lipase and alkaline protease

Each enzyme has its specific activity toward different substrate structures. The combination of commercial lipase and alkaline protease could improve the hydrolysis efficiency, as shown in Table 3. The maximum % degradation of PLA, PBS, and PBS/PLA, also found at 10 g/L of substrate concentration, yielded 75.57±1.50, 74.60±1.35 and 76.50±0.89%, respectively. While at 100 g/L, the % degradation down to 37.70±1.23, 32.60±1.15 and 34.87±3.44%, respectively (Table 3). Alkaline protease was reported as the key enzyme for the degradation of PLA. Oda et al. (2000) reported that all alkaline proteases could degrade L-PLA, while Youngpreda et al. (2017) reported that alkaline protease could degrade DL-PLA polymer. Lipase has reported the ability to degrade PBS (Ding et al., 2012), corresponding to the results of this study, which found that commercial lipase has the specificity for degradation PBS more than PLA. The combination of commercial lipase and alkaline protease improved the degradation by synergistic hydrolysis of bioplastics, which could be applied to develop bioplastics degradation at an industrial level.

Table 3 Degradation of PLA, PBS, and PBS/PLA blend by lipase and alkaline protease at pH 9.0, 50 °C for 72 h

Substrate concentration (g/L)	% Degradation		
	PLA	PBS	PBS/PLA
10	75.57 ± 1.50^{d}	74.60 ± 1.35^{d}	76.50 ± 0.89^{d}
20	66.90 ± 2.59^{c}	56.80 ± 1.38^{c}	59.20 ± 1.84^{c}
50	54.60 ± 2.00^{b}	48.33 ± 1.53^{b}	51.70 ± 0.70^{b}
100	37.70 ± 1.23^{a}	32.60 ± 1.15^{a}	34.87 ± 3.44^{a}

Remark: Different letters within the same column are statistically different at $p < 0.05$

3. Optimum temperature and pH for degradation

The effects of temperature and pH on bioplastics degradation by mixed enzymes of commercial lipase and alkaline protease are shown in Fig. 1. The maximum percent degradation PLA, PBS and PBS/PLA (100 g/L) at 37.67±1.89, 32.83±1.26 and 34.93±2.00%, respectively, were found when incubated at 50°C (Fig. 1a). From our knowledge, most of the bioplastic degrading enzymes showed an optimum at thermophilic condition (40-70°C); Itävaara et al. (2002) and Apinya et al. (2015) also reported that reaction at high temperature increased the water adsorption to the polymers, which stimulated the chemical hydrolysis. In the case of pH, the optimum pH was found when using the pH buffer at 9.5, which yielded 39.13±0.71, 35.77±1.94 and 37.90±1.99%, respectively (Fig. 1b). Bioplastics of PLA, PBS and PBS/PLA have reported the optimum pH for hydrolysis at alkaline conditions, which stimulated the hydrolysis of chemical bonds (Lomthong et al., 2019). The optimum temperature and pH were further used to investigate the effect of UV irradiation in the further experiment.

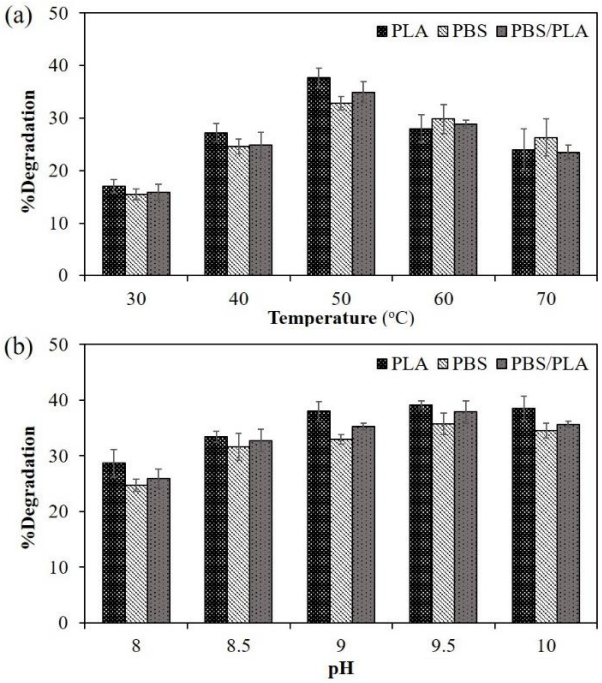

Fig. 1 Effects of temperature (a) and pH (b) on the degradation of PLA, PBS, and PBS/PLA blend polymers

4. Effect of UV irradiation on the degradation of bioplastic polymers

The effect of UV irradiation on bioplastics degradation in combination with hydrolytic enzymes is shown in Fig. 2. The%degradations of PLA, PBS and PBS/PLA were increased from 39.41±4.15, 35.75±3.55, and 37.11±2.90% up to 48.45±2.85, 42.8±2.56, and 44.1±1.75%, respectively, after exposing each bioplastic to UV light for 36 h and subsequent hydrolysis with mixed of enzymes for 36 h (Fig. 2b). This showed that UV- irradiation affects the degradation of these bioplastics compared to the experiment without UV irradiation (Fig. 2a). UV irradiation was mentioned as a photodegradation process that broke the backbone's chemical bonds, cracking the structure and decreasing the melting temperature of bioplastic samples (Janorkar et al., 2007; Cai et al., 2018). However, using photodegradation (UV irradiation) solely may not complete the hydrolysis of bioplastics and cause environmental problems from the micro and nano plastic leftover in nature. Podzorova et al. (2017) reported that UV radiation caused a decreasing degree of crystallinity in PLA film. Nevertheless, it required a long time to irridate, i.e. about 100 h of UV radiation, and small plastic particles remained in the experiment. This reason could explain why we elucidated an alternative approach for bioplastic degradation using UV irradiation together with enzymatic hydrolysis in this study. This study revealed that UV irradiation followed by enzymatic hydrolysis of commercial lipase and alkaline protease improved the degradation of PLA, PBS and PBS/PLA samples faster and at a higher degradation rate than the previous studies.

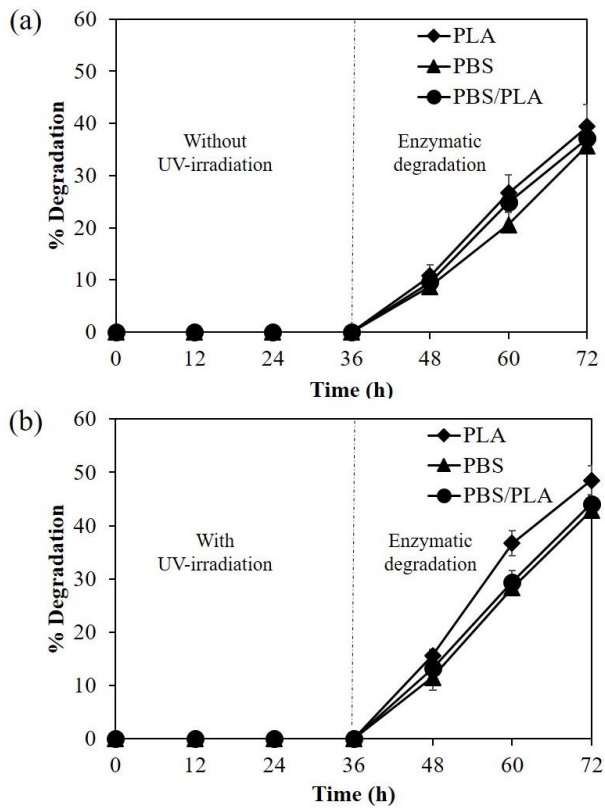

Fig. 2 Effect of UV irradiation on the degradation of bioplastic polymers with or without enzymatic hydrolysis. (a) control without UV- irradiation and (b) UV irradiation for 36 h

5. Degradation of PLA, PBS, and PBS/PLA blends polymers in a 2.0 L stirrer fermenter

Each PLA, PBS, and PBS/PLA were degraded in a 2.0 stirrer fermenter at pH 9.5, 50°C, after being exposed to UV irradiation for 36 h. The agitation rate was set at 200 rpm, as Lomthong et al. (2021) reported, which used a similar bioplastic concentration. The lower agitation rate caused the limitation of enzyme-substrate mixing, while the higher agitation rate affected the stability of enzyme-substrate binding (Lomthong et al.,

2021). The percentage degradation of each bioplastic was 49.50±2.29, 44.33±1.52, and 48.17±3.01%, respectively (Fig. 3), which showed the potential for upscale hydrolysis in the future. The test series of bioplastics without enzymes showed significantly lower percentage degradation than the one with present hydrolytic enzymes in the reactions, which yielded 14.97±3.15, 7.13±0.32, and 6.67±2.02%, respectively (Fig. 3). This study improved the degradation efficiency up to 3.31, 6.22 and 7.23 folds compared to the degradation without enzymatic hydrolysis. Compared with other studies, Olewnik-Kruszkowska et al. (2015) and Podzorova et al. (2017) reported that UV irradiation significantly decreased the molecular weight of PLA polymer. However, low percentage of weight loss has occurred due to UV radiation affects only the mechanical properties of PLA polymer and small, broken particles of the degraded samples remained. Youngpreda et al. (2017) achieved the hydrolysis of PLA polymer at 6.7 g/L after incubating at 60°C for 24 h using alkaline protease produced from *Actinomadura keratinilytica* T16-1. Regarding PBS, Sriyapai et al. (2019) reported of degradation of PBS by PBS depolymerase produced from *Saccharothrix* sp. APL5 using PBS film at 7.0 g/L with a weight loss of 74% after incubating at 37°C for 56 days. The use of photodegradation by UV irradiation in this study coupling with enzymatic degradation could improve the%degradation of these bioplastics, which provides more options for industrial applications.

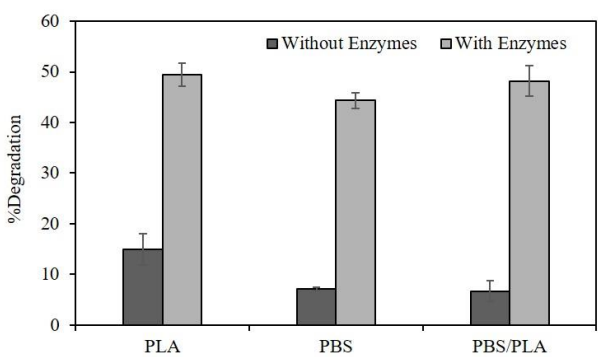

Fig. 3 Degradation of PLA, PBS, and PBS/PLA blend in a 2.0 stirrer fermenter with or without enzymatic hydrolysis at pH 9.5, 50°C after exposed to UV irradiation for 36 h

The SEMs of native and digested bioplastics are shown in Fig. 4, which confirmed the physical change of each bioplastic after being degraded by UV irradiation together with mixed commercial lipase and alkaline protease. The residues of PLA, PBS, and PBS/PLA samples had reduced rigidity, with fractures on the surface structure compared to native samples. Proteolytic enzymes hydrolyzed ester bonds of the bioplastic polyesters due to the similarity in chemical structure between the L-lactic acid unit in PLA and the L-alanine unit in protein (Jarerat & Tokiwa 2001). Shi et al. (2019) reported that lipase enzyme could hydrolyze the ester bonds of PBS polymer due to the analogue structure and substrate specificity between the enzyme active site and PBS polymer.

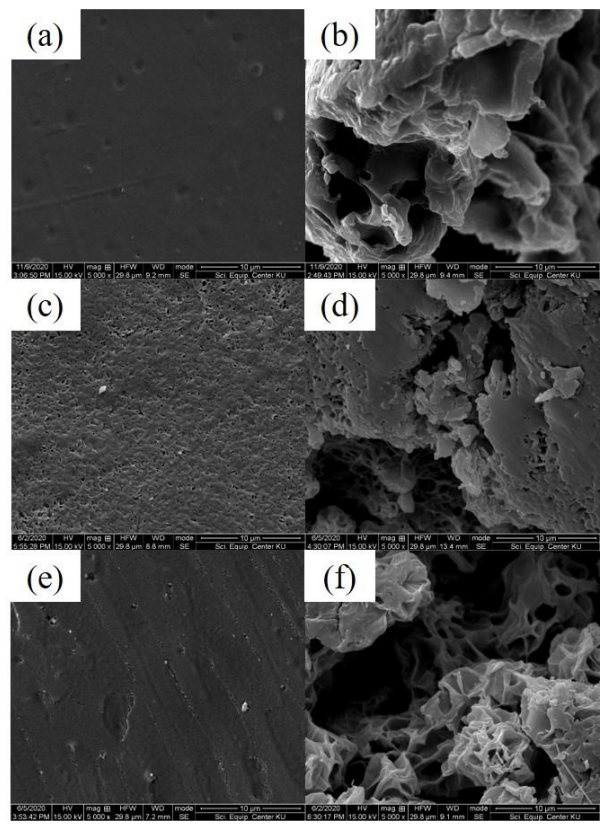

Fig. 4 SEM of native and digested PLA (a, b), PBS (c, d), and PBS/PLA (e, f) after degradation by UV irradiation together with commercial enzymes at pH 9.5, 50°C for 72 h

Conclusion

This study revealed the hydrolytic efficiency of commercial lipase and alkaline protease on the degradation of PLA, PBS and PBS/PLA blend at optimum conditions. The synergistic hydrolysis of commercial enzymes with the photodegradation process has elucidated the potential for the application to reduce the accumulation of bioplastics waste in the future. High

concentrated degradations of PLA, PBS, and PBS/PLA were obtained from this study at 49.50±2.29, 44.33±1.52, and 48.17±3.01 g/L from the initial substrate at 100 g/L, which improved the degradation efficiency up to 3.31, 6.22 and 7.23 folds as compared to the degradation without UV-assisted enzymatic hydrolysis. This study is the first report to develop three types of bioplastic degradation using UV irradiation and enzymatic hydrolysis at high concentrations. This research showed the progress for hydrolysis bioplastics using commercial enzymes at mild conditions which could reduce the accumulation of plastic wastes in the environment.

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Antioxidant Activities, Total Phenolic Compounds and Fucoxanthin of Marine Benthic Diatoms *Amphora subtropica* BUUC1502 and *Thalassiosira* sp.

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Abstract

This research's aim was to study antioxidant activities, total phenolic compounds and fucoxanthin of crude extract of marine benthic diatoms, *Amphora subtropica* BUUC1502 and *Thalassiosira* sp. These two diatoms were cultured in a Guillard F/2 media, which was prepared from sea water with a salinity at 30 psu for 8 days. It was found that *A. subtropica* BUUC1502 shows better growth than *Thalassiosira* sp. (p<0.05). Whereas the biomass yield of *Thalassiosira* sp. is higher than of *A. subtropica* BUUC1502 (p<0.05). The diatoms were extracted using methanol solvent (99.8%). Crude extracts of *A. subtropica* BUUC1502 and *Thalassiosira* sp. yielded 0.38±0.01 and 0.36±0.00 g/g DW, respectively. The total phenolic compounds and fucoxanthin of them were similar (p>0.05) with 2.92-3.09 mg GAE/g crude extract and 18.85-19.74 mg/g DW, respectively. The IC_{50} of DPPH free radical inhibition of crude extracts from *A. subtropica* BUUC1502 and *Thalassiosira* sp. is 231.75±40.75 and 179.84±27.90 µg/mL, respectively, while the IC_{50} of ABTS free radical inhibition is 68.28±7.31 and 46.90±1.83 µg/mL, respectively. The results of this research show that these two marine benthic diatoms may be an antioxidant source that can be used in various related fields.

Introduction

Diatoms are unicellular algae that live as planktonic or benthic diatoms. They can be found in both freshwater and sea water. Diatoms have many kinds of useful bio-compounds, such as unsaturated fatty acids and pigments. The frustule of diatoms is also used to stimulate blood clotting (Peltomaa et al., 2019; Luo et al., 2021). Diatoms can also be a source of natural antioxidants. During the photosynthesis process in diatom cells, oxygen molecules are released from converting carbon dioxide into starch by using light energy. If diatoms have a high photosynthesis activity, their oxygen production is high, too. Therefore, oxygen may be

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stimulated by ultraviolet radiation or heat from sunlight to form reactive oxygen species, which are toxic to living organisms. Plants including diatoms can create a mechanism to inhibit those free radicals by producing antioxidants (Peltomaa et al., 2019). Rico et al. (2013) reported that the marine diatom *Phaeodactylum tricornutum* contains phenolic compounds such as quercetin, myricetin and rutin. Phenolic compounds and its derivatives contain aromatic rings and hydroxyl groups. Of these, phenolic compounds have a potential to clear the free radicals (Mahfuz et al., 2021). Fucoxanthin is a major type of carotenoids which is an accessory pigment in the chloroplasts and is involved in photosynthesis of diatoms and brown macroalgae. Fucoxanthin has an allenic bond, conjugated carbonyl, epoxide, and acetyl groups in its molecule. Because of this structure, Fucoxanthin is a strong antioxidant (Sies, 1997; Xia et al., 2013). Marine diatoms such as *Cymbella* sp. and *Navicula* sp. produce phenolic compounds that have antioxidant properties and can inhibit oxidation reactions and mutation. Therefore, those compounds from diatoms can be used to prevent various diseases in humans, especially ischemic heart disease and cancer (Natrah et al., 2007). In addition, the diatom *Odontella aurita* contains fucoxanthin, which inhibits cellular oxidation and protects skin cells from UV-B radiation (Mohamed et al., 2012).

Because marine benthic diatoms *Amphora* sp. and *Thalassiosira* sp. have a short production cycle, fast growth rate and high useful biochemical compounds content, they are wildly used in aquaculture hatcheries. Machana et al. (2020) reported that diatom *Amphora* sp. isolated from the East Coast of Thailand comprised ω3-polyunsaturated fatty acids (PUFAs) especially 22: 6n-3 (DHA) and 20:5n-3 (EPA). *Thalassiosira* sp. is rich of PUFAs also (Mai et al., 2021). Khwancharoen et al. (2020) found that Pacific white shrimp postlarvae (*Litopenaeus vannamei*) fed with diatom *A. coffeaeformis* supplemented diet could induce the growth performance of shrimp. Diatom *Thalassiosira* are used as food for shrimp larvae, bivalve larvae, and rotifer (Ortega-Salas & Nava, 2017). *Amphora* sp. and *Thalassiosira* sp. are a great source of natural pigments including chlorophylls and carotenoids (including Fucoxanthin) (Kuczynska et al., 2015). In addition, *Amphora* sp. and *Thalassiosira* sp. are considered for the commercial production (Ortega-Salas & Nava, 2017; Govindan et al., 2021).

In this study, marine centric diatom *Thalassiosira* sp.and pennate diatom *Amphora subtropica* BUUC150 were cultured in the laboratory. Then, diatoms were harvested for crude extracts and determined for total phenolic compounds and fucoxanthin. The diatoms' crude extracts were tested for antioxidant activity, DPPH radical scavenging activity (DPPH) and ABTS radical scavenging activity (ABTS). Therefore, the results of this study can provide information about the potential of diatoms regarding possible uses in medicine and for cosmetic products.

Materials and methods

1. Diatom culture

Diatom *A. subtropica* strain BUUC1502 was isolated from Pacific white shrimp (*Litopenaeus vannamei*) pond, located in Chanthaburi Province (Chenchakhan, 2015) and identified using a partial 18S rRNA sequence analysis. Diatom *Thalassiosira* sp. was obtained from the Center of Excellence for Marine Biotechnology, Department of Marine Science, Faculty of Science, Chulalongkorn University. Diatoms were cultured in axenic Guillard F/2 media (Guillard, 1975), which were prepared from seawater with a salinity at 30 psu. Each of them was cultured at 5 L with 5 replicates. The cultures were placed in a laboratory condition. Temperature was controlled at 26±1˚C. The cultures were exposed to continuous illumination at light intensity of 42.25 µmol/m^2/s using fluorescent lamps and aerated with 0.2 µm filtered air over 8 days. The diatom cells were counted every day using Haemacytometer. Cell density and specific growth rate (µ) were calculated by using the equation (1) of Roleda et al. (2013).

$$\mu\ (day^{-1}) = \ln(N_2\text{-}N_1)/t_2\text{-}t_1 \qquad (1)$$

When N_1 and N_2 equal cell density (cells/mL) at time t_1 and t_2 (day), respectively.

On the final day of the experiment, the diatoms were in stationary phase and then harvested by soaking at 3.4˚C for 16 hours. At this low temperature, the diatom cells take a short time to settle to the bottom. The supernatant was then removed. The concentrated diatoms were centrifuged at 10,000 rpm for 5 minutes and dried by using the lyophilization method.

2. Crude extraction

Diatoms were extracted using absolute methanol with a purity at 99.8% by modifying the method of Sachindra et al. (2007) with a dry diatom and methanol ratio at 1:20 (w/v). Weighed 0.5 g of dry diatoms, then extracted with

10 mL of methanol and soaked overnight for 24 h. The solution was filtered with filter paper and solid residue was extracted with methanol for two times. The methanol solution was dried with a rotary evaporator until the crude extract was formed. The crude extract was weighed and then stored at -20˚C for further study.

3. Total phenolic content determination

The total phenolic content was determined by the Folin-Ciocalteu Colorimetric Method (Sukjamnong & Santiyanont, 2012) by preparing a standard solution of gallic acid (Sigma-Aldrich, United States) at a concentration of 0.625-200 µg/mL and a solution of the sample extract at a concentration of 800 µg/mL. Then 20 µL of the solution was placed in a microtiter plate 96-well and 100 µL of 10% Folin-Ciocalteu solution was added and mixed in. After 6 minutes, 80 µL of 7.5% sodium carbonate were added and left in the dark at room temperature for 30 minutes. Absorbance was determined at 765 nm with a microplate reader (Thermo, Finland). The total phenolic content was calculated as milligrams gallic acid equivalent (mgGAE/g) by using a gallic acid calibration curve.

4. Fucoxanthin content determination

The method of Seely et al. (1972) was improved by using 50 mg dry diatom cells, adding 10 mL of 80% acetone solution, incubating in the dark for 24 hours and centrifuging at 10,000 rpm for 5 minutes. The absorbance of the extracted liquid was determined at 420 nm using the UV–Visible spectrophotometer (Metertech, Taiwan). The fucoxanthin content of the diatom crude extracts was calculated by comparison with the standard fucoxanthin curve (Sigma-Aldrich, United States).

5. Antioxidant activity test of crude extracts from diatoms

5.1 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method

The DPPH radical scavenging method was adapted from Fenglin et al. (2004). Diatom crude extract was diluted to a concentration at 3.125-400 µg/mL with methanol in a 96-well microtiter plate. Then, 0.2 mM of DPPH solution (in methanol) was added at a ratio of 1:1 (v/v) and mixed, incubated in the dark at room temperature for 30 minutes. The absorbance of the extracted liquid was determined at 517 nm by using a microplate reader (Thermo, Finland). The absorbance values were collected and calculated as percentage of DPPH inhibition (% inhibition) at different concentrations as shown in equation (2) with methanol as a blank and DPPH solution as a control unit. The IC_{50} (the concentration of substrate that causes 50% reduction in the DPPH) were calculated to compare with a positive control substance, i.e., vitamin C (Ascorbic acid) and a vitamin E derivative (Trolox).

5.2 2, 2'-azino-bis 3-ethylbenz thiazoline-6-sulfonic acid (ABTS) free radical cation decolorization activity method

The ABTS radical scavenging method was conducted following the method described by Yang et al. (2011), i.e., by mixing 2 mL of 7 mM ABTS with 35.5 µL (in distilled water) of 140 mM potassium per sulfate ($K_2S_2O_8$) and left at room temperature in the dark for 16 hours to get ABTS radical cation ($ABTS^{\bullet+}$) stock. Before testing, $ABTS^{\bullet+}$ stock was diluted to an absorbance in the range of 0.700±0.02 at a wavelength of 734 nm. The diatom crude extract was prepared with a concentration at 3.125-400 µg/mL in a 96-well microtiter plate of 50 µL. Then 100 µL of $ABTS^{\bullet+}$ solution was added and left in the dark at room temperature for 10 minutes. The absorbance was measured at 734 nm with a microplate reader (Thermo, Finland). The absorbance values were collected and calculated as percentage of $ABTS^{\bullet+}$ inhibition with an $ABTS^{+\bullet}$ as control unit. The IC_{50} was calculated to compare with vitamin C and Trolox, which is a standard solution. The percentage of free radical inhibition (% inhibition) was calculated according to the equation (2):

$$\% \text{ inhibition} = [(A_{control}-A_{sample})/A_{control}] \times 100 \quad (2)$$

$A_{control}$ = The absorbance of the control unit (DPPH or ABTS solution)

A_{sample} = The absorbance of crude extract / the standard solution mixed with the DPPH or ABTS solution

6. Data analysis

The mean values of the total phenolic compounds, Fucoxanthin content and antioxidant activity of DPPH• and $ABTS^{+\bullet}$ were calculated. Then, the mean difference was analyzed by T-test at 95% confidence level with SPSS version 15.

Results and discussion

1. Growth and biomass yield of diatoms

From 8 days of diatoms culturing, it was found that A. subtropica BUUC1502's specific growth rate and cell density were higher than *Thalassiosira* sp. ($p<0.05$). However, after harvesting those diatoms on the final day

of this experiment, it was found that *Thalassiosira* sp. had a dry weight at 0.62±0.07 g/L which was significantly higher than *A. subtropica* BUUC1502 (p<0.05) (Table 1). This may be due to *Thalassiosira* sp. having a cell diameter more than 10 µm and a cell volume about 1226-2214 µm^3 (von Dassow et al., 2006), which is greater than *A. subtropica* BUUC1502. de Viçose et al. (2012) reported that *Amphora* have a frustule length and width approximately at 10 and 4 µm, respectively. In addition, during the harvesting process the diatoms were kept at low temperature. This indicates that *Thalassiosira* sp. may precipitate better than *A. subtropica* BUUC1502. Although, some of *A. subtropica* BUUC1502 may have been lost during the harvesting of the cells.

Table 1 Mean±SD (stand deviation) of specific growth rate, maximum cell density and dried weight of marine diatoms, *A. subtropica* BUUC1502 and *Thalassiosira* sp.

Diatom	Specific growth rate (day^{-1})	Maximum cell density (x10^4 cells/mL)	Dried weight (g DW/L)
A. subtropica BUUC1502	0.72±0.07^a (day 0-3)	117.33±11.47^a (day 7)	0.38±0.06^b (day 8)
Thalassiosira sp.	0.45±0.11^b (day 0-3)	45.00±7.05^b (day 8)	0.62±0.07^a (day 8)

Remark: Values in each column followed by different letters denote significant difference at p<0.05

2. Diatom crude extract, total phenolic compounds and fucoxanthin content

When diatoms were extracted with methanol, it was found that *A. subtropica* BUUC1502 had a higher crude extract content than *Thalassiosira* sp. (p<0.05) but total phenolic compounds and fucoxanthin of them were similar (p>0.05) (Table 2). This may be due to the fact that both diatoms belong to the same group of benthic diatoms. Using methanol (absolute methanol, purity 99.8%) to extract diatoms resulted in total phenolic compounds of *A. subtropica* BUUC1502 up to 3.09 mg GAE/g crude extract. When Lee et al. (2008a) extracted *Amphora coffeaeformis* with an 80% methanol solution, a total phenolic compound with 1.05 g GAE/g crude extract was found. This indicates that using methanol with a purity of 99.8% may have caused higher total phenolic compounds than using 80% methanol. The extraction of diatoms by using enzyme is another method that had similar results to this research. According to Lee et al. (2008a), *A. coffeaeformis* has total phenolic compounds at 2.88 mg GAE/g crude extract when extracted with food enzymes in a carbohydrase group with the commercial name Ultraflo, whereas *Thalassiosira* sp. has total phenolic compounds at 2.92 mg GAE/g crude extract. However, Hemalatha et al. (2015), using methanol to extract *Thalassiosira subtilis,* have reported diatoms with a total phenolic compound at 0.48 mg GAE/g DW, which was 6 times less than the results of this study. This indicates that apart from solvents and enzymes, there may be other factors that effect the total phenolic compounds content i.e. certain species or the diatom's growth stage (Rahman et al., 2020).

Fucoxanthin is primary carotenoid pigment in xanthophyll. It is a powerful antioxidant and found in macroalgae such as *Laminaria japonica* and *Undaria pinnatifida* and also in microalgae such as diatom *Phaeodactylum tricornutum,* which has fucoxanthin up to 24.2 mg/g DW (Sies, 1997; Eilers et al., 2016). *A. subtropica* BUUC1502 and *Thalassiosira* sp. have fucoxanthin about 18.85-19.74 mg/g DW (Table 2), which is less than *P. tricornutum.* Hence, there may be other factors that can effect on the Fucoxanthin accumulation as well, i.e., species, nutrients, and culture conditions (Eilers et al., 2016; Rahman et al., 2020). However, *A. subtropica* BUUC1502 and *Thalassiosira* sp. had a similar Fucoxanthin content. This may be because they were cultured under the same conditions and harvested at the same stationary phase as well. Rahman et al. (2020) reported that when microalgae, including diatoms, grow from exponential phase to stationary phase, it has an effect on the antioxidants' accumulation, too (carotenoids, total phenolic compounds and fatty acids).

Table 2 Mean±SD of crude extract, total phenolic compounds and Fucoxanthin contents in marine diatoms *A. subtropica* BUUC1502 and *Thalassiosira* sp.

Diatom	Crude extract (g/g DW)	Total phenolic compounds (mg GAE/g crude extract)	Fucoxanthin (mg/g DW)
A. subtropica BUUC1502	0.38±0.01^a	3.09±1.40^a	19.74±2.46^a
Thalassiosira sp.	0.36±0.00^b	2.92±0.27^a	18.85±2.75^a

Remark: Values in each column followed by different letters denote significant difference at p<0.05

3. DPPH antioxidant activity of diatoms crude extract

DPPH antioxidant activity results of crude extracts from diatoms are shown in Fig. 1. It indicates that DPPH antioxidant activity is increasing when more *A. subtropica* BUUC1502 and *Thalassiosira* sp. crude extracts were added. The concentration of 100 µg/mL crude extract showed the best antioxidant activity, i.e., *A. subtropica* BUUC1502 at 29.32% and *Thalassiosira*

sp. at 53.63%, while 25 µg/mL of Ascorbic and Trolox (vitamin E derivative) inhibited DPPH up to 91.24% and 90.99%, respectively. Although, total phenolic compounds and Fucoxanthin of diatoms' crude extracts from *A. subtropica* BUUC1502 and *Thalassiosira* sp. were similar, but they have different DPPH antioxidant activities. This may be because they contained different compounds such as carotenoids, and the amount and type of fatty acids in the polyunsaturated fatty acid group were different, which affected their antioxidant ability (Rahman et al., 2020). Correlation of crude extract concentrations of *A. subtropica* BUUC1502 and *Thalassiosira* sp. and DPPH radical scavenging values provided linear regression line y = 0.1973x+9.0055, R^2 = 0.9732 and y = 0.3943x+14.124, R^2 = 0.9929, respectively (Fig. 1). Of these results, both linearity models are satisfied regression line. In a previous report, DPPH radical interacts with other radicals, and the time response curve to reach the steady-state is not a good linear with different ratios of antioxidant/DPPH (Brand-Williams et al. 1995; Sanchez-Moreno et al. 1998). In addition, DPPH assay was interfered with other contaminants in the sample such as metal (Lee et al., 2008b). So, in this research, our samples are crude extract form diatoms.

Table 3 shows *A. subtropica* BUUC1502 and *Thalassiosira* sp crude extract which has an IC_{50} value at 231.75±40.71 µg/mL and 179.84±27.90 µg/mL, respectively. Due to complicated composition of crude extract, both crudes show the lower inhibition efficiency of DPPH radicalscavenging activity including pure antioxidant of ascorbic acid and Trolox. The reason why ascorbic acid and Trolox are highly effective in inhibiting DPPH is due to their strong antioxidant properties. Ascorbic acid is a strong natural antioxidant, while vitamin E derivative is a strong synthetic antioxidant with 1,3,4-oxadiazole ring that gives it its antioxidant properties (Rabie et al., 2016). Moreover, ascorbic acid and vitamin E derivative have been purified, while the extract of diatom is only a crude extract. According to Coulombier et al. (2020) microalgae extract has a low DPPH antioxidant capacity. Additionally, if green microalga, *Nephroselmis* sp., was cultured at a low light intensity at 250 µmol/m^2/s, the IC_{50} value (395.93 µg/mL) was better than with a high intensity at 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The diatoms *Thalassiosira weissflogi* which were cultured at a low light intensity, the IC_{50} (939.31 µg/mL) was better than others cultured at high light intensity (IC_{50} more than 1,000 µg/mL). In comparison, the results of this research, where *Thalassiosira* was cultured with a light intensity at 42.25 µmol/m^2/s, its extract had a 5.2 times higher DPPH inhibitory ability than the extract prepared during the experiments of Coulombier et al. (2020). This shows that different light intensities in diatom culture conditions and different microalgae species can influence the DPPH antioxidant activity as well.

4. ABTS antioxidant activity of the diatom crude extract

The ABTS antioxidant activity of the diatoms' crude extracts were proportional to the concentration of the extracts. The crude extracts from *A. subtropica* BUUC1502 and *Thalassiosira* sp. at a concentration of 75 µg/mL were able to inhibit ABTS free radicals at 76.30% and 76.25%, respectively (Fig. 2). Linear relationships between ABTS antioxidant activity and crude extract concentrations of *A. subtropica* BUUC1502 and *Thalassiosira* sp. are satisfied. With ABTS assay, good correlation is usually reported with bioactive compounds, and regression factor (R^2) at more than 0.8 (Sadeer et al., 2020). The IC_{50} on ABTS antioxidant of ascorbic acid and Trolox were 5.92 and 11.77 µg/L, respectively. *Thalassiosira* sp. crude extract has an IC_{50} at 46.90±1.83 µg/mL, which was better than *A. subtropica* BUUC1502, which has an IC_{50} at 68.28±7.31 µg/mL (Table 3). However, ascorbic acid and Trolox were able to inhibit free radicals of ABTS better than *A. subtropica* BUUC1502 and *Thalassiosira* sp. extracts. Ascorbic acid is inhibiting free radicals of ABTS better than both diatoms at 12 and 8 times, while Trolox is higher than 6 and 4 times, respectively. Regarding the amount of phenolic compounds and Fucoxanthin, it was found that *Thalassiosira* sp. contains the same amount of these compounds as *A. subtropica* BUUC1502. Therefore, it is possible that the compound may not have a positive effect on the antioxidant activity of ABTS. According to Rahman et al. (2020) the ABTS radical-scavenging activity of diatoms has a positive effect depending on the amount of lutin, beta-carotene and zeaxanthin.

The results of this experiment showed that the extracts from diatom, *A. subtropica* BUUC1502 and *Thalassiosira* sp. had better IC_{50} higher antioxidant effects on ABTS than DPPH. This may be because ABTS is highly soluble in both water and organic solvents, and then quickly reacts and also reacts well at a wide pH range while DPPH free radicals react with antioxidants that are soluble only in solvents such as ethanol. In addition, free radicals ABTS are positively charged free radicals. Thus,

it loses free protons to other molecules with proton receptors, i.e., antioxidants or plant extracts (the extracts from both diatoms). Therefore, it is possible that the extracts from diatoms are highly electronegative which effects their ability to accept protons from free radicals ABTS (Coulombier et al., 2020). Moreover, DPPH is a stable free radical and is not sensitive to reactions like free radicals that occur in the human body. Therefore, the reaction was slow, and the results of the antioxidant activity analysis are less than in reality (Sadeer et al., 2020).

Conclusion

Crude extract of marine benthic diatoms *Thalassiosira* sp. and *A. subtropica* BUUC1502 had similar total phenolic compounds and Fucoxanthin. Additionally, they also showed similar DPPH inhibition. However, crude extraction of centric diatom *Thalassiosira* sp. showed a better ABTS inhibition than pennate diatom *A. subtropica* BUUC1502. This indicates that extracts from marine benthic diatoms, *A. subtropica* BUUC1502 and Thalassiosira sp. produce an antioxidant activity which can be used as a source of natural antioxidants in related fields such as food, cosmetics and medicine.

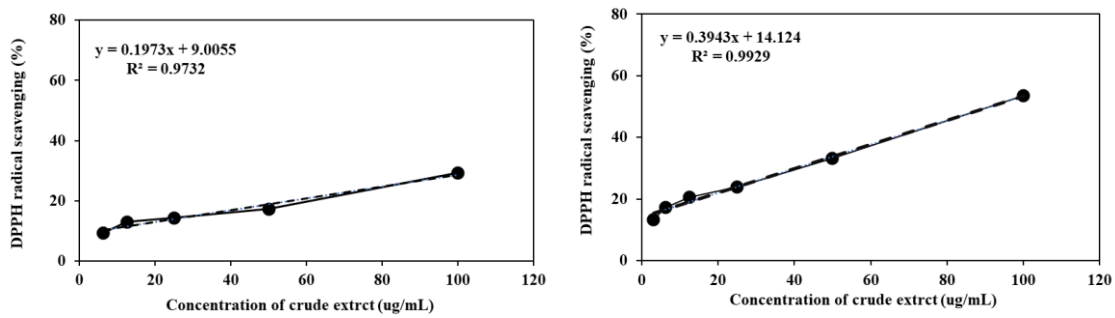

Fig. 1 DPPH radical-scavenging activity of crude extracted from *A. subtropica* BUUC1502 (A) and *Thalassiosira* sp. (B)

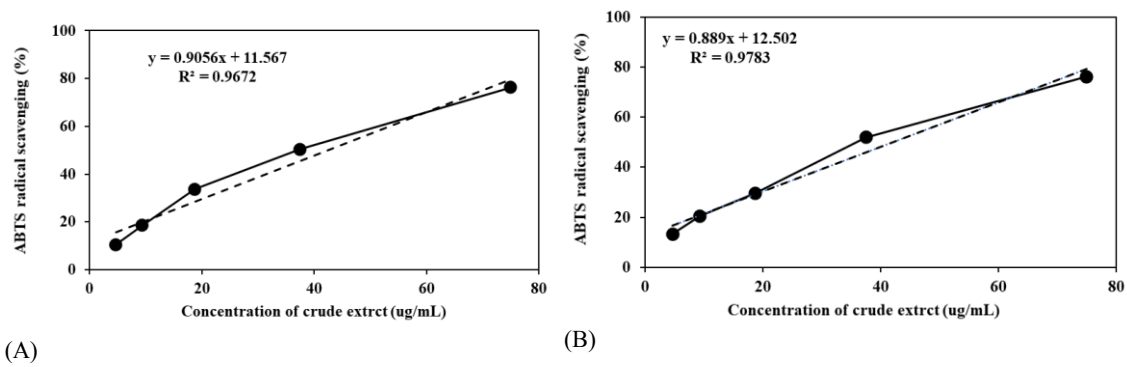

Fig. 2 ABTS radical-scavenging activity of crude extracted from *A. subtropica* BUUC1502 (A) and *Thalassiosira* sp. (B)

Table 3 Mean±SD of the IC_{50} values in the DPPH and ABTS radical-scavenging activity assay of ascorbic acid, Trolox, crude extracted from marine diatoms

Free radical	Ascorbic acid (µg/mL)	Trolox (µg/mL)	Crude extract	
			A. subtropica BUUC1502 (µg/mL)	*Thalassiosira* sp. (µg/mL)
DPPH	5.22±0.42[b]	6.30±0.79[b]	231.75±40.71[a]	179.84±27.90[a]
ABTS	5.92±0.33[d]	11.77±0.41[c]	68.28±7.31[a]	46.90±1.83[b]

Remark: Values in each row followed by different letters denote significant difference at $p<0.05$

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Effect of Malva Nut Gel as Fat Replacer on Sponge Cake

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Abstract

Malva nut originated in Southeast Asia and is cultivated in the Eastern part of Thailand. Gel prepared from its mature seed coat containing high water-soluble dietary fiber was used as a fat replacer in this research. Part of the butter (25-50%) and milk (10-20%) for the sponge cake were replaced with malva nut gel. Physical, chemical and organoleptic analyses were conducted to evaluate the effects of malva nut gel on the properties of the sponge cake. For specific volume, all samples were not significantly different ($p>0.05$); moreover, the roughness, lightness (L*) and yellowness (b*) of the crust increased while the values of the crumb decreased. In the final product obtained from this research 10% of the milk and 25% of the butter were replaced with malva nut gel, which was the highest replacement content of butter and milk with a high customer acceptance. Its chemical composition consisted of 22.72% moisture, 6.28% protein, 16.20% fat, 0.90% ash, 53.90% carbohydrate and 3.76% dietary fiber. Cholesterol and total calories were 114.22 mg and 386.52 Kcal, respectively. The content of fat, carbohydrate, cholesterol, and total calories of this product were lower than the basic formula ($p \leq 0.05$). In addition, 120 consumers accepted this cake in terms of appearance, color, aroma, taste, softness and rated it overall, as 'very much liking' and the aftertaste as 'moderate liking'.

Introduction

Malva nut is a seed of Scaphium scaphigerum (Wall. ex G. Don). G. Planch. It is originated in Southeast Asia and its seed is used as traditional medicine. In Thailand, it is cultivated in the East, especially in Chantaburi province. Its mature seed can form gel when it is soaked. The seed containing water soluble dietary fiber can absorb about 40-45 ml/g water and swell (Piyatrakul, 2013; Sukhasem & Kinkajorn, 2017) to produce brown jelly.

The nutrition composition of dry malva nut

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contains 12.0% moisture, 5.40% protein, 2.40% fat, 75.30% carbohydrate and 67.10% fiber (Disthai, 2019). Due to the high amount of dietary fiber, its swollen and fragmented structure can hold moisture, fat, sugar and other substances (Pramualkijja et al., 2016). Malva nut gel is able to remove fat and toxin in the intestine. Also, the gel can reduce suffering from constipation and control the body weight. Additionally, consuming two grams of fiber extract of malva nut twice a day results in increasing fiber intake and decreasing energy, and waist circumference (Chaitokkia & Panomai, 2018). When this gel was studied regarding a possible use as a fat replacer in food and bakery products (Health Benefits Times, 2019; Pramualkijja et al., 2016; Chaosomboon, 2018). Chajeamjen et al. (2017) found that added malva nut gel increases the moisture and crude fiber in cookie bars.

Nowadays, cake is a popular dessert. There are two types of cake: butter and foam. In a foam type cake, fat provides softness and flavor (Godefroidt et al., 2019). The aim of this research was to test malva nut gel as a partial substitute for butter and milk in a sponge cake, which is a foam type cake. The physical quality and sensory evaluation of the cake were examined. Also, nutrition and consumer acceptance were a focus. The product of the research could not only reduce the fat in the cake, but also show the benefits of using malva nuts.

Materials and methods

1. Materials

Dried malva nuts were from Chantaburi province, Eastern part of Thailand. Cake flour (Royal Fan, 7.0-8.3% protein, UFM Food Center Co. Ltd., Thailand), Double acting baking powder (Best Food, Unilever Thai Holdings Ltd., Thailand), fresh eggs, evaporated milk (Carnation, F&N Dairies (Thailand) Co. Ltd., Thailand), sugar (Mitr Phol, Mitr Phol Sugar Corporation, Ltd., Thailand), salt (Prung Thip, Thai Refined Salt Industrial Co. Ltd., Thailand), cake emulsifier (SP, American Baker Co. Ltd., U.S.A.) including Sorbitol, Ester of Fatty Acid, Monoglyceride, Sucrose Ester of Fatty Acid, Glycerine and Propylene Glycol, vanilla essence (Winner, Greathill Co. Ltd., Thailand) and unsweetened butter (Orchid. The Thai Dairy Industry Co. Ltd., Thailand) were used to make sponge cake.

2. Determination of color and chemical composition of prepared malva nut gel

2.1 Malva nut gel preparation

Dried malva nuts (Fig. 1) were washed in tap water. The top and bottom were cut and soaked in filtered water for 90 minutes at room temperature (30±2°C). The ratio of malva nuts to water was 1:20 (w/w). The swollen gel was drained and squeezed on a 40-mesh sieve. This gel was cleaned until the water was clear. The gel was drained and sealed in a plastic bag. It was kept at 4±2°C to be analyzed later (applied from Paseephol et al., 2016).

Fig. 1 Dried malva nuts

2.2 Color measurement

The color was investigated using a handy colorimeter (Nippon Denshoku NR-3000, Japan) which had been calibrated using a standardized black and white standard plate. The sample of 100 g prepared gel was put into a clear plastic tray for color measurement. Ten recordings were noted on average for the sample.

2.3 Determination of chemical composition

The chemical composition of malva nut gel was determined through proximate analysis, namely moisture, protein, fat, ash, dietary fiber and calculated carbohydrate including crude fiber with the standard methods (Association of Official Analytical Chemists, 2016). The experiment was done in duplicate with triplicate measurement.

3. Replacement of butter and milk in sponge cake with malva nut gel

The basic formula of sponge cake used in this study is shown in Table 1 (Suan Dusit International Culinary Center, 2017). Cake flour was mixed with baking powder (part 1). Eggs, evaporated milk, sugar, salt, cake emulsifier and vanilla essence were mixed together and defined as part 2. The cake batter was prepared by mixing part 1 with part 2, and then adding melted butter (or butter with malva nut gel). The batter was poured into a 5 cm. diameter paper cup with a case (25-26 g/cup).

These cupcakes were baked at 180-190°C for 18-20 minutes. They were left to cool completely on a wire rack.

Table 1 Ingredients of sponge cake (basic formula)

Ingredient (%)	Concentration (%)						
	Basic formula (control)	RM0/ RB25	RM0/ RB50	RM10/ RB25	RM10/ RB50	RM20/ RB25	RM20/ RB50
Cake flour	24.04	24.04	24.04	24.04	24.04	24.04	24.04
Baking powder	0.47	0.47	0.47	0.47	0.47	0.47	0.47
Egg	24.03	24.03	24.03	24.03	24.03	24.03	24.03
Evaporated milk	7.21	7.21	7.21	6.49	6.49	5.77	5.77
Sugar	24.03	24.03	24.03	24.03	24.03	24.03	24.03
Salt	0.19	0.19	0.19	0.19	0.19	0.19	0.19
Cake emulsifier	1.81	1.81	1.81	1.81	1.81	1.81	1.81
Vanilla essence	0.19	0.19	0.19	0.19	0.19	0.19	0.19
Melted butter	18.03	13.52	9.02	13.52	9.02	13.52	9.02
Malva nut gel	0.00	4.51	9.01	5.23	9.73	5.95	10.45
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

The content of malva nut was varied using a factorial in completely randomized design (Factorial in CRD). A cake made by basic formula (RM0/RB0) was used as the control. This gel was used to replace the three levels of evaporated milk weight (RM) at 0, 10 and 20% and two levels of butter weight (RB) at 25% and 50%. There were seven formulas for this experiment as shown in Table 2. These levels were determined based on the appearances of mixture and cake (fluffy, spongy, and moist characteristics) which were compared to the control by visual observation. The treatments which provided a suitable product were selected for measurement of their physical qualities and sensory evaluation. The cake samples were analyzed on day 1 post-baking.

3.1 Physical quality

Diameter and height of the product sample was measured by Vernier calipers. The specific volume was determined by sesame seed displacement (Lee et al., 1982). The cake was weighed (W0). The empty container was filled with sesame seeds and the volume of the seeds was determined by a graduated cylinder (V1). The cake was placed in the container. The sesame seeds were poured over the sample in the box and leveled with a spatula. The seed volume was determined using a graduated cylinder (V2). The difference between V1 and V2 was defined as the sample volume (V0). The specific volume was calculated as the ratio of the volume to weight (V0/W0) or cubic centimeters per gram (cm^3/g). These measurements were done in triplicate.

The color of the crust and the crumb of samples were measured separately. The samples were analyzed on day 1 post-baking. The L*, a* and b* values of the crust and crumb of the cake were determined using a handy colorimeter (Nippon Denshoku NR-3000, Japan), which had been calibrated using a standardized black and white standard plate. Ten readings were recorded on the top part of the cake, Then the top of the cake was cut horizontally, and ten readings were recorded on the crumb color of the cake.

Texture profiles of products were analyzed by a texture analyzer (Stable Micro System TA-XT2i, England). A 3x3x3 cm^3 sample was pressed using a cylinder probe (100 mm. diameter, P/100), 3.0 mm./sec pre-test, 0.5 mm/sec test speed, 10.0 mm./sec of post-test speed, 20% strain, 60 sec and 20 g of trigger type. Hardness, adhesiveness, and springiness of the cake samples were reported. Five measurements were taken to obtain an average value of all texture parameters of each sample.

3.2 Sensory evaluation

Sensory evaluation of the cake with a randomized complete block design (RCBD) was carried out by 55 untrained panelists. Each panelist was presented with individual cupcakes (5 cm diameter) which were placed on a tray in a plastic bag coded with a three-digit random number and served in a randomized order. Drinking water was provided as a palate cleanser before tasting the next sample. The degree of liking was used on a 9-hedonic scale (1=extremely dislike and 9=extremely like) to rate different parameters such as appearance, color, flavor, taste, softness, aftertaste, and overall liking. The formula which provided a soft, good flavor and received the highest scores was accepted for this study.

4. Analysis of chemical composition of malva nut gel sponge cake

The chemical composition of the selected product was determined and compared with the control product by proximate analysis with the standard methods described in Association of Official Analytical Chemists (2016). The contents of moisture, protein, fat, ash, and dietary fiber were determined. Carbohydrate content (including crude fiber) was obtained by calculation. Additionally, cholesterol in both products was determined according to the Compendium of Methods for Food Analysis Thailand (Thailand National Bureau of Agriculture Commodity and Food Standards, 2003).

5. Evaluation of consumer acceptance of malva nut gel sponge cake

Consumer acceptability of the selected malva nut gel sponge cake was performed using central location test (CLT) by 120 consumers at Suan Dusit University,

Bangkok, Thailand. Each consumer received a cupcake (5 cm diameter) which was placed on a tray in a plastic bag with a glass of drinking water at room temperature and a questionnaire. The questionnaire consisted of general information, attitude, sensory evaluation with 9-point hedonic scale (1 = extremely dislike and 9 = extremely like) to evaluate 7 types of attribution: appearance, color, flavor, taste, softness, aftertaste, and overall and channel of distribution.

6. Statistical analysis

All formula developments were arranged in treatments and experiments with factorial in completely randomized design (Factorial in CRD). The experiment of sensory evaluation was performed applying the randomized complete block design (RCBD). The data obtained from this study were analyzed statistically using a t-test for 2 samples and analysis of variance (ANOVA) and the differences between average values were compared by Duncan's new Multiple Range Test (DMRT) for more than 2 samples at the level of significance ($p \leq 0.05$). This statistical analysis was performed using SPSS (Statistical Package for the Social Science).

Results and discussion

1. Color and chemical properties of malva nut gel

The color of the gel, which was from the mucilage in the outer of malva nut seed coat, was dark brown as shown in Fig. 2. Its chemical compositions were shown in Table 2. The water holding capacity of the gel was also measured as 46.40±0.12 times of dry weight. It was composed of high amount of water-soluble dietary fiber presenting a gelling property. The gel was able to form a viscous substance without heat (Piyatrakul, 2013; Sukhasem & Kinkajorn, 2017; Vinyoochareonkul, 2012) which mimicked the rheology of fats (Kathryn et al., 2018); therefore, it was used to substitute butter in a cake in this research. Moreover, the gel was able to hold a high content of water, replacing not only butter but milk also.

Fig. 2 Malva nut gel

Table 2 Color value and chemical composition of malva nut gel

Quality	Malva nut gel
Color value	
L*	26.18±0.20
a*	20.54±0.25
b*	39.01±0.45
Chemical composition	
Moisture (%)	97.89±0.07
Protein (%)	0.44±0.01
Fat (%)	0.01±0.00
Ash (%)	0.09±0.01
Carbohydrate (%)	1.57±0.02
Dietary fiber (%)	0.29±0.00
Total calories (Kcal)	8.13±0.17

2. Effects of butter and milk replacement with malva nut gel

Seven samples of sponge cake from different formulae are presented in Fig. 3. The visual characteristics of cakes replacing milk (RM) at 0%, 10% and 20% of milk weight and butter (RB) at 25% and 50% of butter weight in the basic recipe with malva nut gel were compared. It showed that increasing the gel adding caused a fade color of both on the surface and inside of the cake with brown spots, a rough surface, less butter odor, little sweeter, thicker and less fluffy. It was because of high

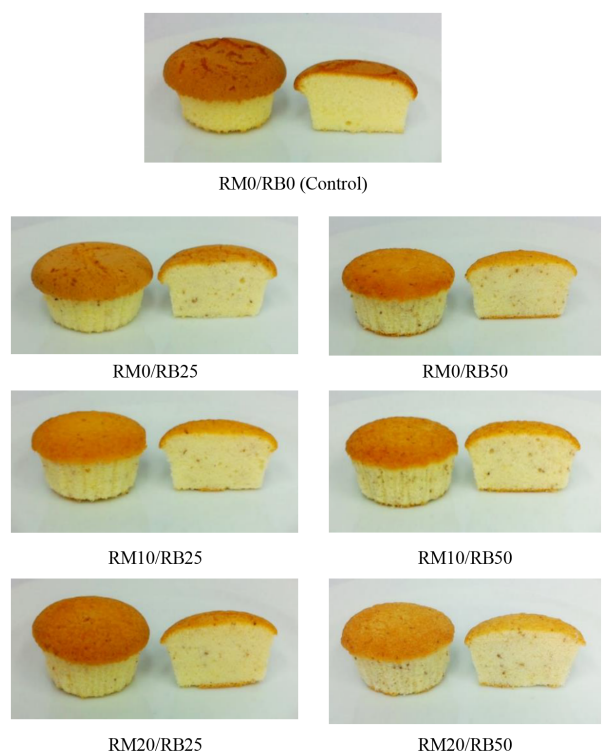

Fig. 3 Sponge cake with malva nut gel replacing milk and butter at different levels

moisture absorption and gum-like properties of the gel. Moreover, reducing fat content led to a weak structure of the cake (Pachekrepapol et al., 2009; Chajeamjen et al., 2017). However, the cake emulsifier in the sponge cake helped to make the mixture compatible. The cake with malva nut gel replacing milk by 10% and 20% and butter by 50% (RM10/RB50 and RM20/RB50) was less fluffy than the others.

2.1 Physical quality

The physical qualities of the selected products are shown in Table 3. Although there was no significant difference in specific volume among the samples ($p>0.05$), those of the experiment were higher than the control ($p \leq 0.05$); while the height of RM0/RB25 and RM0/RB50 cakes (only butter was replaced with malva nut gel) was not different from the control ($p>0.05$). It was caused by the water holding capacity of malva nut gel which accumulated more water in the cake; therefore, adding the gel without reducing milk or liquid materials made the cake formula unbalanced.

As regards the structure of the control cake, a stronger cake structure was found in RM0/RB25 because reducing butter caused more rising and less density (Gray, 2020), while a weaker structure was found in RM0/RB50 because reducing too much butter caused an unbalanced formula; in addition, water in gel replacing butter led to obtain too much gluten; consequently, the cake was too tight. However, the cake batter should be viscous enough to trap gas bubbles during mixing and the bubbles will further be retained when baking. On the other hand, if the batter is too viscous, the cake will have less height with low volume and quality (Huang & Yang, 2019; Zhiguang et al., 2019).

The crust color of the cake with malva nut gel replacing milk and butter is shown in Table 3. Increasing amounts of malva nut gel led to more lightness (L*) and yellowness (b*) of the studied crust cakes but less redness (a*) ($p \leq 0.05$). This was because with less milk, there was also less lactose and protein available to create the color of the product through caramelization and Maillard reaction. Due to a reduction of sugar dissolution in the mixture, there was a recrystallization of sugar causing brown spots on the top of cake; consequently, the crust color of the baked cake was uneven (Gisslen, 2016; Chemmek & Naivikul, 2017). The crumb color of the cakes with malva nut gel had the lightness (L*) near the control ($p>0.05$) except for the formula RM20/RB25. The redness (a*) of all samples was not different ($p>0.05$), but the yellowness (b*) of the RM0/RB25 and RM0/RB50 cake was significantly lower than that of the control ($p \leq 0.05$). The results led to darker and less yellow crumb because butter, which provides yellow color in cake, was reduced. The milk and butter replacement with malva nut gel, which contains more water, caused wheat flour to absorb more water and increased the gluten content. Consequently, the mixture was viscous, and the cake was less rising. These are consistent to the report of Phimolsiripol et al. (2017), revealing that the lightness (L*) and loaf specific volumes of the bread decreased with increased crude malva nut gum levels.

Texture profile analysis presented the hardest and softest textures in the RM0/RB25 and RM20/RB25, respectively. The hardness of RM0/RB50 and RM10/RB25 were not significantly different from the control. In addition, the replacement of milk and butter resulted in a softer cake. Hardness increased when malva nut gel was used to replace 20% milk and 25% butter ($p \leq 0.05$) because of an unbalance of butter and liquid in the mixture. In adhesiveness value, the RM0/RB25 was not significantly different from the control ($p>0.05$) but the RM0/RB50, RM10/RB25 and RM20/RB25 were significantly different ($p \leq 0.05$) in which these values were higher than the control. In contrast, Paseephol et al. (2016) found that butter and/or sugar replacements with malva nut gel resulted in decreasing hardness and increasing firmness and cohesiveness in chocolate cake, which was a butter type of cake.

Table 3 Physical qualities of the products replacing butter and milk with malva nut gel (mean ± standard deviation)

Quality	Replacing butter and milk by malva nut gel				
	Control (RM0/RB0)	RM0/RB25	RM0/RB50	RM10/RB25	RM20/RB25
Height (cm)	3.71^{h}±0.04	3.67^{h}±0.04	3.63^{h}±0.05	3.81^{a}±0.07	3.72^{ah}±0.04
Specific volume ^{ns}	3.67±0.47	3.73±0.29	3.22±0.59	3.47±0.06	3.39±0.20
Color value					
Crust					
- Lightness (L*)	50.16^{c}±0.61	55.26^{ah}±0.77	56.78^{a}±0.49	53.91^{bc}±1.01	54.12^{b}±1.84
- Redness (a*)	15.94^{a}±0.40	15.08^{a}±0.92	13.16^{c}±0.66	13.50^{bc}±1.23	14.73^{ab}±0.59
- Yellowness (b*)	29.92^{b}±1.63	30.91^{b}±0.58	33.99^{a}±1.45	33.93^{a}±0.96	33.10^{a}±1.04
Crumb					
- Lightness (L*)	67.93^{a}±0.71	66.37^{ah}±1.19	67.22^{ah}±1.11	64.08^{ah}±2.44	60.39^{b}±7.56
- Redness (a*) ^{ns}	1.22±0.93	1.64±0.30	1.72±0.51	1.14±0.60	1.06±0.30
- Yellowness (b*)	16.97^{a}±0.40	14.90^{b}±0.23	14.32^{b}±0.44	15.56^{ab}±0.78	15.18^{ab}±1.97
Texture					
Hardness (g)	558.79^{b}±50.95	443.32^{c}±20.91	505.17^{bc}±37.64	532.67^{bc}±18.83	845.15^{a}±9.52
Adhesiveness (N.s)	-5.05^{b}±1.31	-5.25^{b}±0.60	-2.41^{a}±1.64	-1.30^{a}±0.56	-3.02^{a}±1.03
Springiness ^{ns}	1.002±0.001	1.002±0.002	1.002±0.001	1.004±0.001	1.003±0.002

Remark: ^{abc} = Means followed by the same letter in the same row which are significantly different ($p \leq 0.05$)
^{ns} = Means in the same row which are not significantly different ($p > 0.05$)

2.2 Sensory evaluation

The sensory evaluation of the products from the 4 treatments evaluated with the preference test (9-point

hedonic scale) by 55 untrained panelists are shown in Table 4. The result shows that an increased replacement of milk and butter with malva nut gel affected the liking scores of appearance and aftertaste scores ($p \leq 0.05$). The appearance and aftertaste of RM0/RB25 obtained more liking scores than the others ($p \leq 0.05$) with the moderate liking. Moreover, the liking scores of flavor, taste, and overall preference of RM10/RB25 were higher than the others ($p \leq 0.05$). On the contrary, there were no significant ($p>0.05$) differences in color and softness as a moderate liking. Replacing milk and butter with malva nut gel in sponge cake resulted in a decreased milk and butter flavor; however, the odor and aftertaste liking scores were not affected by only milk and butter, but also by eggs in the recipe. (Suppavorasatit, 2014; Gisslen, 2016).

The selection criterion of formula was based on the highest amount of malva nut gel replacing butter and milk with the high panelist preference. Therefore, the product which had replaced 10% of milk and 25% of butter with malva nut gel (RM10/RB25) was the best product from this research. It contained 24.04% of cake flour, 24.03% of eggs, 24.03% of sugar, 13.52% of melted butter, 6.49% of evaporated milk, 5.23% of malva nut gel, 1.81% of cake emulsifier, 0.47% of baking powder, 0.19% of salt and 0.19% of vanilla essence.

Table 4 Average liking score of 55 untrained panelists regarding the sponge cake with butter and milk replaced with malva nut gel

Quality	Replacing butter and milk with malva nut gel			
	RM0/RB25	RM0/RB50	RM10/RB25	RM20/RB25
Appearance	7.20^{a}±1.01	6.74^{b}±0.82	7.15^{ab}±0.76	6.84^{ab}±0.63
Color ^{ns}	6.98±0.53	6.68±0.85	6.94±1.04	6.86±0.87
Flavor	7.02^{ab}±1.03	6.68^{b}±0.86	7.24^{a}±0.94	6.66^{b}±0.22
Taste	7.18^{ab}±0.83	6.90^{b}±0.66	7.40^{a}±0.43	6.94^{b}±1.02
Softness ^{ns}	7.06±0.76	7.10±0.74	7.22±0.98	7.00±1.01
Aftertaste	6.86^{a}±0.85	6.40^{b}±0.55	6.54^{ab}±0.43	6.82^{a}±1.02
Overall	7.18^{ab}±0.83	6.94^{ab}±1.05	7.26^{a}±1.04	6.88^{b}±1.00

Remark: ^{ab} = Means followed by the same letter in the same row which are significantly different ($p \leq 0.05$)
^{ns} = Means in the same row which are not significantly different ($p > 0.05$)

3. Chemical composition of product

The chemical composition of the final product from this research (replaced 25% butter and 10% milk with malva nut gel) and the according values of the control sample are shown in Table 5. Moisture and dietary fiber contents of the developed formulation were higher than the control ($p \leq 0.05$) by 33.10 and 31.80% respectively because mucilage in malva nut seed coats contained a high amount of dietary fiber causing a high moisture absorption (Piyatrakul, 2013). However, protein, fat, carbohydrate, cholesterol content and total calories of this product were lower than the control ($p \leq 0.05$) by 2.18, 23.84, 0.85, 8.35 and 11.06% respectively. The results are in accordance with Chajeamjen, et al. (2017) who reported that cookie bars with malva nuts had less fat and calories than the control ($p \leq 0.05$). In addition, its energy was reduced by up to 11%.

Table 5 Chemical composition of selected sponge cake with malva nut gel

Composition	Replacing butter and milk with malva nut gel	
	Control (RM0/RB0)	RM10/RB25
Moisture (%)	17.07^{b}±0.02	22.72^{a}±0.00
Protein (%)	6.42^{a}±0.02	6.28^{b}±0.01
Fat (%)	21.27^{a}±0.02	16.20^{b}±0.03
Ash ^{ns} (%)	0.87±0.01	0.90±0.02
Carbohydrate (%)	54.37^{a}±0.04	53.90^{b}±0.01
Dietary fiber (%)	2.83^{b}±0.01	3.76^{a}±0.04
Cholesterol (mg)	124.62^{a}±0.00	114.22^{b}±0.03
Total calories (Kcal)	434.59^{a}±0.25	386.52^{b}±0.23

Remark: ^{ab} = Means followed by the same letter in the same row which are significantly different ($p \leq 0.05$)
^{ns} = Means in the same row which are not significantly different ($p > 0.05$)

4. Consumer preference on malva nut gel sponge cake

The preference of consumers in RM10/RB25 tested by 120 consumers are shown in Table 6. Most consumers were 20-29 years old (49%), female (75%), students (35%) and their income was less than 10,000 baht (47%). The consumers preferred this product in appearance, color, flavor, taste, softness and overall characteristics "with a 'very much' liking score". The aftertaste had a moderate liking. Almost all of them used to consume sponge cake (95%), and they did it 1-2 times per month (35.96%) and the places to buy were bakery shops (75%) and department stores (50%). Moreover, 68% of consumers were interested in buying it.

Table 6 Average liking score of 120 consumers to the selected sponge cake with malva nut gel

Quality	Average liking score	Level of liking score
Appearance	7.51 ± 1.37	Like very much
Color	7.64 ± 1.00	Like very much
Flavor	7.60 ± 1.04	Like very much
Taste	7.54 ± 1.56	Like very much
Softness	7.71 ± 1.05	Like very much
Aftertaste	7.08 ± 1.27	Like moderately
Overall	7.66 ± 1.13	Like very much

Conclusion

Malva nut gel was a good ingredient to replace milk and fat in a sponge cake. 10% milk and 25% fat could be substituted with the gel causing a reduction of

energy (calories) up to 11% of the basic formula. Even if the gel affected the changes of the color and texture of a cake, the customers' preference score was still high. The findings from this research were commercially useful for healthy bakery products. To improve the quality of the product, cooperation with other hydrocolloids might be considered.

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Wastewater Treatment in the Brewing Industry Using *Chlorella vulgaris*

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Abstract

We studied the wastewater treatment efficiency of brewing industry effluents using the growth and biomass of the microalgae *Chlorella vulgaris*. Wastewater treated at% concentrations of 0, 20, 40, 60, 80 and 100 was examined and evaluated using the parameters of Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solids (SS), Phosphate (PO_4^{3-}) as ascorbic acid), TKN Nitrogen (Total Kjeldahl Nitrogen) and Total Dissolved Solids (TDS). Treatment reductions noted were 52.48% BOD, 52.42% COD, 79.12% SS, 40.82% Phosphate, 98.72% TKN and 43.96% TDS. During treatment, fats and oils measured as Oil and Grease (OG) increased to a maximum of 70.55% and *Chlorella vulgaris* at day 5 had the highest growth at $7.8x10^5$ cells per mL cultured in wastewater in a concentration of 100%. A maximum of 0.1541 g/L of dry biomass was obtained in 80% wastewater concentration. The autoclave method was the best method to extract oil by *Chlorella vulgaris* with an extraction value at 6.3%.

Introduction

Today, the problem of water pollution is becoming more and more serious due to population growth and industrial production required to meet the demand of the expanding population. Wastewater effluents from increased industrial activity pose risks to human health and the environment. Energy demands to operate treatment facilities (e.g., power for pumps and oxygenation equipment) are increasing every year (Chia et al., 2018) while fossil fuels are rapidly being depleted and are also losing favor because of their contribution to climate change. Renewable energy options available in Thailand have the potential to be developed as substitutes for petroleum-based fuels. Solar energy, biomass, biogas and energy from wind turbines are good examples. Biodiesel is one excellent source of sustainable energy as long as it does not use biomass from agriculture. The production of biodiesel usually requires the extraction of oil from food plants which is an ineffective use of resources since it reduces the food supply in Thailand and also increases the costs of biodiesel. Microalgae offer a good alternative to produce biodiesel since they contain nutrients such as nitrogen and phosphorus and can be produced with fatty acids stored in the cells in large quantities (de Alva et al., 2013). Small algae can

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be easily cultured due to their relatively rapid growth rate and low space requirements. Microalgae are able to photosynthesize and convert solar energy into biomass, fat and protein for use as algae biomass as food and for extracting oil to produce biodiesel. The beer fermentation industry uses large amounts of water, which also produces a large amount of wastewater. Wastewater effluent has major impacts on the environment and typically contains many nutrients that are necessary for the growth of microalgae. Using brewery effluent for microalgal cultures is beneficial for minimizing the use of freshwater, reducing the cost of nutrient addition, removing the remaining nitrogen and phosphorus, and producing microalgal biomass as bioresources for biofuel or high-value by-products (Schneider et al., 2013). We studied and report the wastewater treatment efficiency of brewing industry effluents using the growth and biomass of the microalgae *Chlorella vulgaris*.

Materials and methods

1. Wastewater sampling and analysis

Samples of wastewater were collected from a brewery (TAP Brewery, which brews Heineken, among others) in the SaiNoi, Nonthaburi Province. Samples were taken from a holding tank with treated wastewater prior to discharge from the brewery. The wastewater samples were autoclaved and analyzed to determine the quality of the wastewater prior to their use in experiments. Table 1 lists the parameters examined.

Table 1 Parameter analysis method

Parameters	Method
pH	pH meter (HANNA, HI98128)
TDS	TDS meter (HANNA, HI98312)
SS	Gravimetric analysis (Skoog et al., 1996)
BOD	Azide modification method (APHA, 2012)
COD	Closed reflux method (APHA, 2012)
Oil and Grease	Gravimetric analysis (Skoog et al.,1996)
TKN	Kjeldahl method (APHA, 2012)
Phosphate	Ascobic acid method (APHA, 2012)

2. Algae cultivation

Samples of microalgae (*Chlorella vulgaris*) were taken from the Aquatic Animal Feed Research and Development Division, 5th Floor, Department of Fisheries, Chatuchak, Bangkok, to cultivate with community wastewater. In this study, the starting cells were 5x10^5 cells/mL with a total amount of microalgae suspension of 350 mL. The cells were placed in 18 flasks of 500 mL each and then shaken every day. Afterward, the experiment was divided into 6 treatments (3 replications). The ratio of wastewater: deionized water was 100:0, 80:20, 60:40, 40:60, 20:80, 0:100. Microalgae was cultured indoors at room temperature in covered flasks, light intensity 4,000 lux, 12 h day: 12 h night for 7 days (Verma et al., 2020).

3. Algae biomass analysis

Microalgae was harvested in the stationary growth 350 mL of cultured wastewater samples were separated and filtered through CF/C filter paper, and then treated. The algae filter was dried at 105°C for 2 h. The algae were weighed and calculated according to the following equation.

$$\text{Dry weight of algae (g/L)} = \frac{(A - B) \times 1{,}000 \text{ mL}}{\text{Total algae}}$$

Where: A = after-filter fiber weight
B = pre-filter fiber weight

4. Algae oil extraction

The algal oil was extracted by the following three methods: manual, sonication, and autoclave.

4.1 Manual method

Algae was separated by using a centrifuge at 8,000 rpm, 2 min at 4°C, dried at 80°C for 17 h, dried algae were minified by crushing with medicinal mortar, and placed in 500 mL separator hoppers using 10 g of dried algae in 10 mL of hexane solution, shaken for 2 min. After that, the solvent was filtered and separated from the algae oil by evaporating on a hot water bath at 70°C. The evaporation bowl and samples were placed in the desiccant jar for 1 h.

4.2 Sonication method

Algae was separated by using a centrifuge at 8,000 rpm, 2 min at 4°C and mixed with 10 mL of DI water, then put into a 250 mL beaker. Sonication with a sonicator at 2 KHz for 20 min, then centrifuge again. The algae were then placed in a 500 mL separator funnel using 1 g of algae in 1 mL of hexane solution and shaken for 2 min. After that, the solvent was filtered and separated from the algae oil, and then the evaporation was carried out in a 70°C water bath.

4.3 Autoclave method

300 mL of algae was autoclaved at 125°C at 1.5 pounds/square inch for 5 min, and then the algae were placed in a 500 mL separator funnel using a 1 g of algae in 1 mL of hexane solution and shaken 2 min. After that,

the solvent was filtered and separated from the algae oil by evaporating in a hot bath at 70°C, and then the evaporation bowl and samples were weighed.

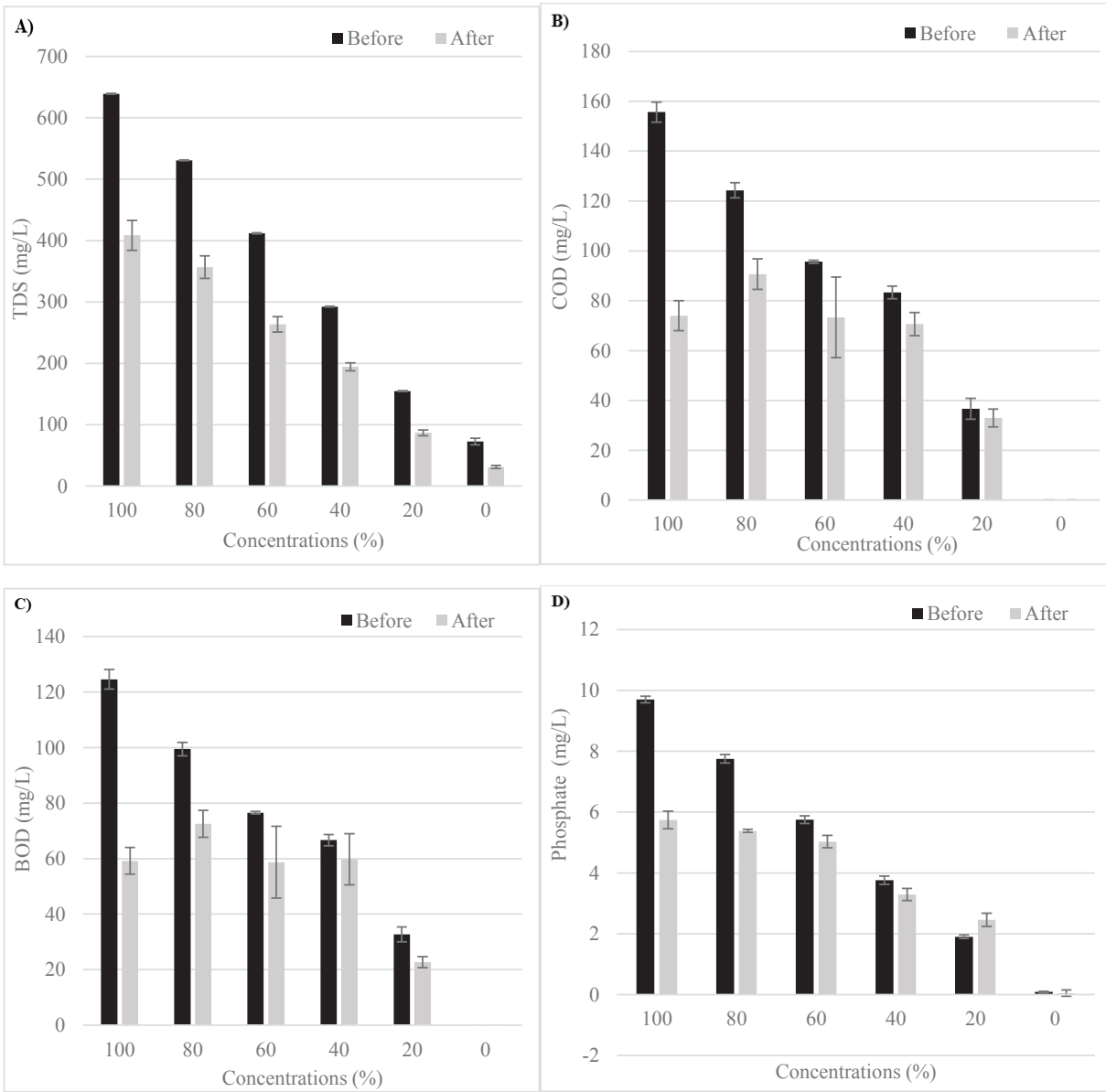
5. Statistical analysis

Data were analyzed by using One-Way ANOVA and compared with Duncan's New Multiple Range Test (DMRT) method at 95% using SPSS version 20.

Results and discussion

1. Wastewater quality analysis

Wastewater quality before and after microalgae culturing for 7 days in the concentrations 100%, 80%, 60%, 40%, 20%, 0% shown in Fig 1.



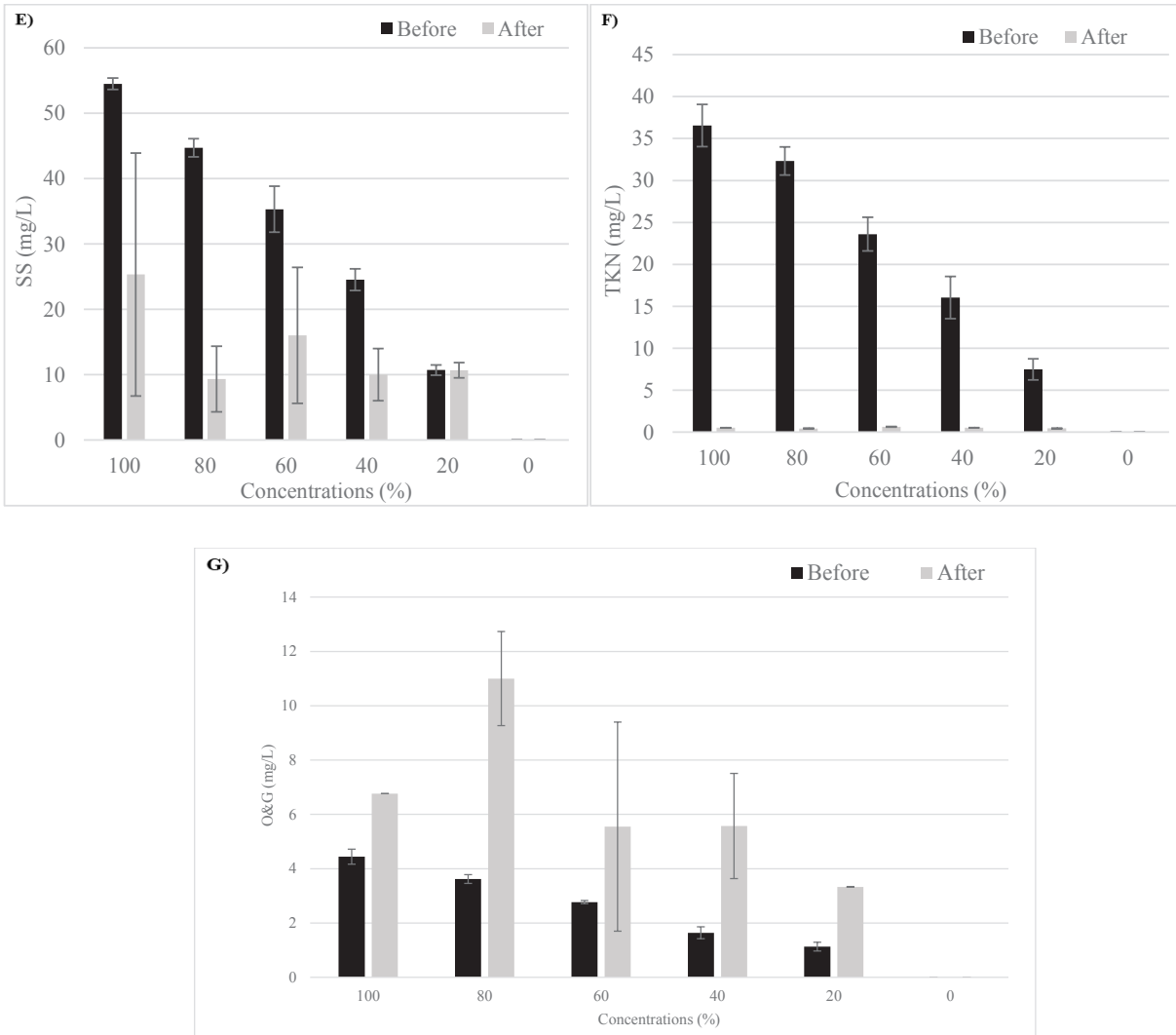


Fig. 1 Wastewater quality before and after microalgae culturing for 7 days A) TDS B) COD C) BOD D) Phosphate E) SS F) TKN G) O&G

2. Analysis of removal of physico-chemical parameters

The physico-chemical parameters of wastewater before and after microalgae culturing were shown in Fig 1. The selected parameters, such as TDS, COD, BOD, SS, PO_4^{3-}, TKN of wastewater decreased except for O&G which increased after *Chlorella vulgaris* culture. The study of all parameters after algae culture for 7 days showed that TDS was significantly reduced because part of the organic matter that plants use for their growth come from the dissolved form of organic matter. This was consistent with the research of Murugesan et al. (2010) culturing microalgae with wastewater of poultry farms, with the highest reduction in total dissolved solids in different trials at 38.92%. COD was significantly reduced clearly because algae can absorb the COD that is a source of carbon to algae for photosynthesis. This was consistent with research of Lin et al. (2007) that algae can absorb organic carbon as an energy source as well. BOD was significantly reduced because BOD contains a large number of dissolved organic compounds. Algae can make the biodegradation to be used as a source of energy that is consistent with the research of Praditwatthanakit (2012) with the average BOD in the range of 15.02-18.53 mg/L. SS was significantly reduced because suspended solids are the major source of nutrients present in brewery wastewater and were taken up during

Chlorella growth. Suspended solids consist of organic matter and many types of inorganic substances which are used during algae culture and its related anaerobic metabolism. Kotteswari et al. (2012) reported that total suspended solids decreased to 74.37% when dairy farm effluent was treated with *Spirulina platensis*. Phosphate (PO_4^{3-}) decreased the most with 40.82 percent because phosphorus is an essential element for algae growth which plays a role in various processes in cells, especially energy transfer and the process of creating nucleic acids including acting as a buffer to help stabilize the pH in cells, which was consistent with the study of Phromya et al. (2001) who cultured spirulina in various mediums. It was found that the syringe formula had a better growth risk because it contained a carbon source, nitrogen source, phosphorus and potassium. Phosphate (PO_4^{3-}) values are reduced because phosphorus is an essential nutrient for algae growth which plays a role in various processes in cells, especially energy transfer and the process of creating nucleic acids. It also acts as a buffer to stabilize the pH in cells, which was consistent with a study by Henkanatte-Gedera et al. (2015) who examined the BOD: N: P ratio in community wastewater by microalgae. The BOD: N: P value of 16.5: 13.4: 1 was able to reduce phosphate values in community wastewater by 98% over 3 days. *Galdieria sulphuraria* can be treated with 99% phosphate in 7 days. TKN was reduced by 90% because TKN is a source of nitrogen for algae and is also the main nutrient for algae. Algae cannot fix nitrogen in the atmosphere for use, therefore, nitrogen in organic and inorganic form in the water supporting growth is consistent with research by Feng & Zhang (2011) microalgae can reduce ammonia (nitrogen) in wastewater by 97%. Lipid and oil analysis (O&G) showed an increase in fat and oil contents due to stress because of the reduction of nutrients in wastewater. Likewise Mujtaba et al. (2012) found *Chlorella vulgaris* increased oil content from 20% to 40% of dry weight when the algae were under unsuitable growth conditions. The pH value selected is important in the cultivation of microalgae as unfavorable pH values will stress the microalgae, which ultimately affects the efficacy of nutrient absorption and metabolites produced (Morais et al., 2015). The pH values in the holding tank of brewery wastewater in 100% concentration was 8.8. In experiments, the wastewater samples were autoclaved to eliminate algae and other microorganisms that were not part of the experiment, thus the pH value in the experiment was 9.92 increasing because of oxygen decay, with the hydrogen value increasing slightly. The alkaline pH after culturing microalgae was 10.68 due to the positively charged micronutrients used by algae and the nutrients in the water contain phosphates, which are negatively charged micronutrients. The phosphates increasing pH after culturing algae is consistent with that reported in Saekoo & Panich-pat (2020) using microalgae cultured in piggery farm wastewater which increased pH from 8.11 to 8.84 at a wastewater concentration 100%. Gong et al. (2014) demonstrated the potential use of *Chlorella vulgaris* to mitigate the toxic effects of cadmium in the water and found under normal cultivation, the optimum pH for the growth of *Chlorella vulgaris* falls between 10.0 and 10.5 and that constant pH adjustment to optimum pH enhances the growth of microalgae and prevents contamination.

3. Growth of *Chlorella vulgaris* algae

The growth of microalgae cultured in wastewater from the brewing industry was studied in different concentrations of 100%, 80%, 60%, 40%, 20%, and 0% that measured the growth for 7 days. It was found that the concentration of 100 percent wastewater supported the highest growth of algae. At day 5, wastewater contained more nutrients than seen in other experiments, and the nutrients were sufficient to both meet the required needs of the algae (Lam et al., 2017) and to support the number of cells equal to 7.8 x 10^5 cells/mL which is the best growth concentration (Fig 2). Thus, the 100% concentration supported the highest live algae biomass when compared to lower concentrations of effluent.

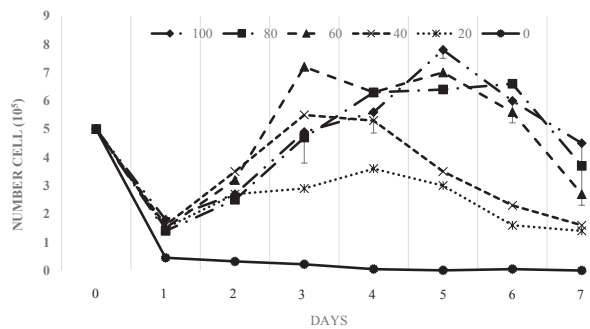


Fig. 2 Growth of *Chlorella vulgaris* in different concentrations for 7 days

4. Amount of biomass obtained from algae culture

The results showed that biomass yields cultured in different concentrations ranged from 0.0165-0.1541 g/L, with the highest concentration of biomass being 80% as followed 100%, 60%, 40%, 20%, 0%, equal to 0.1541, 0.1157, 0.0984, 0.0546, 0.0165 and 0 g/L, respectively,

which was consistent with the work of Feng & Zhang (2011) that microalgae can be cultured in treated wastewater. The value of dried algae was 0.5-0.28 g/L. The concentration of biomass at 80% was not statistically significantly different at $p>0.05$ but 100% and 60% were statistically significantly different ($p<0.05$) as same as 40%, 20%, 0%. Dried algae at a concentration of 80% should be used to extract oil further.

5. Amount of oil extraction obtained from algae culture

In the study, it was found that the extraction of oil from algae at the concentration of 80% by the autoclave method had the highest percentage yield, followed by the sonicate and manual methods with extraction rates of 6.3%, 5.05% and 4.29%, respectively. These three methods of oil extraction are the most widely used ones. The autoclave method was the best oil extraction technique because the pressure during the autoclave treatment destroyed cell walls of the algae which facilitated oil release. One study using HPLC testing for saturated and unsaturated fats to determine the quality of biodiesel production in cultured microalgae reported that the resulting fat was in a saturated form that increased the quality of biodiesel (Rinna et al., 2017). For this study, the extracted algae oil was insufficient for biodiesel extraction because the extraction of biodiesel depends on the amount of algae that need to be cultured in larger quantities by using larger scale cultures than the laboratory scale. Lipid production in *Chlorella vulgaris* increases under nitrogen starvation conditions but this also reduces the total biomass of microalgae which reduces the overall lipid yield (Prîbyl et al., 2012). Hence, in order to solve this problem without affecting the overall lipid yield, it is suggested that microalgae are cultured in nitrogen-rich conditions initially to enhance growth and increase biomass before transferring them to a nitrogen deficient environment to stimulate lipid production (Mujtaba et al., 2012).

Conclusion

Chlorella vulgaris was very effective in reduction of chemical concentrations (BOD, COD, SS, Phosphate, TKN and TDS). TKN removal was achieved up to 98.72%. The maximum growth of algae at day 5 was $7.8x10^5$ cells/mL at 100% concentration of wastewater. The highest algal biomass was 0.1541 g/L at 80% concentration of wastewater. The results of microalgae oil extraction showed that the autoclave method was the best algae oil extraction method with 6.3%. Although the autoclave method was the best oil extraction, the sonicate method is probably the most cost-effective way of oil extraction in terms of energy consumption.

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Product Development of Sweet Fish Sauce from Dried White Shrimp: Sensory Evaluation, Physical and Chemical Quality and Nutrition

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Abstract

This research aimed to study the product development of sweet fish sauce from dried white shrimps (shrimp weight 5.0-6.7 g). All of the samples were compared. Data showed that the modified recipe containing 30% palm sugar, 25% unbleached sugar cane, 9% fish sauce, 13.8% water, 5.5% dried white shrimp, 16% shallot and 0.7% chili had a higher satisfaction and acceptance score than the basic recipes, with a statistically significant difference at $p< 0.05$. The CIE LAB values of both the basic recipes and the modified one had statistically significant differences at $p< 0.05$ as the modified recipe had a lighter color. At the same time, there were no statistically significant differences at $p\geq0.05$ in terms of the viscosity of liquid and a_w. The Guideline Daily Amounts (GDAs) of the modified recipe indicate that one package (five servings) of 230 g consists of 600 kcal, 115 g of sugar, 0 g of fat and 1,050 g of sodium. The shelf life of the modified recipe was 60 days. The CIE LAB values, viscosity of liquid and a_w had no statistically significant differences at $p\geq0.05$. The modified recipe also had an overall acceptance level of 7.98 on the nine-point hedonic scale, which means that the 100 consumers who tasted the final products were highly satisfied. With regard to acceptance of this newly modified recipe (MC1), 98% and 95% of consumers would be highly likely to be willing to buy the products when they were released. Most of the consumers agreed that the selling price was reasonable (86%) and 82% were satisfied with the packaging. The enterprise would gain 40% more income, the compensation for members would increase by 24% and 175 kg of small white shrimps would be purchased from shrimp farmers for drying and producing the sweet fish sauce.

Introduction

Sweet fish sauce is one type of Thai dipping sauce with a unique intense sweetness and a slightly salty and spicy taste. This sauce is not too thick and not too watery, and is generally served with sour or sweet and sour fruits. Sour and unripe mango with sweet fish sauce is the classic combination. However, in the present, sweet fish

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sauce is modernized and adapted to be served with both Thai seasonal fruits and foreign fruits such as santol, salacca, rose apple, guava, plango, apple and strawberries. Sweet fish sauce is a simple dipping sauce with simple preparation. Each recipe has its own distinctive details, yet main ingredients are usually similar, consisting of palm sugar, cane sugar, fish sauce, shallot, dried shrimp and chili. Currently, sweet fish sauce is now distributed and sold in superstores or various souvenir shops depending on packaging. It is generally produced in glass packaging and sealed tight to prolongate its shelf life and make it easy to store if unconsumed (Phungbunhan, 2016; Songsuk, 2008). This is correlated with the rising trends in entrepreneurs or food industry dealers taking an interest in sweet fish sauce, which could become a supplementary vocation or profession and lead to selling both offline and online. More than 150 packages of some sweet fish sauce brands could be sold per day (Chumkam, 2019; Sentangsedtee Online, 2021; PPTV Online, 2021). Due to the rising demands in the market, ingredients required to produce sweet fish sauce are highly sought-after, resulting in higher earnings for farmers, e.g. chili, shallot and shrimp farmers.

Dried white shrimp is one of the essential ingredients for sweet fish sauce since dried shrimp adds texture and thickness to the sauce. Also, the full-flavored, balanced and rich taste of the sauce comes from dried shrimp. *Litopenaeus vannanmei* comes from shrimp farms in Ongkharak District, Nakhon Nayok Province. According to the white shrimp farming statistics from Nakhon Nayok Provincial Fisheries Office (2021), there are approximately 424 shrimp farmers in Ongharak District with roughly 8,670.50 rai of farming area, total producing 2,030.45 tons of shrimp annually. Upon interviewing the director of shrimp processing community enterprise, Buranasuont (2020) on the shrimp farming context and sweet fish sauce processing issues, it became obvious that there are two categories of shrimp farming in Ongkharak District: farming with Nile Tilapia fish, and sole and shrimp farming. Combined farming with Nile Tilapia fish usually produces shrimp four times per annum and shrimp sizes differ in each catch. It is due to the fact that this type of farming relies on natural correlation of the fish and shrimp. Shrimp collect oxygen from fish and consume fish feed products and fish waste, while fish consume shrimp waste and dead shrimp, keeping the water clean. Therefore, shrimp caught from such farming differ in size and shrimp weighing 5.0-6.7 g are usually unwanted in the market. Generally, shrimp farmers use such shrimp as fertilizer or process it into other animal feed products. The shrimp processing community enterprise acknowledges the issue and purchases such shrimp from local farmers to produce small dried white shrimp for sweet fish sauce.

Due to that reason, the researchers focused on developing sweet fish sauce using dried shrimp weighing 5.0-6.7 g. The developed recipe was investigated and modified to create a stable and standard taste in every product. Consequently, the researchers analyzed the sensory evaluation of each sweet fish sauce recipe in order to select the most satisfying recipe based on data collected from research samples. Not only the sensory evaluation was tested, but the researchers also conducted a study on physical and chemical quality, nutrition, shelf life and consumer approval of the modified sweet fish sauce, consistent with the previous research on sweet fish sauce, e.g. the study of Khumwachirapithak et al. (2017). According to Khumwachirapithak et al. (2017), the optimum recipe of sweet fish sauce consisted of palm sugar, fish sauce, dry shrimp, shallot, dry chili and water to produce this sweet fish sauce, dry shrimp are put in boiling water for 10 seconds, palm sugar and fish sauce are then added, controlling the temperature at 160°C during the process, then cool down to 80°C. The total soluble solid should be 75°Brix. Then added dry chili, shallot, ground dry shrimp, and boiled dry shrimp and mix well. Check total soluble solids again; the designated value is 75°Brix. After that, fill in glass bottles.

Hence, this research produced a ready-to-sell sweet fish sauce product, evaluating product processing from community materials and creating a new product for the community enterprise. This product is considered as one of Nakhon Nayok Province's OTOP products, creating career opportunities, increasing members' revenue and supporting local shrimp farmers. This corresponds to the RMUTT Flagship Strategy on Agro-food Innovation, aiming to develop innovation, provide academic output and develop agricultural and processing skills for those who are interested. By doing so, value could be added to local materials (Policy and Planning Division, RMUTT, 2020). Not only is it correlated with the RMUTT plan but it is also in line with the National Strategy 2018-2037 on developing competitive skills in agro-processing using agricultural products to create new products (Economic and Social Development Board, 2018).

Materials and methods

1. Raw material preparation

The ingredients used to produce sweet fish sauce, which were purchased from supermarkets and market stores, included palm sugar (Mitr Phol, Mitr Phol Group., Thailand), unbleached sugar cane (Mitr Phol, Mitr Phol Group., Thailand), shrimp paste (Trachang, Tang Thai Chiang Fish-sauce Manufacturing Co.Ltd., Thailand), fish sauce (Trachang, Tang Thai Chiang Fish-sauce Manufacturing Co.Ltd., Thailand), dried shrimp (community enterprise in the group of shrimp processing, Nakhon Nayok, Thailand), shallot (fresh market., Thailand), dried chili (fresh market, Thailand) and water (Nestle, Perrier Vittel (Thailand) Co.Ltd.).

2. Preparation for drying small white shrimp

The white shrimp weighing 5.0-6.7 g, purchased from shrimp farmers, were thoroughly washed as the first preparation step. The heads were then peeled off and the peeled shrimps were washed again. The shrimp were boiled in boiling water for 5 minutes. After boiling, the boiled shrimp were rinsed and added to the Chaichana 12-storey gas dryer, produced by the Chaichana company, Thailand. The drying process took 30 minutes at 100°C. After that, the heat was lowered to 70°C and the process was continued for 3 h, each time with 30 kg of boiled shrimp. Once all the shrimp were completely dried, it was found that approximately 3 kg of small dried white shrimp had been produced, as displayed in Fig. 1.

Fig. 1 Dried white shrimp

3. The study on the standard recipe

The research investigated three recipes, as shown in Table 1. The research was conducted as follows. Sugar was added to fish sauce and water and gently mixed. Then it was brought to the boil. Shrimp, shallot and dry chili were added to the thickened mixture and stirred until it boiled. The samples were stored at room temperature. The results were analyzed in terms of sensory evaluation by following the nine-point hedonic scale criteria (1 = dislike extremely and 9 = like extremely). The sweet fish sauce was evaluated on its appearance, color, smell, salty flavor, sweetness, spiciness, viscosity and overall acceptance. Testers for this research were 30 trained panelists from the Department of Food and Nutrition, Rajamangala University of Technology Thanyaburi.

Table 1 Basic sweet fish sauce recipes

Ingredients	Basic recipe 1 (%) (BC1)	Basic recipe 2 (%) (BC2)	Basic recipe 3 (%) (BC3)
Palm sugar	30	40	40
Unbleached sugar cane	25	-	5
Shrimp paste	-	6	5
Fish sauce	8	12	15
Water	15	20	19
Dried shrimp	7	10	5
Shallot	14	9	6
Dried chili	1	3	5

Remark: Basic recipe 1 (BC1) was modified from Khumwachirapithak et al. (2017), Basic recipe 2 (BC2) was modified from Manuntapong (2019) and Basic recipe 3 (BC3) was modified from Chumkaew (2018)

4. Development of the basic recipes and the production process

The basic sweet fish sauce recipe (BC1), which received the highest satisfaction scores in the sensory evaluation test, was selected, and the recipe was developed and modified resulting in a new recipe (Table 2) which was based on the evaluation results and suggestions. The novel recipe was then evaluated in comparison to the basic one as follows.

Table 2 Sweet fish sauce recipes

Ingredients	Basic recipe 1 (%) (BC1)	Modified recipe (%) (MC1)
Palm sugar	30	30
Unbleached sugar cane	25	25
Fish sauce	8	9
Water	15	13.8
Dried shrimp	7	5.5
Shallot	14	16
Dried chili	1	0.7

4.1 Sensory evaluation of the basic sweet fish sauce and the modified recipe

The basic sweet fish sauce recipe, which received highest acceptance score, was later analyzed in comparison with the modified recipe by using the nine-point hedonic scale (1 = dislike extremely and 9 = like extremely). The plain sweet fish sauce was

evaluated on its appearance, color, smell, salty flavor, sweetness, spiciness, viscosity and overall acceptance scores. Testers for this research were 30 trained panelists from the Department of Food and Nutrition, Rajamangala University of Technology Thanyaburi. All of samples were served with unripe mango. During the tasting test, panelists are required to drink water prior to the next sample testing. Results were used to further develop the recipe.

4.2 Analysis of the physical and chemical quality of the basic and modified recipes

The physical quality of the plain sweet fish sauce was analyzed under the CIE LAB L* a* b* system by using a Minolta colorimeter CR-300 model (Minolta Co., Ltd., Osaka, Japan) and the viscosity analysis was performed five times with a CSC Scientific Bostwick consistometer model 1-800-458-2558. The chemical quality analysis on water activity (a_w) was performed five times with an Aqualab water activity meter and the basic recipe was compared with the modified one.

4.3 Nutrition study of the modified recipe

The modified recipe was analyzed based on the criteria of one serving weighing 100 g. The nutritional criteria consisted of total energy and energy from fat (Ralph, 1995), total fat (AOAC, 2019), saturated fat and cholesterol (in-house method) (Bureau Veritas AQ Lab (Thailand) Limited, 2021), sodium (in-house method TPT-FS-252TM)(Bureau Veritas AQ Lab (Thailand) Limited, 2021), total carbohydrate (Ralph, 1995), dietary fiber (AOAC, 2019), sugar and protein (in-house method)(Bureau Veritas AQ Lab (Thailand) Limited, 2021), vitamin A (in-house method TPT-FS-262TM) (Bureau Veritas AQ Lab (Thailand) Limited, 2021), vitamin B1 and vitamin B2 (in-house method TPT-FS-271TM) (Bureau Veritas AQ Lab (Thailand) Limited, 2021), calcium and iron (in-house method TPT-FS-252TM) (Bureau Veritas AQ Lab (Thailand) Limited, 2021) and moisture and ash (AOAC, 2019)

5. The study on sweet fish sauce shelf life

The sweet fish sauce in glass packaging stored at a room temperature of 30°C was tested for its shelf life according to physical quality. The plain sweet fish sauce was analyzed by using the CIE LAB L* a* b* system and the color analysis was performed by using Minolta colorimeter CR-300 model (Minolta Co., Ltd., Osaka, Japan), and the viscosity analysis was performed five times with a CSC Scientific Bostwick consistometer model 1-800-458-2558. The chemical properties were tested five times with an Aqualab water activity meter at 0, 10 ,20, 30, 40, 50 and 60 days after production to define the shelf life of the sweet fish sauce.

6. The study on consumer acceptance of the sweet fish sauce product

The study was conducted by simple random sampling from 100 consumers in Rajamangkala University of Technology Thanyaburi and by using a questionnaire. The questionnaire consisted of three parts: Part 1 Personal Information, such as gender, age, profession, income and frequency of sweet fish sauce consumption; Part 2 Sensory Evaluation, performed using the nine-point hedonic scale (1 = dislike extremely and 9 = like extremely). The plain sweet fish sauce was evaluated on its appearance, color, smell, salty flavor, sweetness, spiciness, viscosity and overall acceptance. The sample preparation is similar to part 4.1. Part 3 of the questionnaire was based on attitude toward the sweet fish sauce product: approval and acknowledgment, purchase decision, proper selling price and proper packaging.

7. The study on production and distribution of sweet fish sauce products from the shrimp processing community enterprise

The researchers instructed the shrimp processing community enterprise on the production process of the sweet fish sauce and shared technology and innovation with them in order that entrepreneurs could later use such knowledge to produce their products and distribute them as Nakhon Nayok Province's OTOP products. The researchers collected selling data from October 2020-July 2021 on the following categories; the amount of small dried white shrimp for sweet fish sauce (kg), total sold (packages), total income (baht), increase in income from sweet fish sauce distribution (%) and increase in compensation for the shrimp processing community enterprise members (%).

8. Statistical analysis

Both the study on basic sweet fish sauce recipes and the study on shelf life involved analysis using descriptive statistics: mean and standard deviation. Analysis of Variance (ANOVA) and average comparison using Duncan's New Multiple Range Test (DNMRT) at a 95% confidence interval were also conducted. Furthermore, the sensory evaluation and physical and chemical quality of both the basic recipe and the modified one were analyzed by Dependent T Test at a 95% confidence interval. The study on consumer approval of sweet fish sauce was carried out by using SPSS program, version 22, in terms of statistical analysis. (SPSS, 2018).

Results and discussion

1. The study on basic sweet fish sauce recipes

According to the sensory evaluation results of three basic sweet fish sauce recipes, as illustrated in Table 3, basic recipe 1 (BC1) was highly accepted by the 30 panelists in all characteristics with statistically significant differences at $p< 0.05$. Further comments indicated that the panelists found the taste of basic recipe 1 (BC1) most likable due to the lack of shrimp paste, since shrimp paste usually produces an unpleasant smell and sweet fish sauce with shrimp paste normally has a darker shade of color than those without it. In addition to these arguments, shrimp paste causes issues in quality control. Therefore, the researchers selected this basic recipe (BC1) to adapt and develop further.

Table 3 Sensory evaluation scores of three basic sweet fish sauce recipes (n = 30)

Characteristics	Basic recipe 1 (BC1)	Basic recipe 2 (BC2)	Basic recipe 3 (BC3)
Appearance	$8.56^{a} \pm 0.50$	$6.96^{b} \pm 0.61$	$6.23^{c} \pm 0.72$
Color	$8.53^{a} \pm 0.50$	$7.06^{b} \pm 0.69$	$6.50^{c} \pm 0.93$
Smell	$8.43^{a} \pm 0.50$	$6.40^{b} \pm 0.96$	$6.16^{b} \pm 0.94$
Salty Flavor	$8.56^{a} \pm 0.50$	$6.33^{b} \pm 0.80$	$5.90^{c} \pm 0.95$
Sweetness	$8.50^{a} \pm 0.50$	$5.96^{b} \pm 1.09$	$5.33^{b} \pm 0.75$
Spiciness	$8.06^{a} \pm 0.69$	$6.33^{b} \pm 0.99$	$5.63^{c} \pm 0.92$
Viscosity	$8.13^{a} \pm 0.68$	$6.70^{b} \pm 0.83$	$5.80^{c} \pm 0.99$
Overall acceptance	$7.90^{a} \pm 0.66$	$6.30^{b} \pm 0.70$	$6.33^{b} \pm 0.66$

Remark: The *a, b,* and *c* superscripts in the same row indicate the order of ac ceptance with regard to each characteristic ($p< 0.05$), All values are shown as mean ± S.D.

2. The results of recipe development and the production process

According to the sensory evaluation results, basic recipe 1 (BC1) was highly accepted. Thus, basic recipe 1 (BC1) was selected and developed into a new modified recipe (MC1) following suggestions from panelists, by focusing on the production process to create a stable and standard quality product. The adaptation and modification of the recipe was done by adding more fish sauce, reducing the amount of small dried white shrimp (some were finely blended, others were whole shrimp), adding more shallot (peeled, thoroughly rinsed, then finely chopped into 1 mm pieces and baked in air dryers at 100°C for 30 minutes), reducing the amount of chili (rinsed and dried, then chopped into 2 mm pieces) and reducing the amount of water.

The production process was also developed in order to create a stable recipe and standard taste, as shown in Fig. 2. Palm sugar, unbleached sugar cane, and fish sauce were mixed with water and simmered over low heat. After the sugar had dissolved, the mixture was heated over a high heat until it thickened at a temperature controlled at 112°C using a thermometer in order to create a standard viscosity in every production. Once the sauce was stable, whole dried shrimp and blended shrimps, dried shallot and dried chili were added and it was stirred for 3 minutes. The sauce was poured equally into pasteurized glass packages, each containing 230 g, and allowed to cool in order to seal the package. The appearance of the modified sweet fish sauce in comparison to the sauce of the basic recipe is displayed in Fig. 3.

Fig. 2 Sweet fish sauce production process (a) Add palm sugar, unbleached sugar cane, fish sauce and water to the pot; (b) Stir over a low heat until the sugar dissolves; (c) Heat over a high heat at 112°C until it thickens; (d) Add small dried white shrimp, shallot and chili; (e) Stir thoroughly for 3 minutes; (f) Pour into pasteurized glass packaging, each containing 230 g, and seal tightly for later distribution

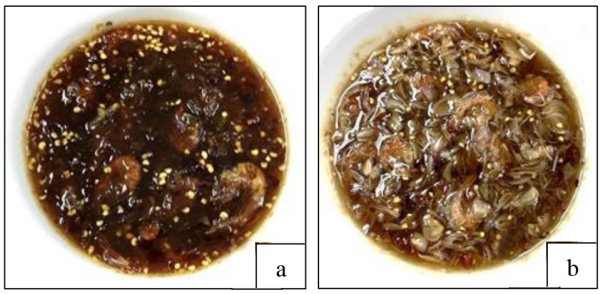

Fig. 3 Sweet fish sauce, basic recipes and modified recipes (a) Basic recipe (BC1) (b) Modified recipe (MC1)

According to the analytical results of the comparison of basic (BC1) and modified recipes (MC1) of sweet fish sauce by 30 panelists, as shown in Table 4, the samples preferred the modified recipe over the basic one with statistically significant differences at $p<0.05$. The scores according to each characteristic indicate that MC1 has higher sensory test scores in all attributes (appearance, color, smell, salty flavor spiciness and overall acceptance). However, the sweetness and viscosity scores of both recipes were slightly different with no statistical significance. Hence, it could be assumed that the modified recipe of sweet fish sauce was preferred over the basic one. Therefore, the researchers adopted the modified recipe (MC1) for further development.

Table 4 Sensory evaluation of basic and modified sweet fish sauce recipes (n=30)

Characteristic	Basic recipe (BC1)	Modified recipe (MC1)
Appearance	$6.43^{b} \pm 0.97$	$8.10^{a} \pm 0.71$
Color	$6.20^{b} \pm 0.84$	$8.50^{a} \pm 0.50$
Smell	$6.13^{b} \pm 1.04$	$8.06^{a} \pm 0.82$
Salty Flavor	$7.26^{b} \pm 0.78$	$7.90^{a} \pm 0.85$
Sweetness^{ns}	7.60 ± 1.06	7.80 ± 0.66
Spiciness	$6.93^{b} \pm 1.01$	$7.53^{a} \pm 0.77$
Viscosity^{ns}	7.83 ± 1.17	7.73 ± 0.58
Overall acceptance	$6.00^{b} \pm 1.11$	$8.36^{a} \pm 0.55$

Remark: $^{a, b}$ superscripts in the same row refer to the order of acceptance regarding characteristics with statistically significant differences at a confidence interval with $p<0.05$

^{ns} refers to no statistically significant differences ($p\geq0.05$)

The comparison of physical and chemical qualities of basic and modified sweet fish sauce recipes, as illustrated in Table 5, reveals that the L* (lightness) a* (redness) and b* (yellowness) of both recipes had statistically significant differences at $p<0.05$ as the L* of the modified recipe was at 17.72, which was higher than the 13.84 of the basic recipe, signifying a lighter shade of color compared to the basic recipe. This was due to the fact that more processes had been added to the modified recipe, the amount of water had been lessened and the diced shallot had been air-dried before being added to the sauce to reduce simmering time and to allow the sauce to reach a constant heat of 112°C 10 minutes faster than the basic recipe. Temperature control is the core factor in producing a constant and stable color of sauce in every production. Without temperature control, the color and viscosity will not be constant since the temperature is likely to differ in every production. For example, the higher the heat or the longer the simmering period, the more likely the sweet fish sauce is of a darker shade. This corresponds to one of the factors of the Maillard reaction, the temperature, due to the fact that the Maillard reaction increases once the temperature rises and the color changes according to the heat (Rattanapanone, 2002). It is also in line with Andrew et al. (2002) statement that the longer the simmering period was, the darker the shade of the sample would be. Furthermore, a process was developed where palm sugar, cane sugar, fish sauce and water are stirred together, dissolving the sugar before turning on the heat in order to reduce the brownish shade of color from the Maillard reaction (Sitachitta, 2012; Khumwachirapithak et al., 2017). By doing so, caramelization from using high heat with sugar is also reduced since caramelization occurs once sucrose is exposed to temperatures higher than 120°C (Fadel & Farouk, 2002; Zhang et al., 2013). The a* and b* color values of the modified recipe were also higher than the basic recipe. The a* value of the modified recipe was 5.18 and the b* value was 10.76, indicating more red and yellow shades compared to the basic recipe. At the same time, the viscosity and water activity (a_w) of both recipes could barely be distinguished, with no statistically significant differences at $p\geq0.05$. This might be due to the fact that temperature control was performed using a thermometer during production; therefore, viscosity and water activity (a_w) of both recipes showed no significant differences.

Table 5 The physiochemical properties comparison of basic and modified sweet fish sauce recipes (n=5)

Characteristic	Basic recipe (BC1)	Modified recipe (MC1)
L*	$13.84^{b} \pm 0.68$	$17.72^{a} \pm 0.61$
a*	$4.10^{b} \pm 0.30$	$5.18^{a} \pm 0.57$
b*	$5.78^{b} \pm 0.40$	$10.76^{a} \pm 1.46$
Consistency (cm./second)^{ns}	0.60 ± 0.06	0.61 ± 0.02
$a_w{}^{ns}$	0.650 ± 0.04	0.670 ± 0.02

Remark: $^{a, b}$ superscripts in the same row refer to the order of acceptance regarding characteristics with statistically significant differences at a confidence interval with $p<0.05$.

^{ns} refers to no statistically significant differences ($p\geq0.05$)

Upon studying the nutrition of the modified recipe, as displayed in Table 6, it was revealed that the modified recipe contained energy equal to 250 kcal per 100 g package and 120 kcal per serving. Energy from fat was equal to 3.24 kcal per 100 g package or 0 kcal per serving. The total amount of fat was equal to 0.36 g per 100 g package and 0 g per one serving. The amount of protein was equal to 4.58 g per 100 g package and 2 g per serving, while the amount of carbohydrate was 57.1 g per 100 g or 26 g per serving. Dietary fiber amounted to 0.88 g per 100 g or 0 g per serving, and the amount of sugar was equal to 50.7 g per package or 23 g per serving. Sodium amounted to 458 mg per 100 g or 210 mg per serving. It could be assumed that one serving of sweet fish sauce (or 46 g), approximately three tablespoons, consisted of nutritional amounts at levels not exceeding the Thai Recommended Daily Intakes (Thai RDI) for people over six years of age based on a daily energy requirement of 2,000 kcal (Ministry of Health, 1998). Thus, it is safe to consume. However, frequency of consumption is a concern and it is not recommended to consume it regularly due to the high amount of sucrose in sweet fish sauce (50.7 g per 100 g). This is correlated with the research of Khumwachirapithak et al. (2017) which established that there was a high amount of sucrose in sweet fish sauce (44.71 g per 100 g of the sauce). Hence, consumers with a hyperglycemia condition need to be cautious and consumers are advised to exercise regularly (Bureau of Nutrition, Department of Health, 2011).

Table 6 Nutrition labeling-Thai RDI

Test Item	Per 100 g	Per Serving (46 g)	%Thai RDI
Total energy (kcal)	250	120	-
Energy from fat (kcal)	3.24	0	-
Total fat (g)	0.36	0	0
Saturated fat (g)	ND	0	0
Cholesterol (mg)	ND	0	0
Protein (g)	4.58	2	-
Total carbohydrate (g)	57.1	26	9
Dietary fiber (g)	0.88	0	0
Sugar (g)	50.7	23	-
Sodium (mg)	458	210	11
Vitamin A (µg)	ND	-	0
Vitamin B1 (mg)	ND	-	0
Vitamin B2 (mg)	ND	-	0
Calcium (mg)	18.7	-	0
Iron (mg)	0.48	-	0
Moisture (g)	35.0	-	-
Ash (g)	2.94	-	-

3. The study on sweet fish sauce shelf life

As displayed in Table 7, regarding the results of the shelf life of sweet fish sauce in glass packaging and its physical and chemical qualities over a period of 0-60 days, it was established that the L* (lightness), a* (redness), b* (yellowness), viscosity and water activity (a_w) values barely indicated any statistical significant differences at $p \geq 0.05$. However, such values are highly likely to increase. The L* value was recorded at 16.68-18.04, the a* at 3.60-4.28 and the b* at 9.88-11.65, while the viscosity was recorded at 0.60-0.65 cm/second. One factor which might affect sweet fish sauce's physical and chemical qualities is the glass packaging since glass packaging prevents air permeability and steam, leaves contained food unaffected and is heat resistant (Soroka, 2002; Petchwattana, 2020). Thus, sweet fish sauce contained in such containers is well preserved, since a fish sauce packaging process which uses glass bottles preserves the quality and color of fish sauce better than one which uses plastic bottles (Kongpun, 2010). In addition to the mentioned factors, the study is also consistent with Bacigalupi et al. (2016), who found that glass packaging could decelerate oxidation in fruit juice better than PET bottles and preserve more antioxidants.

Furthermore, the water activity (a_w) was at 0.67-0.71, befitting of the standards for community products, which state that the water activity (a_w) of sweet fish sauce may not exceed 0.80 (Community Product Standards, 2003). This is also consistent with the research of Khumwachirapithak et al. (2017), which found that the water activity (a_w) of a modified recipe sweet fish sauce was 0.74 and aerobic plate counts at 3.7 x 10^2 with the amount of yeast and mold at < 10 cfu/g. The data in this research, similar to our study, indicated that the number of microbial growths in sweet fish are likely not to affect the quality of product. The water activity value (a_w), however, affects the microbial growth in which water activity (a_w) lower than 0.7 decelerates the microbial growth (Rattanapanone, 2006).

Table 7 Physiochemical properties of the modified recipe affecting its shelf life at 0, 10, 20, 30, 40, 50 and 60 days (n=5)

Day	L^{*ns}	a^{*ns}	b^{*ns}	Consistency (cm./second)^{ns}	$a_w{}^{ns}$
0	17.08 ± 0.66	3.60 ± 0.49	9.88 ± 0.83	0.60 ± 0.05	0.670 ± 0.03
10	16.68 ± 1.21	3.72 ± 0.53	10.30 ± 0.46	0.61 ± 0.05	0.670 ± 0.08
20	16.86 ± 1.35	3.92 ± 0.44	10.70 ± 1.74	0.62 ± 0.02	0.680 ± 0.03
30	17.24 ± 0.75	4.08 ± 0.98	10.80 ± 1.19	0.62 ± 0.02	0.690 ± 0.05
40	17.42 ± 0.99	4.10 ± 1.07	11.05 ± 1.36	0.63 ± 0.02	0.700 ± 0.06
50	17.76 ± 0.86	4.28 ± 1.65	11.40 ± 1.24	0.64 ± 0.02	0.700 ± 0.34
60	18.04 ± 0.36	4.16 ± 0.27	11.65 ± 1.46	0.65 ± 0.03	0.710 ± 0.02

Remark: ^{ns} refers to no statistically significant differences in a column ($p \geq 0.05$)

4. The results of the overall acceptance of consumers

The collected personal information from 100 consumers showed that 79% were female and 21% were male. The dominant age group was 16-20 years of age (52%), followed by age 21-25 (20%), age 26-30 (13%), age 31-35 (10%) and age 36 or above (5%), respectively. In total, 65% of the consumers were students, while 26% were university staff and 9% were lecturers. Consumers' monthly income was categorized as follows: 56% had an average monthly income of 5,000-10,000 baht, 27% had 10,000-20,000 baht of monthly income and 17% had a monthly income higher than 20,000 baht. Moreover, regarding the frequency of sweet fish sauce consumption, 45% consumed it 2-5 times a month, 38% consumed it once a month and 17% consumed it 2-3 times a month.

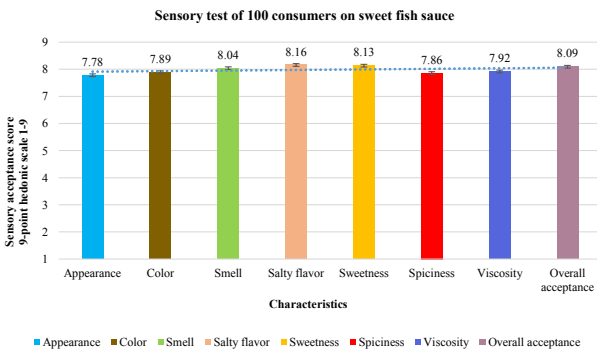

Fig. 4 The results of a sensory test of 100 consumers on sweet fish sauce

According to Fig. 4 the results of a sensory test of 100 consumers on sweet fish sauce, the data proposes that consumers accepted sweet fish sauce in all attributes (7.78-8.16). With regard to consumers' attitude toward sweet fish sauce, analysis showed that 98% of consumers accepted this modified recipe and 95% would be very likely to purchase it once the product was officially released. Most of the consumers agree that a selling price of 65 baht per package is favorable (86%) and 82% see the packaging as suitable and appealing. This is consistent with the research of Chumkaew et al. (2019); Chumkaew (2019); Punfujinda et al. (2019); Chumkaew et al. (2020) on product development. They argued that most consumers are likely to accept and purchase products once they are on the market. However, questionnaire respondents have suggested that there should be a wider variety of packaging and more than one level of spiciness to meet the demands of a range of consumers and the expanding market.

5. The results of sweet fish sauce production and distribution by the shrimp processing community enterprise

According to the shrimp processing community enterprise's financial statement, as displayed in Table 8, the total value of the fish sauce sold was 109,200 baht, or approximately 10,920 baht of monthly income. The income increased by 40% and members' compensation also increased by 24%. Besides, the price is 10-15% cheaper compared to the commercial products. However, there are some commercial products having the same price or within similar price range with our product. This is due to the ingredients added to each product and different qualities.

Table 8 The results of sweet fish sauce production and distribution by the shrimp processing community enterprise

Details of sweet fish sauce production and distribution October 2020-July 2021	Total
Amount of small dried white shrimp for sweet fish sauce (kg)	17.50
Total sales (packages)	1,680
Total sales (baht)	109,200
Increase in income (%)	40
Increase in compensation for members (%)	24

Remark: Community Enterprise in the Group of Shrimp Processing (2021)

Conclusion

The research established and developed modified sweet fish sauce products from small white shrimp consisting of 30% palm sugar, 25% unbleached sugar cane, 9% fish sauce, 13.8% water, 5.5% dried white shrimp, 16% shallot and 0.7% dried chili in 230 g in glass packaging. One package contains five servings (46 g per serving). As indicated by the GDAs, one serving would consist of 120 kcal, 23 g of sugar, 0 g of fat and 210 ml of sodium and the shelf life would be 60 days. The L* (lightness), a* (redness), b* (yellowness), viscosity and water activity (a_w) values barely indicated any statistical significance at $p \geq 0.05$. The a_w was relatively low at 0.67-0.71 and was in line with the standards of community products, which state that it should not exceed 0.80. The sensory evaluation of the final pre-distributed sweet fish sauce resulted in an average acceptance score of 7.89, which means that the testers liked the product extremely, according to the criteria. Furthermore, data collection from October 2020-July 2021 revealed that small white shrimp weighing 5.0-6.7 g were processed into 17.50 kg of dried shrimp, or 175 kg of raw shrimp. This illustrates the economic support for shrimp farmers, which helps

to reduce the problem of low prices for small shrimp. It also shows that it is profitable for the community enterprise to process these shrimps into other products and gain increasing incomes from a wider selection of products, in this case, sweet fish sauce.

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Evaluation of Antimicrobial Activity of *Rhinacanthus nasutus* (L.) Kurz and *Acanthus ilicifolius* L. Extracts

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Abstract

The aim of this research was to evaluate the antimicrobial properties of medical plants *Rhinacanthus nasutus* (L.) Kurz and *Acanthus ilicifolius* L. that were extracted with water/aqueous (AqER, AqEA) and ethanol (EtER, EtEA). The extracts were tested for activity and evaluated based on the effectiveness against three strains of microorganism: Gram-positive bacteria such as *Staphylococcus aureus,* Gram-negative bacteria such as *Escherichia coli,* and fungal such as *Candida albicans* by using the agar well diffusion and broth dilution method. The extracts from *Rhinacanthus nasutus* and *Acanthus ilicifolius* with ethanol showed the effect of inhibiting all microbes. The most effective against *Candida albicans* with the similar MIC and MFC values of 18.75 and 37.50 mg/mL. Meanwhile, extracts with water of *Rhinacanthus nasutus* and *Acanthus ilicifolius* with MIC values of 37.50 and 75 mg/mL and MFC values of 75 and 150 mg/mL, respectively. Conversely, these extracts showed no effect to inhibit *Escherichia coli.* This could be due to the capabilities of the solvents extractive and using part of the plant. Likewise, a combination of the extracts with ethanol of *R. nasutus* and *A. ilicifolius* to evaluate the efficacy of synergistic herbs can be considered from the MIC value. The antimicrobial synergy was evaluated in terms of FIC obtained from multiple-combination bactericidal/fungicidal assays. FICi value was interpreted as synergy only in ethanol extract *R. nasutus+A. ilicifolius* (EtRA) of 0.26.

Introduction

Rhinacanthus nasutus (L.) Kurz belongs to Acanthaceae family, having the common name "white crane flower" and is a medicinal shrub that is widely distributed in Southeast Asia. In Thailand the local name is also referred to as "Thong phan chang" (Puttarak et al., 2010; Brimson et al., 2020). Several reports have revealed that traditional medicinal uses for the treatment of diverse diseases that include diabetes, hepatitis, tuberculosis, hypertension, inflammation, psoriasis, eczema, ringworm, antioxidant, neuroprotective, aphrodisiac, anticancer, and antimicrobial (Puttarak et al., 2010) also showed significant larvicidal activity against

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four larvae of mosquitos (Komalamisra et al., 2005). The extract of *R. nasutus* (Rn) has shown to have an effective antibiotic activity against Gram-positive strain such as *Bacillus cereus, Bacillus subtilis* and *Staphylococcus aureus* (Sendl et al., 1996; Puttarak et al., 2010; Kumar et al., 2021) and against Gram-negative bacteria such as *Klebsiella pneumoniae,* followed by *Enterobacter aerogenes, Proteus mirabilis* and *Escherichia coli* of ethanol extract (Kumar et al. 2011; Sheikh & Reshi 2020). The major bioactive active compound to antimicrobial was the naphthoquinone esters, namely rhinacanthin-C, -N, -Q from ethanol and aqueous extracts (Puttarak et al., 2010; Panichayupakaranant et al., 2021).

The evergreen spiny herb named *Acanthus ilicifolius* L. with the local name "Sea holly" is in the same family of Acanthaceae. It is widely found in mangroves of southern Thailand. It has been used to treat rheumatism, asthma, paralysis, psoriasis and leucorrhoea. The antimicrobial activity of alcoholic, butanolic and chloroform extracts of leaves and roots of *A. ilicifolius* is strong against Gram-positive bacteria such as *Staphylococcus aureus, Bacillus subtilis, Staphylococcus epidermidis* and fungi such as *Candida albicans*, *Saccharomyces cerevisiae, Aspergillus niger, Aspergillus fumigatus, Rhizopus oryzae* while moderate inhibitory action against Gram-negative bacteria such as *Proteus vulgaris, Proteus mirabilis, Pseudomonas aeruginosa, Escherichia coli* (Siripong et al., 2006; Bose & Bose 2008; Kumar et al., 2011; Govindasamy & Arulpriya 2013; Pothiraj et al., 2021). The major bioactive active compound to antimicrobial of *A. ilicifolius* reveals the presence of 2-benzoxazolinone, lignan glucosides, benzoxazinoide glucosides, flavone glycosides and phenylethanoid glycosides (Govindasamy & Arulpriya 2013). In currency, antibiotics are widely used that can cause many bacterial mutations such as *Salmonella* spp. (Onvimol et al., 2020). The use of medicinal herbs for infectious control has started to play an increasingly important role. But the use of antibiotics alone may not be as effective as the combination. The combination of herbal extracts with antibiotics was found to be able to increase the efficiency of infection prevention (Jiang et al., 2021). Clinical studies also exhibited that patients treated with antibacterial combination therapy can obtain good clinical effect and lower mortality rates (Ni et al., 2015). However, the results of this study may reduce the medical uses of antibiotics also in the food, pharmaceutical industry including replacing preservatives in cosmetic products. The aim of this research was to evaluate the antimicrobial properties of medical plants that include *R. nasutus* (L.) Kurz and *A. ilicifolius* L. and were extracted with water/aqueous (AqER, AqEA) and ethanol (EtER, EtEA).

Materials and methods

1. Plant material

The fresh sample of *Rhinacanthus nasutus* was collected from Tatum Subdistrict, Sangkha District, Surin Province. *Acanthus ilicifolius* was collected from Laem Sak Subdistrict, Ao Luek District, Krabi Province, Thailand. The samples were compared with specimens deposited in the Bangkok Forest Herbarium.

2. Preparation of plant extracts

The fresh leaves and stem of *R. nasutus* were extracted with two solvents; aqua and 95% ethanol. For water extraction, 50 g of herbs were boiled with 500 mL of distilled water at 90°C for approximately 2 h and allowed to cool at room temperature. After that, it was filtered through a straining cloth to separate the residue and filtered with filter paper (Whatman No.1). The filtrates were pooled and evaporated on a rotary evaporator under reduced pressure at 50°C to obtain dry powder. For the ethanol extraction, 50 g of the herb was soaked with 50 mL of 95% ethanol for 7 days subsequently filtered through a straining cloth and filtered with filter paper (Whatman No.1), concurrently. The filtrates were pooled and evaporated on a rotary evaporator under reduced pressure at 50°C to obtain a crude residue and concentrated at 45°C approximately 12 h. Similarly with the solvent and procedures for fresh leaves and stem of *A. ilicifolius*. These extracts were kept in the refrigerator until usage (applied from Santos et al., 2002; Puttarak et al., 2010).

3. Microorganisms tested

The standard strain of bacteria and fungus used to evaluate the antibacterial properties of medical herb plants were obtained from the Department of Medical Sciences, Ministry of Public Health, Nonthaburi that include *Staphylococcus aureus* (DMST 8840), *Escherichia coli* (DMST 7948) and *Candida albicans* (DMST 8684). Pure cultures of these bacteria and fungus strains were grown on Mueller Hinton agar (MHA) and Sabouraud dextrose agar (SDA) consequently and maintained on agar slant at 4°C until used.

4. Determination of antibacterial activity

Antimicrobial activity of 95% ethanol and aqueous

extracts of *R. nasutus* (EtER, AqER) and *A. ilicifolius* (EtEA, AqEA) were tested by agar well diffusion method (applied from Jeyaseelan et al., 2012; Chanda et al., 2013) using MHA and SDA medium for bacteria and fungus to screen the plant extracts for antimicrobial activities. Briefly, MHA or SDA plates were prepared by incorporating 1 mL of test bacteria or yeast (0.5 McFarland turbidity standards) into 20 mL of molten medium at 45°C. After solidification of the medium, wells were made using 8 mm diameter of sterile stainless steel cork borer, and 100 µL of each of the test extracts (300 mg/mL), the antibiotic tetracycline (125 mg/mL) for bacteria or ketoconazole (200 mg/mL) for yeast were used as a positive control and negative control (0.2% DMSO) were added into the wells separately. This was the initial concentration of the extract used to check the antimicrobial activities of the extracts from the plant. Plates were incubated at 35-37°C for 24 h. Finally, the antimicrobial activity of the test extracts were determined by measuring the diameter in millimeters of clear zone around the well. Each experiment was performed in triplicate and the average value of inhibition was calculated.

5. Minimum inhibitory concentration (MIC)

Antibacterial activity of *R. nasutus* (EtER, AqER) and *A. ilicifolius* (EtEA, AqEA) extracts were determined by the broth dilution method (Chic & Amom 2014). All dissolved extracts were serially diluted two-folds in Muller-Hinton broth/Sabouraud dextrose broth to give a final concentration ranging from 4.69-300 (4.69, 9.38, 18.75, 37.50, 75, 150 and 300) mg/mL. An aliquot (0.1 mL) of overnight broth culture of test microorganism (approximately concentration 1.5×10^8 cfu/mL of bacteria and 2.0×10^5 spores (yeast cell)/mL for fungal that were adjusted by using 0.5 McFarland standard) in sterile normal saline was introduced into each extract dilution. The mixtures in sterile test tubes were incubated (37ºC, 24 h) and observed for turbidity (signifying growth) or absence of it (signifying inhibition). Tetracycline (antibacterial drug) at the concentrations of 125 mg/mL and ketoconazole (antifungal drug) at the concentrations of 200 mg/mL were used as a positive control agent, and sterile normal saline as negative control. The MIC (Minimum Inhibitory Concentration) is the lowest concentration of an antimicrobial agent or extract solution that inhibits or prevents growth as determined visually after a standard incubation period of 18-24 h at 35-37°C.

6. Minimum bactericidal concentration (MBC)

A loopful (1 µL) of the test mixture was transferred from each MIC tube that showed no growth or no turbidity, inoculated onto Mueller-Hinton/Sabouraud dextrose agar plate, incubated at 37ºC for 24 h, and inspected for the presence of colonies indicating growth. The MBC (Minimum Bactericidal Concentration) and MFC (Minimum Fungicidal Concentration) is the lowest concentration of the antimicrobial agent or extract solution that shows no growth, defined as a 99.9% reduction in the initial inoculum, after a subculture of all the dilutions that showed no bacterial or fungal growth in the MIC test (Magaldi et al., 2004).

7. Fractional inhibitory concentration (FIC) and ΣFIC determinations

According to Doern (2014) and Tiwana et al. (2020) the extracts solution showing appreciable antibacterial activity were tested in combination with each other, to test whether interactions occurred between *R. nasutus* (EtER, AqER) and *A. ilicifolius* (EtEA, AqEA) extracts. Initially, 1:1 ratio of two different extracts (concentration 300 mg/mL) were tested based on the hypotheses of mutant selection window (MSW) and mutant prevention concentration (MPC) (Drlica, & Zhao 2007; Xu et al., 2018; Jiang et al., 2021). Interactions between extracts were examined by determination of the sum of fractional inhibitory concentrations (ΣFIC) for each combination. The FIC values for each component (a and b) were calculated using the following equations where a and b represent the two plant extracts being tested:

FIC (a) = MIC (a in combination with b) / MIC (a alone)

FIC (b) = MIC (b in combination with a) / MIC (b alone)

ΣFIC was then calculated using the formula ΣFIC = FIC (a) + FIC (b). The interactions were classified as synergistic ($\Sigma FIC \leq 0.5$), additive ($\Sigma FIC > 0.5$ to ≤ 1.0), indifferent ($\Sigma FIC > 1.0$ to ≤ 4.0) or antagonistic ($\Sigma FIC > 4.0$ The multiple-combination bactericidal/fungicidal assays were inoculated with the test microorganism to a final approximate concentration of 1.5×10^8 CFU/mL (using 0.5 McFarland standard) and incubated at 37°C for 24-48 h. The inspection for turbidity, and without visual evidence of growth were sub-cultured to MHA or SDA medium and assessed after overnight incubation for 99.9% killing.

8. Statistical analysis

Values are represented as mean ± SD. All analyses were done as three biologically independent experiments.

The student's t test was used to determine the statistical significance of differences between groups in EtER-AqEr and EtEA-AqEA expression. A value of p = 0.05 was considered statistically significant.

Results and discussion

1. Plant extracts

The profiles of *R. nasutus* and *A. ilicifolius* extracts are shown in Table 1. The highest yield of extract was with aqueous (7.66%), whilst the least yield was obtained with ethanol (4.15%).

Table 1 Yield and other physical properties of medical herb plants extracts

Plant name	Solvent extractant	Method to extraction	Yield (%)	Colour and consistency
R. nasutus	Ethanol	Maceration	4.15	Deep green, Gummy solid
	Aqueous	Digestion	6.89	Dark brown almost black, solid in form of dry powder
A. ilicifolius	Ethanol	Maceration	5.40	Deep green, Gummy solid
	Aqueous	Digestion	7.66	Dark brown almost black, solid in form of dry powder

2. Antimicrobial assay

All four extracts of *R. nasutus* (EtER, AqER) and *A. ilicifolius* (EtEA, AqEA) showed different degrees of activity against bacterial and fungal. Preliminary testing to confirm the antimicrobial activity of EtER and EtEA extracts by agar well diffusion method showed that both plant extracts had inhibitory activity against Gram-positive bacteria such as *Staphylococcus aureus* with mean clear zone of inhibition of 29.33 ± 1.15, 23.67 ± 0.58 mm., Gram- negative bacteria such as *Escherichia coli* with mean clear zone of inhibition of 22.33 ± 0.58, 18.00 ± 0.00 mm., and fungal such as *Candida albicans* with mean clear zone of inhibition of 17.67±0.58, 14.33±0.58 mm., respectively, as shown in Table 2. While the antimicrobial efficacy of AqER and AqEA extracts showed only inhibitory activity against *S. aureus* and *C. albicans* without ability to inhibit Gram-negative bacteria such as *E. coli* and had less of the zone of inhibition. It was shown that extraction of *R. nasutus* and *A. ilicifolius* by using 95% ethanol had more antimicrobial effect than aqueous extraction.

3. The MIC and MBC (MFC)

The antimicrobial activity of the four extracts (EtER, AqER, EtEA, AqEA) potency was quantitatively assessed by the MIC and MBC (MFC) values of the extracts. The MIC and MBC (MFC) values were between 18.75 to 37.50 mg/mL and 18.75 mg to 75 mg/mL for ethanol extract of *R. nasutus* and *A. Ilicifolius,* respectively, which were found to be better than the MIC and MBC (MFC) values of aqueous extracts (Table 2). The highest MIC and MBC (MFC) values were recorded of 150 mg/mL for the aqueous extract of both *R. nasutus* and *A. ilicifolius*. We found that the lowest MIC and MBC (MFC) were 18.75 mg/mL and 37.50 mg/mL for ethanol extract of *R. nasutus* (EtER), which showed the activity against Gram-positive bacterial and fungal while the lowest MIC and MBC was 37.50 and 75 mg/mL of both EtER and EtEA showed the activity against Gram-negative bacterial. Conversely, neither AqER nor AqEA were found to be effective against Gram-negative bacteria.

However, EtER and EtEA have shown antimicrobial efficacy with MIC and MBC (MFC) values between 18.75 to 75 mg/mL. Consistent with the experiment of Bose & Bose (2008) that studied antimicrobial activity of *A. ilicifolius* extract by using ethanol and results exhibited strong inhibitory action against Gram-positive bacteria, fungi and showed moderate activity in Gram-negative bacteria. While Govindasamy, & Arulpriya (2013) conclusion that aqueous extract of *A. ilicifolius* included a variety of bioactive components, including alkaloids, saponins, phenolics, flavonoids, steroids, cardiacglycosides, tannins, and terpenoids showed minimum activity against both bacterial and fungal species. Similarly, these phytochemical compounds are found in both *A. ilicifolius* and *R. nasutus* but the naphthoquinone rhinacanthin is not contained in *A. ilicifolius* while the glycosides are not found in *R. nasutus*. Conversely, Pothiraj et al. (2021) reported that the aqueous extract of *A. ilicifolius* was more effective against Gram-negative bacteria than against Gram-positive bacteria of inhibition zone being between 9.6 ±0.01-11.1±0.10 mm and that probably the extract of leaves of polar solvent inhibits Gram-negative bacteria in a dose-dependent manner. This consideration is also compatible, with the observations of Park et al. (2016) who recommended that the action could potentially be explained by changes in the quantities of phytoconstituents and bioactive components restrictive in the crude extract, and the capabilities of the solvent extractive.

For the extractive values as shown in Table 2, the ethanol extract of *R. nasutus* (EtER) and *A. ilicifolius* EtEA indisputably showed antibacterial activity against Gram-positive *S. aureus* more aqueous extract with an inhibition zone of 29.33 ± 1.15 and 23.67 ± 0.58 mm, respectively. Followed by Gram-negative *E. coli* with an

Table 2 Antimicrobial activity of leaf and stem extracts of *R. nasutus* and *A. ilicifolius*

Plants	Extracts	*Staphylococcus aureus*			*Escherichia coli*			*Candida albicans*		
		Inhibition zone (mm)	MIC (mg/mL)	MBC (mg/mL)	Inhibition zone (mm)	MIC (mg/mL)	MBC (mg/mL)	Inhibition zone (mm)	MIC (mg/mL)	MFC (mg/mL)
R. nasutus	EtER	29.33 ± 1.15	18.75	37.50	22.33 ± 0.58	37.50	75.00	17.67±0.58	18.75	37.50
	AqER	14.33 ± 0.58	75.00	150.00	NA	NA	NA	16.67±1.15	37.50	75.00
	EtER - AqEr	15.00 ± 0.82*	-56.25*	-112.5*				1.00±0.67*	-18.75*	-37.5*
A. ilicifolius	EtEA	23.67 ± 0.58	37.50	75.00	18.00 ± 0.00	37.50	75.00	14.33±0.58	18.75	37.50
	AqEA	12.00 ± 1.00	150.00	150.00	NA	NA	NA	14.00±0.00	75.00	150.00
	EtEA- AqEA	11.67 ± 0.74*	-112.50*	75.00*				0.33±0.28*	-56.25*	-112.5*
Control										
Tetracycline		41.00 ± 1.15	31.25	62.50	38.33 ± 0.58	31.25	62.50	NT	NT	NT
Ketoconazole		NT	NT	NT	NT	NT	NT	43.00±1.15	12.50	25.00
DMSO		NA	NA	NA	NA	NA	NA	NA	NA	NA

Remark: EtER= ethanol extract of *R. nasutus,* AqER= aqueous extract of *R. nasutus,* EtEA= ethanol extract of *A. ilicifolius,* AqEA= aqueous extract of *A. ilicifolius,* NA = No activity, NT = Not tested; *Significance level (p = .05)

inhibition zone of 22.33 ± 0.58 and 18.00 ± 0.00 mm, respectively. While EtEA and AqEA showed similar antifungal activity against *C. albicans* with an inhibition zone of 14.33±0.58 and 14.00±0.00 mm, respectively. Although in both plants the alcohol extract showed antimicrobial activity, it was significantly less than that of tetracycline except for EtER. Therefore, this study shows that the extraction of *R. nasutus* and *A. ilicifolius* with alcohol has a greater inhibition effect on microorganisms than the extraction with aqueous.

Previous research (Panichayupakaranant et al., 2009; Maheshu et al., 2010; Puttarak et al., 2010; Bukke et al., 2011; Jayapriya 2015; Antonysamy 2017; Lukiati et al., 2019; Mondal et al., 2021) reported that the phytochemical compounds of *R. nasutus* contained from steroids, alkaloids, tannins, saponins, flavonoids, triterpenes, naphthoquinone rhinacanthin-C, D, N. These chemical compounds have antimicrobial activity that are extracted from different kinds of solvents such as chloroform, methanol, ethanol, ethyl acetate and aqueous, of which ethyl acetate extracts of *R. nasutus* showed maximum metabolites occurrence followed by alcoholic extract. The mechanism of polyphenol which is contained in *R. nasutus* and in *A. ilicifolius* as antibacterial were able to disrupted cell membrane permeability while hydroxyl group could form a hydrogen bond with positively charged nitrogen and oxygen in protein of cell membrane. Hydroxyl group also formed an ionic bond with positively charged amine in protein of cell membrane. The damage of membrane caused increase permeability and cell leak which are followed by intracellular material discharge that causes obstructed cell growth or cell death in microorganisms (Wink, 2015; Singh, 2017). In the same way, this study of 95% ethanol of *R. nasutus* showed the potency of antimicrobial activity more than aqueous and indicates that ethanol could be extracted from the phytochemical compounds of *R. nasutus* greater. Similarly in *A. ilicifolius* no naphthoquinone rhinacanthin was found in this plant. For this reason, *R. nasutus* exhibited more efficacy antimicrobial activity than *A. ilicifolius.* Although in this study the activity of each phytochemical compounds was not studied.

Likewise, Puttarak et al. (2010) found that rhinacanthin-C, -D, and -N in *R. nasutus* extract with ethyl acetate are active to kill *Streptococcus mutans* with MIC and MBC of 4 mg/mL, and potent activity against *Staphylococcus epidermidis, S. aureus* and *Cutibacterium acnes* (*P. acnes*), with the MICs of 8 to 16 mg/mL. Moreover, its extract showed no activity against *C. albicans* at concentration up to 2000 mg/mL. However, Panichayupakaranant et al. (2009) showed that the activity of *R. nasutus* against pathogenic fungal of *Trichophyton* spp., *Microsporum* sp. and *Candida albicans*. The ethanolic crude extract of *A. ilicifolius* (stem bark) dominates many important secondary metabolites, including alkaloids, flavonoids, terpenoids, phenols, glycosides, steroids and tannins. The extract showed moderate antibacterial activity against Gram-positive *Bacillus subtilis* and *B. megaterium* MIC value of 46.875 µg/mL, and Gram-negative *E. coli* MIC value of 750 µg/mL, this result was reported in 2021 by Mondal et al. Similarly, in this study ethanolic crude extract of *A. ilicifolius* showed activity against *E. coli,* though the MIC value had a higher mean of 37.50 mg/mL. This could be due based on the capabilities of the

solvents extractive and using part of the plant.

Based on Table 2, the mean difference of statistics test with t-test, found that the values between EtER-AqEr and EtEA-AqEA were significantly different ($p<0.05$), with EtER-AqEr being higher than EtEA-AqEA. This indicated that that the potency of antimicrobial activity from *R. nasutus* extracts were better than *A. ilicifolius,* in accordance with previous studies as explained above. While water/aqueous is a solvent with higher polarity than ethanol, enabling the extraction of phytochemicals to be greater, which is a bioactive substance that can inhibit many microorganisms as mentioned above. Moreover, aqueous has also been used as a safety or green solvent with optical extraction for polyphenols compared to organic solvents and for different classes of chemical compounds like flavonoid, organic acid, and alkaloid. Dielectric constant or polarity of water can be manipulated by the application of temperature making it suitable for extraction of polar, moderately polar and nonpolar compounds.

4. Fractional inhibitory concentration (FIC) determinations

The antimicrobial synergy between *R. nasutus* and *A. ilicifolius* extract was evaluated in terms of FIC obtained from multiple-combination bactericidal/fungicidal assays. Antagonism or synergism is a negative or positive effect observed when the combined effect of the drugs or agent substance is significantly less/more than expected (Renneberg 1993; Doern 2014; Gan et al., 2020). Interpretation of the fractional inhibitory concentration index (FICi) was followed as described by Doern (2014) and Gopal et al., (2014). We considered a synergistic effect for FICi < 0.5; partial synergy for $0.5 \leq$ FICi < 1; additive for FICi = 1; indifferent for 1 < FICi < 4; antagonism for FICi ≥ 4. In this study, the combination *R. nasutus* and *A. ilicifolius* extract with ethanol and aqueous found that the FICi value showed in Table 3 were interpreted as synergy only in ethanol extract *R. nasutus*+*A. ilicifolius* (EtRA). Our result may be the first FICi report of the combination between *R. nasutus* and *A. ilicifolius.* For the calculation of FICi value was applied from van Vuuren and Viljoen (2011).

FIC of *R. nasutus* (ethanol extract) = MIC of *R. nasutus* in combination with *A. ilicifolius*/MIC of *R. nasutus* alone = (10/18.75 = 0.53)

FIC of *A. ilicifolius* (ethanol extract) = MIC of *A. ilicifolius* in combination with *R. nasutus*/MIC of *A. ilicifolius* alone = (10/37.5 = 0.27)

Table 3 Fractional inhibitory concentration (FIC) value combine of *R. nasutus* and *A. ilicifolius*

Solvent	Plant	MIC (300 mg/mL) alone	MIC (300 mg/mL) combine	*FICi value	Interpretation
95%Ethanol	*R. nasutus*	18.75 (EtER)	10 (EtRA)	0.53	Additive
	A. ilicifolius	37.50 (EtEA)	10 (EtRA)	0.26	Synergy
Aqueous	*R. nasutus*	75 (AqER)	150 (AqRA)	2	Indifference
	A. ilicifolius	150 (AqEA)	150 (AqRA)	1	Additive

Remark: EtER= ethanol extract of *R. nasutus,* AqER= aqueous extract of *R. nasutus,* EtEA= ethanol extract of *A. ilicifolius,* AqER= aqueous extract of *A. ilicifolius,* EtRA= ethanol extract of *R. nasutus*+*A. ilicifolius,* AqRA= aqueous extract of *R. nasutus*+*A. ilicifolius,*
*FICi value: ≤0.5 = Synergy, ≥0.5-1 = Additive, 1-4 = Indifference, >4= Antagonism

Nevertheless, sometimes the use of a single antibiotic or substance does not produce the desired or effective inhibitory effect, and to overcome this, treatment with a combination of drugs or substance may be attempted, their synergistic effect often surpasses their individual execution. In this study, the synergistic effect resulting from the combination of two plant extract both potency antimicrobial with crude plant extract with different solvent was verified against all tested microorganisms and consistent with the experiment above though augmentative research is required to extract and identify bioactive chemicals in order to create new antimicrobial substance from Thai medical plants. The FICi values 0.26 of EtEA+EtRA had shown a synergistic effect, this result confirms that some phytochemicals contained in *A. ilicifolius* may have a better ability against microorganisms and more active compounds than *R. nasutus.* Although MIC alone of EtER is better than EtEA and needs to be investigated in depth.

Conclusion

The medicinal plants *R. nasutus* and *A. ilicifolius* were extracted with aqueous and 95% ethanol. The antimicrobial activity of the four extracts (EtER, AqER, EtEA, AqEA) potency was quantitatively assessed by the MIC and MBC (MFC) values of the extracts. The MIC and MBC (MFC) values were between 18.75 to 37.50 mg/mL and 18.75 mg to 75 mg/mL for ethanol extract of *R. nasutus* and *A. Ilicifolius,* respectively, which were found to be better than the MIC and MBC (MFC) values of aqueous extracts. The lowest MIC and MBC (MFC) was 18.75 mg/mL and 37 mg/mL for ethanol extract of *R. nasutus* (EtER) and showed activity against Gram-positive bacterial and fungal. While the lowest MIC and MBC was 37.50 and 75 mg/mL of both EtER

and EtEA and showed activity against Gram-negative bacterial. Conversely, neither AqER nor AqEA were found to be effective against Gram-negative bacteria. As a result, the action could potentially be explained by changes in the quantities of phytoconstituents and bioactive components restrictive in the crude extract, and the capabilities of the solvents extractive. The antimicrobial synergy between *R. nasutus* and *A. ilicifolius* extract was evaluated in terms of FIC obtained from multiple-combination bactericidal/fungicidal assays. FICi value were interpret as synergy only in ethanol extract *R. nasutus*+*A. ilicifolius* (EtRA) of 0.26. Furthermore, augmentative research is required to extract and identify bioactive chemicals in order to create new antimicrobial substance from Thai medical plants.

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Thailand: Agriculture Outlook and Response during Covid 19

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Abstract

Covid 19 is unprecedented and has had profound impact on the agriculture sector globally. According to the United Nations Thailand Economic Focus, Covid 19 led to contraction in the manufacturing sector in the first quarter of 2020. The three key sectors that faced economic losses during the first quarter of 2020 include agriculture (-5.7%) followed by industry (-1.9%) and services (-1.1%). Reduction in tourists had negative impact on the accommodation and food service activities (-24.1%) on YoY basis. The National Economic and Social Development Council (NESDC) indicated that droughts coupled with pandemic, which act as double disasters, affected a total of 6 million farmers in 2020 impacting their employment or adversely affecting their involvement in agricultural activities. Digital technologies have provided immense support during the period. Microsoft cloud platform has been utilized by the Bank of Agriculture and Agricultural Cooperatives (BAAC) to support Thai farmers. Innovative technology applications have also been used by the private product and service providers. The research methodology includes literature review, interaction and discussion with food industry officials and rice experts to analyze the pandemic influence on agriculture. Apart from the challenges farming households faced during Covid 19 in terms of reduced incomes and increased debts, Covid 19 accelerated the use of digital technologies in the agriculture sector through online platforms, digital payments and digital advisory applications. Covid 19 also poses a great opportunity for Thailand to leverage on technology, innovation and logistics to take the food industry to the next level.

Introduction

Thailand is the world's 11th largest food exporter with a worldwide market share of 2.51 percent, according to the National Food Institute. Thailand had successfully contained Covid 19 cases in the first year with a total of 87 deaths (Worldometers, 2021), but the epidemic has caused several socio-economic impacts which might have devastating effects in the long term. Thailand's government imposed a curfew on the 3rd of April 2020 to discourage people from violating physical distancing and to allow operations of essential businesses. Thailand's economy contracted at 6.1% in 2020, the worst ever since the 7.6% decline during the Asian Financial Crisis in

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1997. In 2021, Siam Commercial Bank (EIC) forecasted growth of 2.2% because of very slow recovery of foreign tourism, depressed export demand and revival of Covid 19 (Deloitte, 2021).

Thailand Labor Force Survey observed as increasing unemployment rate in various sectors during April-May 2020, 0.3% in agriculture, 1.3% in manufacturing, 2.8% in construction, 1.2 % in wholesale and retail and 2.2% in hotel and restaurant businesses and was expected to increase if Covid 19 continued until Q3. The sectors most impacted by unemployment were tourism, restaurants & food shops, hospitality industry, retail & wholesale, logistics and construction. The Social Security Office in Thailand also indicated that foreign tourists declined by 83% in 2020 and were expected to reach 4 million in 2020 and it could take 2-3 years to revive as normal pre Covid levels.

Thailand ranks 51st on the global food security index, 32nd in terms of food affordability and 67th in terms of food availability (The Economist, 2020). NESDC indicated that the real GDP growth rate in the agriculture sector was expected to decline -3.9% in 2020 and bounce back at 2.2% in 2021 as compared to 5.0% in 2018 and 2.0% in 2019, respectively. Although a decline was seen in 2019, Covid 19 slowed down the growth rate significantly (World Bank Group, 2021).

Thai agricultural sector employs 24% of the total (38 million) workforce with 62% of Thai agricultural households dependent on income from general employment outside the agricultural sector (Deloitte, 2021). The agriculture sector generates the lowest value added per worker with the slowest growth amongst other economic sectors accounting for only 10% of GDP in 2019 (United Nations, 2020b). The agricultural sector has been adversely affected by partial lockdown and measures to suppress the epidemic has affected most household income.

Covid 19 is unprecedented and best practices and mitigation measures continue to evolve. The study documents how the pandemic has impacted the various players in agriculture and food systems in Thailand and provides suggestions on how technology can be utilized not only to mitigate its impact but also accelerate the journey towards some of the policy goals. It examines the agro-economic trends post the onset of the pandemic as well as its impact on food security and nutrition, which was further exacerbated by droughts. Further analysis was undertaken to understand its impact on rice and other food systems. Literature review was carried out based on secondary data available from public and private stakeholders. The information collected was further validated through interviews with experts from government, food industry and academia and considers the response from various stakeholders including government measures and subsidies. Noting the important role that digital technology has played during the pandemic, it emphasizes on the importance of technology and shares the emerging trends and application in the agricultural value chain.

Agriculture Sector: Trends during Covid 19

Various studies indicate that Covid 19 had major disruptions in transportation and logistics which led to changing market demands and impacted agricultural households with high debt burden (Aday & Aday, 2020; FAO, 2020). Farmers in the south of Thailand faced maximum decrease in agricultural income by around forty percent (Puey Ungphakorn Institute for Economic Research, 2020). According to BAAC, 54% of Thai farmer households are on debt settlement or debt restructuring programs mostly from the central and metropolitan regions of Thailand and 50% of approximately 4.5 million farmers have debt of more than 200,000 THB (Thai Baht) and 20% of farmers have debt of more than 400,000 THB. With restrictions at both domestic and international level, the middleman merchant has disappeared and the farmers are facing lower price for their product and marketing problems have reduced the income levels as well. Exporting for products like rice, cassava and rubber trees were impacted due to the lockdown. The Trade Policy and Strategy Office (TPSO) reported that exporting products of Thailand in June 2020 valued at US$16,444.3, a decline of 23.17% from July 2009. Major stakeholders impacted were the food companies and several retailers. While big retailers had little negative implications, the small retailers were heavily damaged and many small retailer stores, have been forced to shut down their businesses. According to the Government Saving Bank (GSB) research estimations, the growth of these retailers dropped by 10.0%-13.6% compared to last year.

Besides the agricultural food system, consumption behavior has also impacted the market. For many food businesses, fewer customers led to lay off employees, in order to sustain their businesses. This contributed to increasing unemployment and slowing down the economy creating problems of food security in terms of

access and appropriate nutrition.

Food loss has been a major concern during Covid-19. Logistics disruptions were responsible for the damage of products such as fruits, vegetables and livestocks. The domestic poultry market accounts for 62% of total production where demand of meat products particularly declined due to the closure of restaurants and decline in tourism. Consumers explored alternatives that involved less meat consumption and were more economical. Also, chicken price had declined from 33-36 THB in March to 30 THB per kg in April (Agroberichten Buitenland, 2020).

Thailand's Q3/2020 experienced a slight recovery as the GDP contracted by 6.4% as compared to 12.1% in Q2/2020. This was a result of improvements in total exports of goods and services, private investment, and private final consumption expenditure. During this period, the government expanded the final consumption expenditure and public investment (The Nation, 2021).

Perception of people clearly influenced market behavior and demand. Surveys conducted by Suan Dusit Poll and GroupM Thailand reported that consumers preferred plant-based protein, immune boosting foods and other natural local ingredients either because they believed natural food were healthier or due to the limited opportunity caused by lockdown (FFTC-AP, 2020). Covid 19 has impacted overall economic slowdown, disrupted supply chains and increased inequalities, further impacting food prices, change in demand and production and shifting food patterns and environment that could lead to food insecurity issues (Fig.1).

The Committee on World Food Security through the HLPE Global Narrative report highlighted six dimensions of food security including availability, access, utilization, stability, agency and sustainability (Committee of Food Security HLPE, 2020). These issues discussed with the agricultural experts and consumers clearly highlighted the impact of Covid 19 to all dimensions especially sustainability as they witnessed reduced attention to environment and climate change issues, rise in food losses and waste, rise in plastic waste through packaging and overall social and economic losses affecting food systems (Table 1).

Covid 19 and droughts acting as double disaster

Covid 19 further worsened the impact on households that have already been affected by droughts, stagnant wages and increasing poverty in 2016 and 2018 (The World Bank, 2020). During April 2020, about 6,255

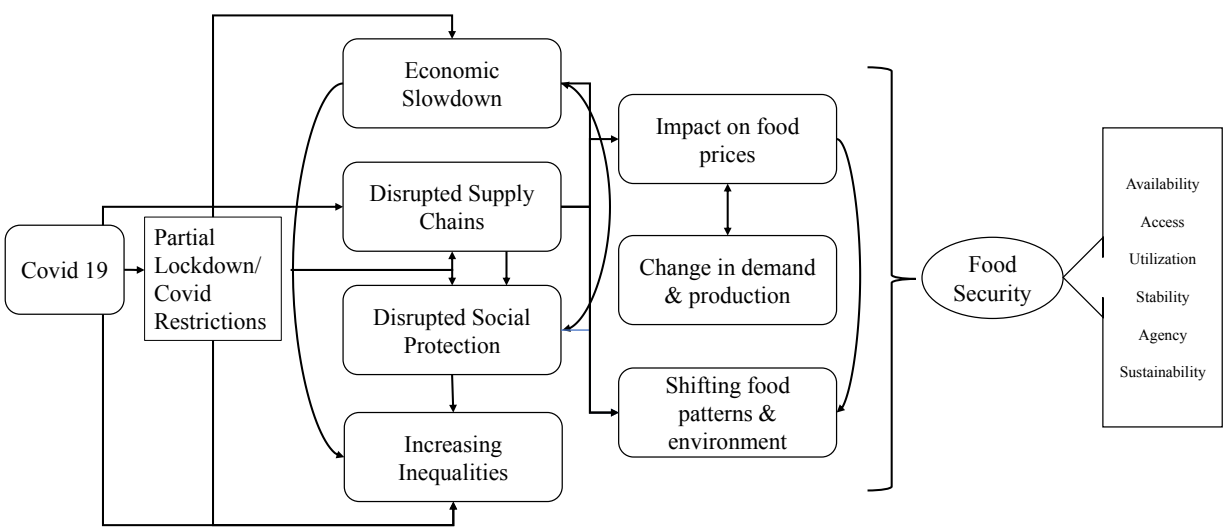

Fig. 1 Linkages between Covid 19 and agricultural landscape
Source: Adapted from Moseley et al. (2020)

Table 1 Impact of Covid to all dimensions of food security and nutrition

Dimensions of food security and nutrition	Criteria
Availability	• Disrupted supply chains • Shortage of labour • Closure of high-risk locations such as restaurants, food stalls and processing plants • Farmers shifting to lower risk crops
Access	• Jobs and income loss • Increasing food prices • Disruption of school meal programme • Reduced safety nets or access to them • Closure of informal and local nearby markets
Utilization	• Shift to cheaper / less healthy diets • Shift towards processed and shelf stable food • Link between malnutrition and Covid -19
Stability	• Access to markets and inputs • Disrupted supply chains • Restrictions on exports • Unpredictable price
Agency	• Access to ICT • Loss of economic and social empowerment • Loss of jobs and affiliations • Restrictions to meet, demonstrate and organize • Weakening farmers' and producers' organization
Sustainability	• Rise in food loss and waste • Rise in plastic waste through packaging • Reduced attention to environment and climate change issues • Social and economic losses affecting food systems

Source: Adapted from Committee of Food Security HLPE (2020)

villages in 24 provinces had been declared as drought affected areas and the dam and reserves had 49% of its water capacity (Bangkok Post, 2020a). The forest fire further created health concerns in the north of Thailand. The vulnerable labor migrants returned back to their country. Over 120 thousand workers returned to Myanmar from Thailand during March-October 2020 (FAO, 2021), more than 90 thousand returned to Cambodia and about 120 thousand returned to Laos during March-June 2020 (ILO, 2020). This crisis was very different from the Asian financial crisis where reverse migration created opportunity for laborers to work in their homeland. Droughts and other structural problems in the same period created challenges in fulfilling the employment requirements in the agricultural sector.

According to TMB analysis, food products in Thailand were categorized as positive, moderate and slow recovery products. Positive recovery product included frozen meat and canned fruit and vegetables that projected increase both in domestic and export markets. Moderate recovery products included rice and grains, palm oil and seafood that projected increase domestically with high competition in the region and slow recovery products included rubber, tapioca and sugar (TMB, 2020). Neither of the demands increased due to Covid 19 and severe drought, as it depended on foreign markets.

Because of the pandemic, healthy, functional and processed food were seen to have higher demand as consumers were preparing for a lockdown, or stocking for possible major outbreak. In addition, livestock has seen a surge in demand in the international market according to the Office of Agricultural Economic (OAE). The high rate of informal consumption debt in Bangkok had already created insufficient income for a group of people even before Covid 19 (United Nations, 2020a) and the lockdown further reduced the income for many families involved in the informal sector and lowered its ability to purchase basic necessities. The most vulnerable were the migrant workers from the rural north-eastern provinces that returned home with less money than they previously earned adding stress to their local food resources.

Rice market during Covid 19

Thailand's rice market had positive and negative impacts from Covid 19. Positively, because other major rice exporting countries such as India, Vietnam and Cambodia were concerned about their supplies during the pandemic and imposed restrictions on rice export between late March to April. This temporarily led to increase in Thai rice export inflating the price up to 570 USD/MT that is 43 percent higher compared to the same period in 2019. But after Vietnam removed their restriction, Thai rice export price dropped to 470 USD/MT (Fig. 2a, c). However, the price was 18% higher than in 2019 because the off-season rice production declined by 40% due to severe drought. After Vietnam resumed its exports in May and with the return of other competitors, Thailand's rice exports declined. The rice market was further negatively impacted as many countries introduced restriction policies on imports creating further difficulties for agricultural exports and decreasing the income of exporting companies directly. A decrease in rice prices were seen reflecting the decline in the purchasing power in the country (Fig. 2b).

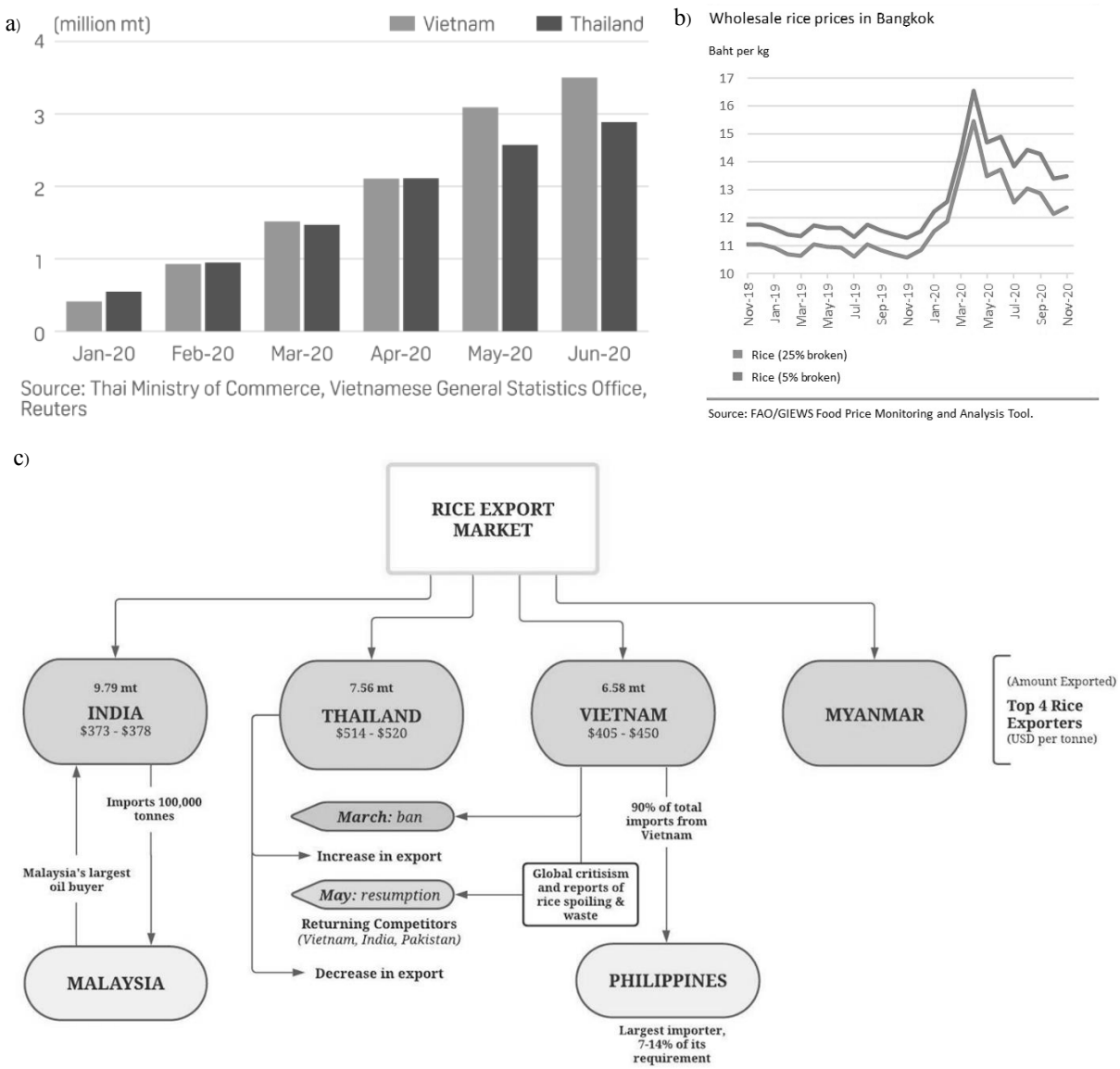

Fig. 2 Rice market during Covid 19 (a) Vietnam and Thailand rice exports during Covid 19 (b) Wholesale rice prices in Bangkok (c) Analyzing rice export scenario during Covid 19

Source: Adapted from USDA (2020); TRIDGE (2020); NIKKEI Asia (2020); (Bangkok Post, 2020b); Reuters (2020); IMF (2020); The Nation (2021)

Meat, eggs, milk and fisheries

Local demand for several food products declined in Thailand such as chicken meat, pork, fishery products, milk, wheat-based products and fruit. Small wheat flour mills were heavily impacted by the government's lockdown enforcement in the first half of the year which caused a reduction of domestic wheat consumption. Decrease in the wholesale prices for livestock, eggs and fishery products were seen (Fig. 3).

Ministry of agriculture and cooperatives (MOAC) also indicated that shrimp prices declined 10.6% YoY basis in 2020. Hog and shrimp prices saw a dip during April and the prices shot up again in May (USDA, 2020). Egg price saw a rise as the lockdown was announced but soon the government realized people were hoarding eggs and a ban on the exporting of eggs was introduced to ease the domestic consumption. Similar decline was

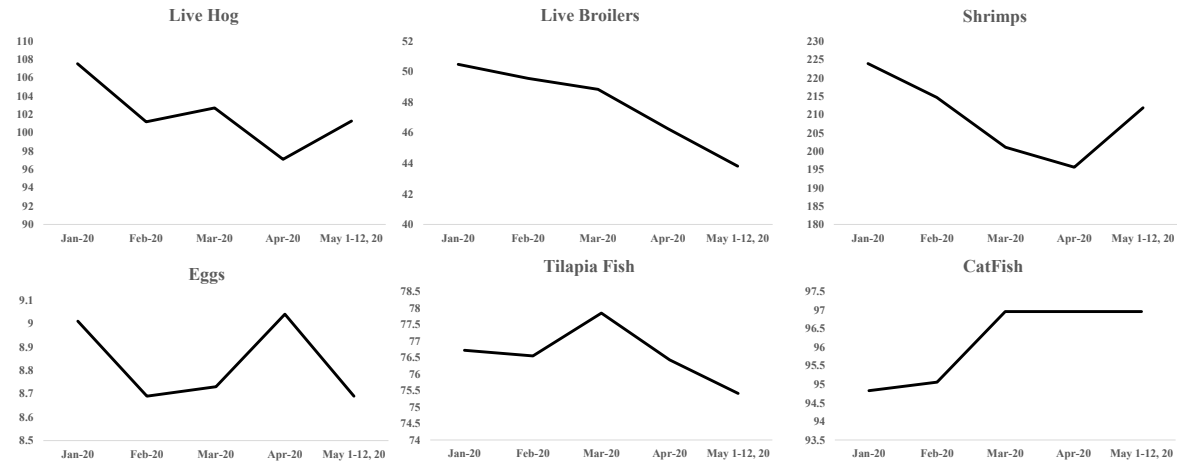

Fig.3 Wholesale prices of various food items in Bangkok
Source: Data adapted from Ministry of Commerce, 2020 (USDA, 2020)

observed with broilers and tilapia fishes. The worst hit was broiler farms and chicken meat proccessing factories as they had stock piled and had to somehow disperse it reducing the price by more than 13% from January to May. At the same time, milk production industry was also hit as the schools were closed due to the lockdown. As half of milk consumption is from government schools and the other half from commercial sales that was also disrupted in the lockdown period (Pattaya Mail, 2020).

Impact on agricultural stakeholders

All stakeholders across the agricultural value chain were seen to have varied impact. Major stakeholders impacted were the food companies and several retailers. While big retailers had little negative implications, the small retailers were heavily damaged and many retailer stores have been forced to shut down their businesses. Examples of some organizations/stakeholders having positive and negative impacts have been highlighted across the value chain (Fig. 4). Central Food Retail reported 710 million USD sales revenue, 4% increase from previous year. Siam Makro also reported an increase in sales, 9% growth rate for the first quarter at 1.7 billion USD. Covid 19 also indicated growth on retailers online shopping platforms, although it may only be around 10-15% of the total revenue.

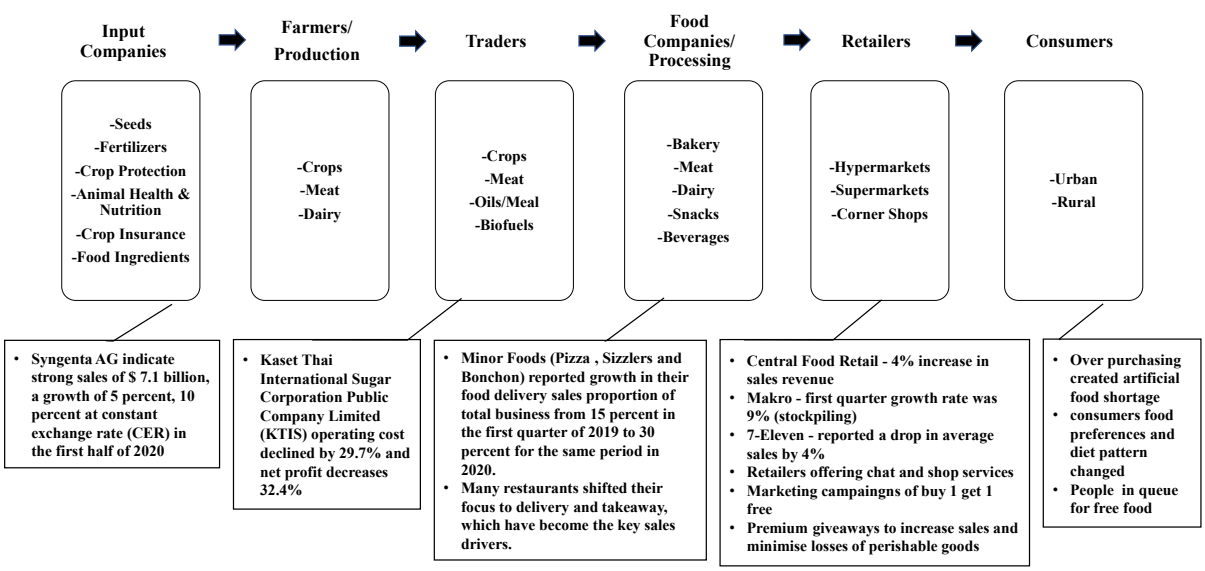

Fig. 4 Highlights of positive and negative impacts on agriculture stakeholders in Thailand

1. Input Industry: Thai Seed Trade Association (THASTA) and the Seed Association of Thailand (SAT) together contribute significantly to the Thai seed industry where the seed market was valued approximately US$ 565 million in 2016. Thailand has been an attractive hub for seed production not only for the domestic varieties but also exporting vegetables seed to India, Vietnam, China, US. Approximately, 20% of corn production in Thailand is kept for domestic consumption, the rest is exported to Vietnam, Myanmar, Indonesia and other neighboring countries (Mordor Intelligence, 2020). Syngenta AG, Bayer AG, East West Seed (Thailand Co.), Pioneer Hi-Bred (Thailand Co.) are amongst the top seed market players in the region. Syngenta AG indicate strong sales of US$ 7.1 billion, a growth of 5%, 10% at constant exchange rate (CER) in the first half of 2020, where the crop protection sales improved by 6% (12% at CER) and seed sales improved by 2% (9% at CER) (Syngenta Group, 2020).

2. Farmers/ Production: Thailand's domestic markets accounts for 62% of poultry production. About 85% of fisheries and seafood production goes for export and fruits exports are worth 88,000 million baht per year (Agroberichten Buitenland, 2020). Production has been stable while huge decline in exports have been observed. More than direct impacts from Covid 19, farmers face indirect impacts such as decline in production because of droughts, while income from non-farm activities (60% of their total income) and remittances have also declined. Besides, decline in tourism has reduced the food demand by 66.2% on YoY basis (Thailand Development Research Institute, 2020).

3. Traders: Thailand rice export forecasts were slashed to 6.5 million tons, the lowest in 20 years because of the early year drought and also the rise in prices making it uncompetitive in global market. Although export of Thai jasmine rice increased by 63% because of the panic purchasing done by countries during Covid 19 (Reuters, 2020).

4. Food Companies/Industry: Thai food industry currently comprises of around 30 thousand food processing factories generating an annual income of about US$25 billion according to the National Food Institute. Thailand food industry sources 80% raw material locally and is expected to grow by 2.6% annually. Some of the multinational leaders are Nestle, Thai Union, Charoen Pokphand Group, Dole Thailand, Betagro, P&G and Ajinomoto. Nestle pet foods experienced 9% growth in 2020 as compared to last year, partly because during the Covid 19 consumers spent more time with their pets. Unilever was impacted by the disruption in transportation and logistics. As an alternative, it produced sterilization products for hospitals, schools, and other venues. It included product donations, collaborations, and legitimate handwashing education programs through government agencies and independent organizations to help communities in great need. Apart from that, Unilever's suppliers and customers provided financial liquidity for 17.5 billion baht to support the livelihoods of individuals in the value chain (UNICEF, 2020).

5. Retailers: Siam Makro Public Company Limited, a wholesale business under the Charoen Pokphand Group (CP) was impacted in both positive and negative ways. Panic buying especially in the dry food segment during March 2020, positively affected sales of the Makro business in Thailand and abroad. While the food business service was severely hit, declining mid-February 2020 onwards because of the widespread impact on the tourism industry. However, revenue, service fees, and other income of Makro totalled 1,149 million baht. The company's total revenue was 56,308 million baht, an increase of 4,509 million baht or 8.7% compared to the same quarter last year indicating a growth of 10.7% within a year (The South African, 2020). TESCO believes that the impact of Covid 19 will last for another three years. In this period TESCO is focusing on opening small branches like TESCO Express in the EEC or Eastern Zone gaining benefits from local nearby logistics.

6. Consumers: The National Statistics Office (NSO) suggests that Thai household spent about 33.9% of their expenditure on foods and beverage based on their preferences and taste rather than cleanliness and nutrition (FFTC-AP, 2020). Post Covid 19, consumers are more concerned regarding the health, food safety and hygiene making the consumers more conscious and aware engaging more digitally. Mckinsey & Company research indicated that consumers seemed worried about the increasing meal cost because of the takeout, delivery and ready meal consumption preferences. 41% respondents suggested that grocery spending has increased and 92% respondents who have switched stores will move back to their original stores post Covid 19 situations (McKinsey & Company, 2020).

In the initial days of the lockdown, artificial shortage of food made some food items more expensive. Consumers also panicked and over purchased and stocked up on food items leading to empty racks at various retailers in Thailand. The poor could not access various

items as they did not have enough resources to purchase food items and were seen in queues for free food distribution because of the rising food prices. With reference to commercial sales, the McKinsey survey indicated that people in Thailand have been getting more conservative with their money since the pandemic and they are also cautious in going out to buy groceries. Thailand's consumers have found a way to avoid their fear of going to stores through E-marketplaces and food/ groceries delivery services practicing social distancing. Their preference of cooking at home increased by 57.48 percent (HFocus, 2020).

Measures and Policies by Ministry of Agriculture and Cooperatives (MOAC)

Thai government supported various initiatives such as Kon La Khrueng campaign, Rao Mai Thing Kun campaign, Shop Dee Mee Kuen campaign, etc. to promote buying and selling of agricultural products. The Ministry of Agriculture and Cooperatives (MoAC) and the State Bank for Agriculture and Agricultural Cooperatives (BAAC) established financial aid, an exemption for repayment of loans to restore and balance the effects of the pandemic. Thailand planned a new large-scale cash transfer programs to support vulnerable individuals that were not registered in any social protection database earlier (Table 2). This scheme supported almost half of the employed informal workers in Thailand.

There have been three main categories of beneficiaries:

(a) Informal off-farm workers received 5,000 baht per month for 3 months (April to June) under the 'No-One Left Behind' scheme. At about 37% of monthly GDP per capita, this amount is close to the median monthly income for informal workers in most sectors and is higher than the global average of cash transfers provided in response to Covid 19. To date, over 15 million recipients have received this assistance (almost

Table 2 Thailand key fiscal measures under the 1 trillion-baht emergency decree

	Planned amount (Bln baht)	% GDP	Approved amount (Bln baht)	Disbursed amount (Bln baht)	Target recipients (Mln)	Total recipients (Mln)	Timeframe
1) Healthcare measures	**45.0**	***0.27***	**2.56**	**0.52**			
2) Relief measures for households, farmers, entrepreneurs	**555.0**	**3.29**	**365.66**	**310.59**			
- 5,000 Baht cash transfer to the informal workers for 3 months – "No-One Left Behind" 15	170.0	1.01	170.0	n/a	16.00	15.30	Apr – Jun 2020
- Farmer assistance of 5,000 Baht for 3 months	150.0	0.89	150.0	114.31	10.00	7.59	May – Jul 2020
- 1,000 Baht cash transfer to the state welfare card holders for 3 months	3.49	0.02	3.49	3.49	1.16	1.16	May – Jul 2020
- 1,000 Baht cash transfer to the vulnerable groups for 3 months	20.35	0.12	20.35	20.35	6.78	6.78	Jun – Oct 2020
- Top-up of the state welfare card holders of 500 Baht for 3 months	20.92	0.12	20.92	n/a	13.95	n/a	Oct – Dec 2020
- 15,000 Baht cash transfer to the formal workers by Social Security Office	0.89	0.01	0.89	0.89	0.059	0.059	Aug – Oct 2020
- Available for additional (as yet unannounced) measures	189.34	1.13					
3) Recovery and rehabilitation measures	**400.0**	**2.37**	**120.07**	**9.73**			
- "We Travel Together" Program	20.0	0.12	20.0	n/a			Jul 2020 – Jan 2021
- Uplifting large agricultural plots with new technology and market integration	13.9	0.08	13.9	n/a	0.26		Aug 2020 – Sep 2021
- Co-payment program of not more than 3,000 Baht/person	30.0	0.18	30.0	9.22	10.0	8.77	Oct – Dec 2020
- Promotion of employment on new graduates in public and private sectors	19.46	0.12	19.46	n/a	0.26		Oct 2020 – Oct 2021
- Other approved measures	36.69	0.21	36.71	n/a			FY2021 onward
- Available for additional (as yet unannounced) measures	279.93	1.66					

Source: NESDC, Fiscal Policy Office, Thailand (World Bank Group, 2021)

all those eligible), at a total cost of 230 billion baht (about 1.4% of GDP). The program was not extended beyond June.

(b) Farmers also benefited from a similar cash transfer of 5,000 baht per month for 3 months (May to July). As of November 2020, around 7.6 million farmers had received this assistance (around three quarters of those eligible), at a total cost of 114 billion baht (about 0.7% of GDP).

(c) Social assistance beneficiaries received top-ups to their regular programs to help them cope with the economic impact of Covid 19. A cash transfer of 1,000 baht per month was paid for 3 months (May to July) for young people up to age 6, older people, and people with disabilities. In the December quarter, state welfare card holders are receiving increased payments totaling 500 baht/person/month; the existing cash transfer is 200-300 baht/person/month.

The major challenge faced by these programs was determining the eligibility. While many of the most vulnerable in Thailand have now benefitted from government support, some vulnerable groups are likely to have missed out, including informal migrant workers. Nevertheless, the expiration of these benefits is already likely to have had significant impacts on the incomes and welfare of these groups, and these impacts may persist in the absence of further targeted support.

Role of Technology

Covid 19 became a major trigger point which forced producers, distributers and consumers to turn towards technology enabled alternatives. This trend influenced food safety and transparency that cannot be ignored for maintaining daily hygiene and dietary preferences. It also gave opportunity to existing stakeholders to explore ways for raising standards in agriculture production and distribution. Kasikorn Research Center forecasted that total number of deliveries in 2020 will increase to 66-68 million times which is about 78.9 - 84.0 percent growth rate compared to last year (Kasikorn Research Center, 2020). According to the research, the growth rate of food delivery platform illustrates trend of consumption which means consumers and producers would benefit from it. The food delivery platform tends to exploit and expand the gap between major and minor actors in the food supply chain.

Online platforms played an important role during the pandemic and created opportunity for the retailers. Many retailers shifted to online selling and home delivery that indicated positive outcomes. TESCO Lotus adapted the marketing strategy of "Chat and Shop" and e-commerce delivery services. In order to address the decline in fruit export during the first quarter of 2020, the Ministry of Agriculture and Cooperatives (MOAC) in collaboration with 1,300 agricultural co-operatives started marketing through online platform (Ministry of Agriculture and Cooperatives (MOAC), 2020) providing promotions and discounts for domestic consumption. Other applications like Grab and TALAD APP were also used to increase sales in all provinces.

Conclusion

Covid 19 is an ongoing crisis and is yet to fully reveal real impacts on all industries. The impact of the pandemic on the agriculture value chain varied across different players. While it led to increased unemployment, enhanced food security risks and greater challenges for the most vulnerable, it also increased adoption of digital technology by various actors in the value chain including input companies, producers, food companies, retailers, and consumers. This shift to use of technology provides a great opportunity to Thailand in building back better through policies and incentives that accelerate the Thailand 4.0 agenda, improve benefits to farm workers and assist in reducing the uncertainties posed by the pandemic. Farmers and producers are facing losses in revenue and contribute to the negative implication of Covid 19 in the long term. At the same time, retailers are the ones who gain the opportunity in the crisis by shifting to online platforms. Some of the future steps and way forward supporting the agriculture system include:

1. Accelerating the use of digital technologies: Apart from the challenges farming households faced during Covid 19 in terms of reduced incomes and increased debts, Covid accelerated the use of digital technologies in the agriculture sector through online platforms, digital payments and digital advisory applications. This will further boost the growth of food industry in accordance with Thailand 4.0.

2. Implement robust direct benefit transfer (DBT): Thailand can benefit by implementing digital payment infrastructure by integrated citizens' digital ID with bank account and mobile system for example application of JAM Trinity in India. Other countries like Togo and Bangladesh were also exploring such opportunities to benefit during Covid 19.

3. Opportunity to develop the agriculture sector: Thailand for years has been producing far more food than it consumes. In 2019, Thailand exported US$ 33 billion worth of products including rice, processed food and tropical fruits. United Nations forecasts that global demand of food will increase by 60% in the upcoming decade in addition to the insecurities created by Covid 19. It could be a great opportunity for Thailand to leverage on technology, innovation and logistics to take the food industry to the next level.

This aligns with Thailand 4.0 program that has identified 'food for the future' and 'advanced agriculture and biotechnology' among the 12 target industries. Eastern Economic Corridor (EEC), which is a key component of the program, should encourage the use of technology and smart farming approaches to increase the competitiveness of Thailand agriculture sector.

Acknowledgement

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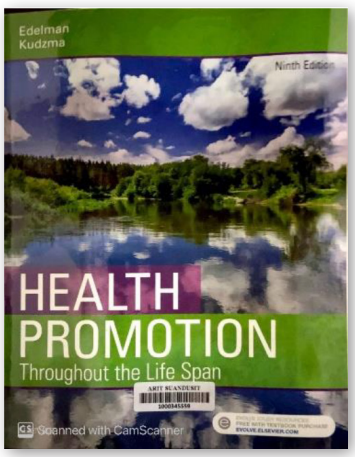
Book Review

Doungnetre Thummakul



Book name: Health Promotion Throughout the Life Span
Authors: Edelman, C.L., & Kudzma, E.C.
Publisher: Elsevier, 2018
Paperback: 696 pages
Language: English
ISBN: 978-0-323-41673-3

"Health Promotion Throughout the Life Span" reflects the earlier assessments of major risks to health, changing public health priorities, and emerging issues related to *Heathy People 2020* in Americans' health preparedness and prevention. The following vision was established: A society in which all people live long healthy lives.

This edition presents health data with related theories and skills that are needed to understand and practice when providing care. This book focuses on primary prevention intervention; its three main components are: (1) health promotion, (2) specific health protection, and (3) prevention of specific diseases. Health promotion is the intervention designed to improve health, such as providing adequate nutrition, a healthy environment, and ongoing health education. Specific protection and prevention strategies, such as massive immunizations, periodic examinations, and safety features in the workplace, are the interventions used to protect against illness.

In addition to primary prevention, this book discusses secondary prevention interventions, focusing specifically on screening and education. Such programs include blood pressure, cholesterol, and diabetes screening and referral. This text is present in five parts, unit 1, *Foundations for Health Promotion,* unit 2, *Assessment for Health Promotion,* unit 3, *Interventions for Health Promotion,* unit 4, *Application of Health Promotion,* and unit 5, *Emerging Global Health Issues.* Throughout these units, the evolving health care professions and the changing health care systems, including future challenges and initiatives for health promotion, are described. Emphasis is placed on the current concerns of reducing health care costs while increasing life expectancy and improving the quality of life for all Americans. This promotes the reader's immediate interest in thoughts about the content of the chapters.

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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.

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Original Articles

Review Article

Book Review

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