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Content

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Effect of Thickness on Qualities of Dried Sweet Bamboo Shoots (*Dendrocalamus asper* Backer) Products

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Abstract

The objective of this research was to investigate the effect of thickness on the quality of dried sweet bamboo shoots (SBS). The SBS were prepared with various thicknesses. The quality of dried SBS was investigated, including weight loss, lightness, hardness, and sodium chloride content. The results indicated that the weight loss and the amount of sodium chloride tended to decrease when the thickness of SBS increased. Conversely, the brightness and hardness varied directly as the thickness of SBS. The effect of thickness on drying time and rehydration time using different methods of SBS were studied. The results showed that the SBS with a thickness of 2 mm had the shortest drying time and rehydration time. The dried SBS was soaked in water at room temperature for 30 min before boiling in hot water. This method had the shortest rehydration time when compared with other methods. The sensory evaluation of dried SBS after rehydration by untrained panelists were investigated. It was found that SBS with a thickness of 2 mm had the highest scores on sensory acceptability in appearance, color, texture, flavor, and overall liking score. The analysis of the chemical composition of dried SBS was high carbohydrate (48.18%), protein (22.52%), and low-fat content (4.18%). The shelf life of dried SBS product was at least 6 months. Accordingly, the knowledge gained from this study can be applied to the production of dried SBS, which can extend the shelf life of SBS and added value of agricultural products.

Introduction

The scientific name of rough giant bamboo is a *Dendrocalamus asper* Backer. It is a kind of rhizome bamboo with large clumps, and straight body pressed together into a rather tight clump. The tip of the body is curved to hang down. There are four species of this bamboo in the group, which are small bamboo, green bamboo, big bamboo and sweet bamboo. The sweet bamboo is a valuable economic crop and popular with consumers because the shoots of this species are large, without burr, sweet in taste, crispy in texture and fine

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white (Chiang Mai Rajabhat University, 2020). Currently, there is an increase in the area of sweet bamboo growing in Thailand. To the fresh shoots, the plant can also be processed into bamboo shoots in a sealed container or dried in sun, which can be sold to foreign countries almost all over the world. The highest improting countries are Japan, followed by the United States, Hong Kong and Saudi Arabia (Bhattarakosol & Seejan, 2020). The bamboo shoots are a source of high-quality protein, low fat and high fiber. The chemical composition of fresh SBS and long sheath bamboo shoots were reported. The moisture, protein, fat and dietary fiber were 91-92%, 1-3.44%, 0.06-0.13% and 2.23-2.60%, respectively. Moreover, there are 18 free amino acids, including all 8 essential amino acids (Kusalaruk & Limsangouan, 2016). The problem for bamboo shoots growers is that in high season, the sale price of fresh bamboo shoots is lower. If it is improperly kept, fresh bamboo shoots will rapidly reduce in quality post-harvest. Therefore, bamboo shoot processing is an option that will help extend the shelf life of bamboo shoots and help add value to agricultural products and provide a variety of options for consumers.

The dried bamboo shoots are obtained by boiling fresh bamboo shoots. They are peeled, then grated into strips or sliced or cut into sheets. It is mixed with salt, and dried using solar heat or other energy. They are rehydrated in boiling water before use (Thai industrial standards institute, 2020). The purpose of making dried bamboo shoots is to extend the shelf life. The water activity is reduced, which influences inhibiting the growth of microorganisms and the activity of enzymes. The drying process helps reduce the weight and volume of bamboo shoots. They are more convenient to store and transport, including, the convenience for off-season consumption.

There are many factors of drying rate, including the characteristic of bamboo, pressure, temperature, relative humidity, wind speed, specific humidity, and the amount of bamboo shoots for drying. The physical properties of bamboo shoots such as size, shape, volume, and surface area are important for drying rate (Fellows, 2017). The effect of different drying temperatures on phytochemistry and antioxidant activity of bamboo shoots was studied. It was found that the use of high temperatures in drying resulted in the reduced phytochemicals and antioxidant activity in bamboo shoots. The optimum drying temperature was 60 °C (Seewaeng & Siriamornpun, 2019). The thickness of sliced bamboo shoot affected the cyanide content in fresh bamboo shoot (Rana et al., 2012). Generally, the thickness influenced on the conductive heat transfer and water droplet evaporation of raw material during drying. Therefore, it is one of the important factors for drying rate and quality of dried bamboo shoots.

The objective of this research was to study the effect of thickness on the quality of dried SBS. Phytochemicals and sensory evaluation were investigated to select the optimum thickness to produce dried SBS.

Materials and methods

1. Preparation of SBS

The SBS were obtained from the agriculture group in Sa Kaeo province. The fresh SBS were cut. The SBS with similar growth stages of equal size and length approximately 30 cm were selected. The shell was peeled off and washed with water.

2. Study on the effect of thickness on quality of dried SBS

The prepared SBS were cut by slicing machine (HH-society, China). The SBS with a thickness of 2, 4, 6, 8 and 10 mm, 5 mm in width, and 100 mm in length were prepared. The SBS were boiled in hot water for 30 min, and were fermented with 10% of salt for 3 hr. It has been washed with water 5 times. It was boiled in hot water for 30 min and was washed with water. It was drained and dried in a hot air dryer at 60°C, dried until the final moisture content of SBS was not more than 10%.

3. Quality analysis of SBS

3.1 Weight loss

The weight loss before drying was measured. The fresh SBS and processed SBS before drying were weighed by balance (Zepper EPS-3001, China). The weight loss before drying values was defined as follows: Weight loss before drying (%) = [(weight of fresh SBS – weight of SBS before drying)/ weight of fresh SBS] x 100 (Kotoki & Deka, 2010).

The weight loss after drying was measured. The SBS before drying and after drying were weighed by balance (Zepper EPS-3001, China). The weight loss after drying values was defined as follows: Weight loss after drying (%) = [(weight of SBS before drying – weight of SBS after drying)/ weight of SBS before drying] x 100 (Kotoki & Deka, 2010).

3.2 Color

The color of SBS was measured by color meter

(Colorimeter, WR10QC, China). The CIE system was evaluated by L * or brightness (0 = black, 100 = white), a * (+ a = red, -a = green) and b * (+ b = yellow, -b = Blue). Browning value was calculated as follows: Browning value = (ΔL * / L0 *) / 100, where ΔL* was the difference of brightness between two measuring points. and L0 * was the reference brightness (Lertwiram & Sawatming, 2020).

3.3 Hardness

The hardness of SBS was measured by texture analyzer (Daiichi FG 520K, Japan). The cylinder probe with 8 mm of diameter was used and the unit of force measured was newtons (N).

3.4 Salt content analysis

The rehydration SBS was measured using a digital salinity meter (HANNA, HI96821, USA).

3.5 Rehydration

Rehydration of SBS after drying by different methods as follows: 1) soak in room temperature 2) boil in hot water 3) soak at room temperature for 30 min before boiling in hot water were investigated. The complete rehydration was obtained by observing the appearance, size, hardness and weight of SBS by comparison with SBSs before drying. The optimum rehydration time of SBS with different thicknesses was investigated.

3.6 Sensory evaluation

Sensory evaluation of rehydration SBS by 30 untrained panelists was investigated. The importance of liking of appearance, color, texture, flavor and overall liking were expressed by 9-point hedonic scale (9 = most liked, 1 = most disliked).

3.7 Chemical composition analysis

The chemical composition of dried SBS including moisture, protein, fat, carbohydrates, ash and energy was analyzed (Association of Official Analytical Chemists, 2012).

3.8 Shelf life of dried SBS

The dried SBS were packed in sealed plastic bags and stored at 30°C for 6 months. They were evaluated every 3 months. The moisture content, yeast and mold were investigated.

4. Statistical analysis

The statistical technique one-way ANOVA was used for calculating. Duncan's new multiple-range Test (DMRT) was used to compare the difference in the average values at the 95% confidence level (Duncan, 1995).

Results and discussion

1. Effect of thickness on physicochemical properties of dried SBS

The effect of thickness on weight loss of SBS before drying and after drying was shown in Fig. 1. The weight loss of SBS was decreased when the thickness of SBS increased. The weight loss of SBS before drying and after drying was compared. It was found that the SBS after drying had higher weight loss values than before drying. In general, the moisture of bamboo shoots evaporates during drying, which involves both mass transfer and heat transfer. The heat is transferred to the surface of bamboo shoots and the water in bamboo shoots evaporates by the latent heat of vapor formed. The steam will spread through the air film and be blown away by the movement of the hot air. This condition will cause the vapor pressure on the surface of bamboo shoots to be lower than the steam pressure inside of bamboo shoots, resulting in differences in vapor pressure. The inner of bamboo shoots have high vapor pressure and gradually reduced when bamboo layer approached the dry air. This causes pressure to expel water from bamboo shoots cells (Damondaran & Parkin 2017). Obviously, bamboo shoots of 2 mm thickness have more surface contact with air than other sizes which leads to it having the highest weight loss. The thickness of bamboo shoots affected on the drying rate. The bamboo shoots with a high ratio of surface area to volume will have a lot of water evaporation and have a faster drying rate. Therefore, the drying rate of thick bamboo shoots be slower than thinner ones. The drying rate was decreased when the thickness of bamboo shoots increased (Fellows, 2017). A similar observation had found with dried apples which were reported by Paradkar & Sahu (2018).

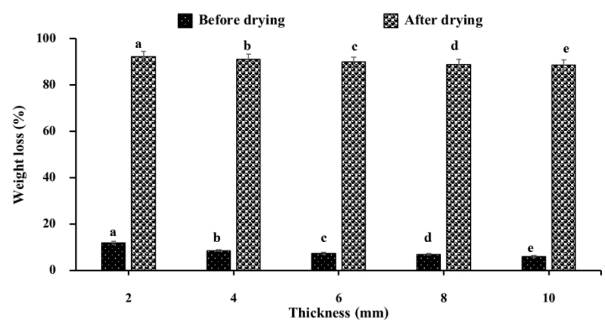

Fig. 1 The effect of different thickness on weight loss of SBS

The unit operation before drying, including size reduction, boiling, salt fermentation affects the weight loss of bamboo shoots. The size reduction of bamboo shoots causes to cells damage that affecting the ratio between the surface area exposed to the environment. If there is a lot of surface area, water will be more likely to spread out than having a small area of contact that can cause high weight loss (Suphamityotin, 2013). The weaken cell membranes of bamboo shoots was found when the bamboo shoots were boiled in hot water. The diffusion of water from the bamboo shoots allowed the sodium chloride to spread more easily into the cell. Moreover, the salt concentration increases osmotic pressure and reduces the water activity of bamboo shoots (Yuenyongputtakal, 2013).As the suitable concentration of NaCl solution, the osmotic pressure of the solution decreases. This simplifies the increased removal of water from bamboo shoots and thereby decreases the cyanide content (Rana et al., 2012).

The effects of the thickness on the color value of SBS before drying, after drying and rehydration is shown in Table 1. The results show that the color values of SBS before drying, after drying and rehydration had significant difference of the 95% confidence interval when $p \leq 0.05$. The brightness (L *) tends to decrease when the thickness of the SBS increases. The a* value indicates the red to green color value. If the a* value is negative in the green range and the a* value is positive in the red range. The a* value tends to increase when the thickness of the SBS before drying, after drying and rehydration increases. Furthermore, the b* value indicates the yellow to blue range. If the b* value is negative in the blue range and the b* value is positive in the yellow range. The b* value tends to increase when the thickness of the SBS before drying, after drying and rehydration increases. The browning value is shown in Fig. 2. It was found that the browning value varied directly as the thickness of SBS before drying, after drying and rehydration. There are many factors for changing of color in SBS before drying, after drying and rehydration. The enzymatic browning reaction occurs at the surface of SBS when exposed to oxygen in the air. In general, bamboo shoots contain polyphenol oxidase (PPO) that is a browning catalyst (Huang et al., 2002). The enzymatic browning reaction is an oxidation reaction. It occurs when the cells of bamboo shoots are bruised, torn, bumped, crushed, sliced or chopped. The enzyme, substrate and oxygen react together. Colorless monophenol is oxidized to colorless diphenol. It is oxidized to o-quinone, which reacts with amino acids or proteins to be brown substances and will form a polymer with large molecules that has a brown color such as melanin (Damondaran & Parkin, 2017). In addition, heating by boiling and drying may result in the Maillard reaction between reducing sugar and amino acids, proteins or other nitrogen compounds with the heat catalyzed (Zhang et al., 2011).

Table 1 The effect of different thickness on color changing of SBS

Color	Thickness (mm)				
	2	4	6	8	10
Before drying: L*	88.60±0.14^{a}	87.23±0.05^{b}	86.46±0.12^{c}	85.27±0.02^{d}	84.49±0.29^{e}
Before drying: a*	-0.67±0.03^{c}	1.76±0.03^{b}	1.81±0.05^{b}	2.45±0.30^{a}	2.32±0.08^{a}
Before drying: b*	6.85±0.08^{e}	7.32±0.47^{d}	10.07±0.02^{c}	11.11±0.07^{b}	12.46±0.18^{a}
After drying: L*	79.66±0.10^{e}	73.56±0.22^{d}	70.30±0.15^{c}	68.78±0.02^{b}	63.11±0.18^{a}
After drying: a*	5.03±0.02^{e}	5.44±0.16^{d}	6.49±0.10^{c}	9.03±0.22^{b}	10.24±0.13^{a}
After drying: b*	18.58±0.11^{d}	18.65±0.38^{d}	23.84±0.05^{c}	24.38±0.12^{b}	30.47±0.36^{a}
Rehydration: L*	85.25±0.03^{a}	81.42±0.15^{b}	78.92±0.60^{c}	75.87±0.39^{d}	74.54±0.36^{e}
Rehydration: a*	0.65±0.02^{e}	2.75±0.03^{d}	5.45±0.31^{c}	6.36±0.39^{b}	8.24±0.32^{a}
Rehydration: b*	11.16±0.05^{c}	19.22±0.24^{d}	22.52±0.26^{c}	23.32±0.17^{b}	25.47±0.13^{a}

Remark: mean±SD, a-e means within each row indicate significant differences ($p \leq 0.05$) using Duncan's multiple range test

The effect of heat also affects the color change of pigment compositions in SBS, such as carotenoids, flavonoids, phenol, chlorophyll and betalains, etc (Bal et al., 2011). Chlorophyll is a green pigment that is in the chloroplast of plants. It is important in the photosynthesis process of plants that turns into glucose. It is a nutrient that provides energy in the cells of plants. Chlorophyll is unstable when exposed to heat. It turns into pheophytin which turns from green to brown color (Erge et al., 2008). The different thickness of SBS affects the processing and chemical composition. The thin SBS has a large surface area. Therefore, the heat transfer from the unit operation occurs faster. Polyphenol oxidase, which is the main cause of the browning reaction, and it is easily destroyed under high temperature. The low-thickness will take shorter time to dry than high-thickness. Resulting in low-thickness having a higher brightness than high-thickness. The color characteristics of SBS before drying, after drying and rehydration was compared. It was found that SBS after drying had a darker brown color than SBS before drying and rehydration due to the change of water which is the main component in SBS. The SBS before drying and rehydration have high water content in the cells. The firm cells, resulting in a high brightness of SBS were observed. As for after drying SBS, the water in the cells

is eliminated, causing the cells to shrink or become wrinkled resulting in a darker brown color that the brightness of the SBS decreases.

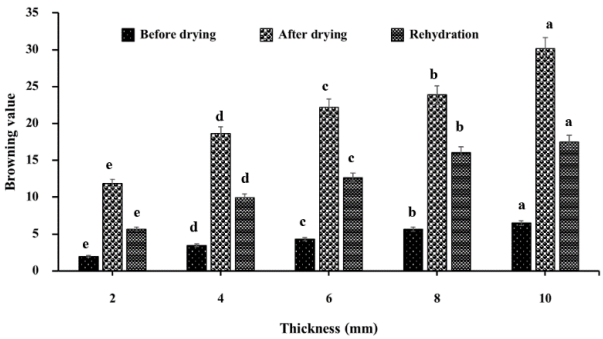

Fig. 2 The effect of different thickness on browning value of SBS

The effect of different thickness on the hardness of SBS is shown in Fig. 3. It was found that the hardness of SBS had significant difference of the 95% confidence interval when $p \leq 0.05$. The hardness was increased when the thickness of fresh, before drying, after drying and rehydration of SBS increased. Apparently, the SBS after drying had the highest hardness, followed by before drying, and rehydration, respectively. The hardness of fresh SBS has the strong structure. In general, the SBS is the main chemical constituents of carbohydrates that is found both digestible and indigestible. Most digestible carbohydrates are starch and sugar. Indigestible carbohydrates are the strong structure in plants tissues, including cellulose (73.83%), hemicellulose (12.49%), lignin 10.15 (%), pectin (0.37%) and other substances (3.16%) (Azeez & Orege, 2020). It gives strength of SBS structure, which directly effect on the SBS texture. The high-thickness has a high hardness, and the structure is stronger than SBS with low-thickness. The texture of SBS that through boiling in hot water and fermented with salt were very soft. The SBS boiling in hot water causes some structural changes and the cell membrane weakens. Moreover, fermentation with sodium chloride salt increases the propulsion of osmosis causing the spread of water and solvent (Yuenyongputtakal, 2013). Therefore, the hardness of SBS decreased compared to fresh SBS.

The effect of drying on the hardness of SBS was investigated. It was found that SBS after drying had higher hardness values compared to other procedures. It may be caused by the water in the cells of SBS is eliminated during drying, which causing the structure of SBS to shrink, dry, crispy. The high-thickness has a

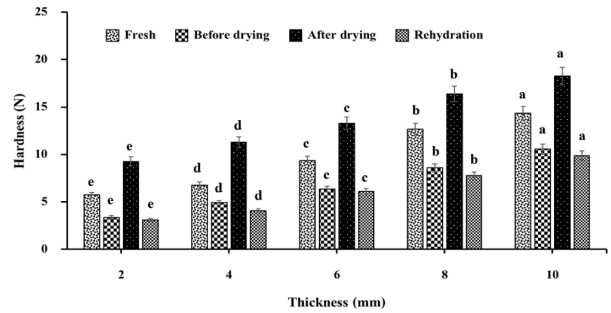

Fig. 3 The effect of different thickness on the hardness of SBS

higher hardness than the low-thickness. Furthermore, the dried SBS is soaked in water that it absorbs water. The structure of the SBS absorbs water, causing the cells to develop better and with more firmness. It is not sticky or hardened, soft, flexible, and similar to SBS before drying. The water molecules penetrate the cells of SBS. The hardness of SBS rehydration decreased compared to SBS after drying.

The effect of different thickness on the sodium chloride content of SBS after rehydration is shown in Fig. 4. It was found that the sodium chloride salt content of SBS after rehydration at various thickness had significant difference of the 95% confidence interval when $p \leq 0.05$. The amount of sodium chloride salt in SBS after rehydration was decreased when the thickness of SBS increased. The sodium chloride salt content in SBS is safe for consumption. According to the Recommended Daily Intakes (Thai RDI) of sodium in the nutrient list, recommended daily for Thais aged 6 and over is 2000 mg (Ministry of public health, 2020). The use of sodium chloride mixed bamboo shoots resulted in the removal of water by the osmosis method that can reduce the amount of water in bamboo shoots. The osmosis factor is related to mass transfer and product quality. Some water is removed from bamboo shoot tissues. The difference in osmotic pressure between bamboo shoots cells and osmotic solutions occurs. It is a driving force that causes the mass transfer between SBS cells and osmotic solutions. In the opposite direction through the cell membrane, which acts as a semi-permeable membrane. The cell wall of bamboo shoots can expand. When the pressure occurs within the cell, the membrane cell acts as a semi-permeable membrane. The water is more permeable than osmotic solutions. The mass transfer is occurring between osmotic system. The water inside the cells of bamboo shoots will spread from the cells to osmotic solutions.

Sodium chloride will spread into the cells of bamboo shoots. Some substances contained within the bamboo shoots cell will spread from the cell to osmotic solution (Yuenyongputtakal, 2013). The thickness of bamboo shoots affects the mass transfer of osmotic material. It is the result of the movement of water from bamboo shoots and the movement of the solvent from the osmotic solution. The ratio between the contact area and the osmotic solution has an effect. The bamboo shoots with a low thickness will have a lot of surface area. Water is more likely to propagate than high thickness bamboo shoots with little contact area. It results in low-thickness bamboo shoots having higher sodium chloride content than high-thickness bamboo shoots.

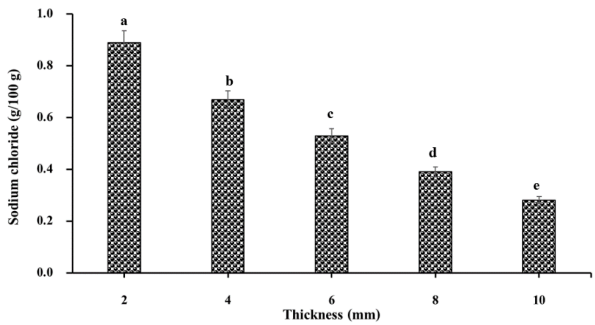

Fig. 4 The effect of different thickness on the sodium chloride content of SBS after rehydration

2. Effect of thickness on drying time and rehydration time using different methods of SBS

The effect of thickness on drying time and rehydration time using different methods of SBS is shown in Fig. 5. It was found that the drying time and rehydration time varied directly as with the thickness of SBS. The thickness, size, shape, volume, surface area of SBS is a physical property that affects the drying time. The low thickness SBS have a high ratio of surface area to volume, it will have more area for water evaporation and faster drying rate. Therefore, the drying rate of high-thickness will be slower than the low-thickness. The drying rate varied directly as with the thickness of SBS.

Rehydration quality is important for dried bamboo shoots. It involves changes in the physicochemical of unit operations of bamboo shoots preparation before drying and during drying (Sirijariyawat et al., 2017). The thickness of bamboo shoots influences rehydration time. The high-thickness is longer to recover water than the low-thickness. Rehydration of bamboo shoots after drying by different methods as follows: soak in room temperature, boil in hot water and soak at room temperature for 30 min before boiling in hot water were studied. The result shows that the combination method between soaking the water at room temperature 30 min before boiling in hot water had the least amount of rehydration time, followed by boiling in boiling water, and soaking the water at room temperature, respectively. Soaking dried SBS at room temperature, it absorbs water into the cells, causing the SBS structure to weaken. When the temperature increases, resulting in the water absorbed during the initial phase. The heat transfer within the cells improves, resulting in the water returning faster than other methods. The rehydration of dried bamboo shoots involves the process of bamboo shoots preparing before drying and during drying, which affects the changes structural of bamboo shoots. There are many factors affecting on rehydration rate such as temperature and drying time. The thick bamboo shoots had a long drying time. It is dry and hard on the surface area, causing fluctuations in air flow rate and difficult to control the moisture transfer from bamboo shoots. Prolonged heating of the bamboo shoots cell walls has been damaged, resulting in decreased water absorption of bamboo shoots (Madan & Pare, 2017).

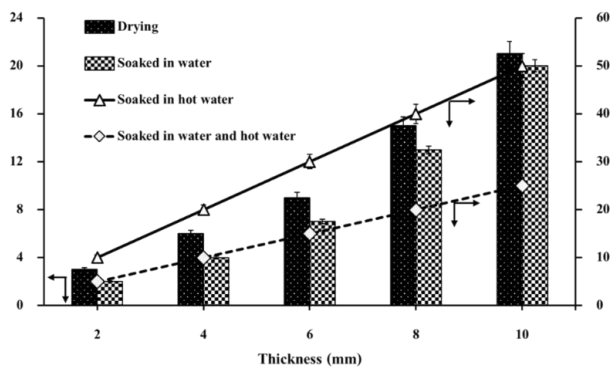

Fig. 5 The effect of different thickness on time for drying and soaking of SBS

3. Effect of thickness and rehydration using different methods on the sensory acceptance of SBS

The effect of thickness and rehydration using different methods on the sensory acceptance of SBS shown in Table 2. It was found that the mean scores of sensory acceptability of SBS with a thickness of 2 mm which rehydration using 3 different methods, had the highest scores on appearance, color, texture, flavor and overall liking. The SBS with a thickness of 10 mm had the lowest average score on appearance, color, texture, flavor

and overall liking. In general, the average liking score trends to decrease when the thickness of SBS increases. The sensory evaluation results were consistent with the obtained values from the physicochemical analysis of SBS. Obviously, the appearance and natural color of the SBS were not dark. The SBS with a thickness of 2 mm had the least brown color. The texture after rehydration was soft, not sticky or hardened. It was like boiled SBS before drying and the flavor was natural of bamboo shoots. Therefore, the SBS with a thickness of 2mm was the appropriate size for further development in the production of dried SBS for commercial production. In addition, the advantages of producing dried SBS with a low thickness, which does not take long to dry compared to using high thickness. It saves energy in drying and results in shorter rehydration times at a high thickness. The quality of the final product with low-thickness had better physicochemical and sensory properties than that of high-thickness.

Table 2 The effect of different thickness and soaked methods on sensory evaluation of SBS

Attribute	Thickness (mm)				
	2	4	6	8	10
Soaked in water (hr)	2	4	7	13	20
Appearance	7.77±0.86^{a}	7.37±0.89^{ab}	6.87±1.20^{bc}	6.40±1.13^{cd}	6.10±1.32^{d}
Color	8.00±0.79^{a}	7.50±0.94^{a}	6.73±1.26^{b}	6.53±1.28^{bc}	6.03±1.61^{c}
Texture	7.67±0.88^{a}	7.37±0.89^{ab}	6.77±1.14^{b}	6.13±1.46^{c}	5.90±1.65^{c}
Flavor	7.83±0.79^{a}	7.43±0.86^{a}	7.40±0.93^{a}	6.63±1.40^{b}	6.30±1.62^{b}
Overall liking	7.90±0.88^{a}	7.33±1.03^{a}	6.63±1.35^{b}	6.17±1.72^{bc}	5.53±1.48^{c}
Soaked in hot water (min)	10	20	30	40	50
Appearance	7.10±1.24^{a}	6.40±1.77^{a}	4.83±2.45^{b}	4.07±2.74^{bc}	3.00±2.02^{c}
Color	7.20±1.27^{a}	6.07±2.02^{b}	4.97±2.30^{c}	3.83±1.95^{d}	2.70±1.84^{e}
Texture	6.83±1.44^{a}	5.73±2.18^{b}	4.47±2.45^{c}	3.37±2.13^{d}	2.13±1.57^{e}
Flavor	7.07±1.41^{a}	6.03±2.08^{ab}	4.90±3.00^{bc}	4.87±2.52^{bc}	4.33±2.64^{c}
Overall liking	7.00±1.39^{a}	5.57±1.92^{b}	4.87±2.56^{b}	3.60±2.46^{c}	2.77±2.03^{c}
Soaked in water and hot water (min)	5	10	15	20	25
Appearance	7.57±0.90^{a}	7.10±0.66^{a}	6.53±1.01^{b}	5.97±0.93^{c}	5.43±1.33^{d}
Color	7.67±0.84^{a}	7.20±0.71^{a}	6.50±1.22^{b}	5.80±1.10^{c}	5.37±1.47^{c}
Texture	7.43±0.73^{a}	7.13±0.73^{ab}	6.57±1.07^{b}	5.73±1.28^{c}	5.03±1.61^{d}
Flavor	7.60±0.62^{a}	7.20±0.66^{ab}	6.97±0.85^{b}	5.83±1.39^{c}	5.20±1.79^{d}
Overall liking	7.63±0.76^{a}	7.07±0.78^{a}	6.33±1.09^{b}	5.77±1.63^{bc}	5.30±1.39^{c}

Remark: Mean±SD, $^{a-d}$ means within each row indicate significant differences ($p \leq 0.05$) using Duncan's multiple range test

4. Chemical compositions and shelf life of dried SBS

The chemical composition of dried SBS with 2 mm was analyzed. It was found that moisture content, protein, fat, total carbohydrate and ash were 11.86 ± 0.92%, 22.52 ± 0.18%, 4.18 ± 0.16%, 48.18 ± 0.86% and 13.26 ± 0.26%, respectively. The energy was 320.24 ± 1.04 kcal. The dried SBS was kept in sealed plastic packaging for 6 months at 30°C. The results show that dried SBS had moisture content not more than 14% by weight. Yeast and mold were not more than 500 colonies per 1 gram samples, which are safe for consumption, according to the standard of dried bamboo shoots products. The standard range, the number of acceptance level from the yeast and mold of dried bamboo shoot products were not more than 500 colonies per 1 gram sample (Thai industrial standards institute, 2006).

Conclusion

The thickness of SBS is important for the drying rate and the quality of dried SBS. Physicochemical properties and sensory evaluation were investigated for selecting the suitable thickness to produce dried SBS. It was found that the weight loss was increased when the thickness of SBS decreased. The unit operations during processing affects weight loss. The SBS during the drying process had higher weight loss values than before drying. The browning value and the hardness of the SBS varied directly as the thickness. The low-thickness had a higher salt content than the high-thickness. The high thickness had longer drying time and longer rehydration time. The SBS with 2 mm received the highest overall liking scores of sensory acceptability. Apparently, the optimum thickness for producing dried SBS was 2 mm. The dried SBS was high fiber, low fat, and protein content. It has a shelf life in sealed packaging for at least 6 months. Therefore, the knowledge gained from this research can be used as a guideline for the development of dried SBS which can be further extended for commercial production. Further development should be studied about the other choice for drying and techniques for reducing the water recovery time of SBS, etc.

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Product Development of Germinated Black Glutinous Rice Drink in a Sachet as Affected by Roasting and Brewing Time

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Abstract

Germinated black glutinous rice (GBGR) drink in a sachet was developed. The effects of roasting time (0, 5, 10 and 15 min) and brewing time (3, 5, 7, 10 and 15 min) on color, chemical compositions and antioxidant capacity were investigated. Prolonged roasting time increased L*, positive b*, C* and h° values, whereas decreased positive a* value, resulting in lighter color. Ash and carbohydrate contents were higher when longer roasting time was applied; however, a_w, moisture and protein contents were lower. GABA contents (61.04 mg/kg) retained in GBGR with 5 min-roasting time. Fat, crude fibers, total phenolic (TPC), total anthocyanin (TAC) contents and DPPH scavenging capacity did not significant change according to roasting time. Regarding brewing time with water at 98°C, L* and h° values of GBGR drink decreased. Higher TPC, TAC and DPPH scavenging capacity of GBGR drink were induced by using short brewing time 3-5 min). Therefore, roasting and brewing time of 5 and 3 min, respectively, were recommended for healthy drink production from GBGR. GABA concentration of 0.17 mg/150 mL was detected in finished product. For consumer acceptability test, GBGR drink was liked moderately and 77% of consumers would like to buy the product. Physical, chemical and microbial changes of the product prototype during storage time of 98 days were accepted following the Thai agricultural standard TAS 4404-2012 Good manufacturing practices for germinated brown rice standard.

Introduction

Rice is not only a stable food for Thai people but also an important economic crop. In Thailand, varieties of rice are cultivated, each of them possesses unique characteristics, especially pigmented rice such as riceberry, aromatic black rice (Hom-nil rice) and black glutinous rice. Pigmented rice is a kind of typical whole grain with colored pericarp. Pericarp is a part that provides protection for the seed coat and give colors to the rice such as light brown, red, purple and black (Ito & Lacerda, 2019). In the past, pigmented rice was

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demanded by consumers because of its specific characteristics including medical properties and exclusive taste (Ahuja et al., 2007). In comparison of white rice, pigmented rice has recently received increasing attention due to higher nutrients and antioxidants.

Black glutinous rice (*Oryza sativa* L.) is a well-known local pigmented rice in Thailand and characterised by dark purple pigment in the hull and pericarp and distinctive smell. The color of riceberry is determined by the different anthocyanins (Abdel-Aal et al., 2006). Anthocyanin is a flavonoid which belongs to polyphenol and responsible for antioxidant capacity. The significant roles of anthocyanins have been reported to alleviate several chronic diseases such as cardiovascular disease, hypertension and cancer, resulting from their antioxidant capacity. Black glutinous rice is commonly processed using steaming method and consumed as main dish. In addition, it has also been applied as ingredient for snacks or desserts (Loypimai et al., 2016). However, black glutinous rice is not cultivated in Thailand as an export crop; therefore, it could be processed as healthy food product in order to add value and increase commercial chances.

In this study, germinated black glutinous rice (GBGR) was used as main ingredient for healthy food development because of its higher nutritive values and larger amount of bioactive compounds, when compared to normal rice. During seed germination, the content and bioactivity of active compounds such as free amino acid, vitamins, and polyphenols increase (Chu et al., 2020). Additionally, gamma-aminobutyric acid (GABA), a natural occurring free amino acid, is greatly synthesized during germination. Several studies have been reported that a neurotransmitter GABA benefits human health by reducing blood pressure, accelerating the metabolism in the brain, inhibiting cancer cells and promoting relaxation and sleep (Klaykruayat et al., 2020). To my knowledge, application of GBGR as healthy food and beverage is very limited. Therefore, rice drink in a sachet using GBGR was developed in this study and the effects of roasting and brewing time on color, chemical compositions and antioxidant capacity were determined in order to obtain a high quality product that is desirable to consumers. The final product made from GBGR might be an alternative functional beverage which is a source of dietary bioactive compounds including phenolics, anthocyanins and GABA. Furthermore, manufacturing processes of this product was also friendly to environment and not complicated. Finally, this study could provide the efficient and suitable process of production of functional drink from GBGR in a sachet and might be useful commercially for healthy food production from black glutinous rice.

Materials and methods

1. Materials and chemicals

Black glutinous rice was purchased from local market in Bangkok in 2018. Folin-Ciocalteu's reagent, gallic acid, 2,2-diphenly-1-picrylhydrazyl (DPPH) and Trolox was purchased from Sigma-Aldrich (St. Louise, USA). Sodium carbonate, potassium chloride, sodium acetate and ethanol were purchased from Merck (Germany). All chemicals used are analytical grade. Sodium hypochlorite used is food grade.

2. Preparation of GBGR

The method was modified from Charoenthaikij et al. (2009). Black glutinous rice seeds were screened, washed with clean water. The seeds were soaked into 0.07% sodium hypochlorite for 30 min and washed with sterile water. In germination process, the seeds were soaked into sterile water with the ratio of 1:5 (rice: water) for 12 h at 35°C. After that, the soaked seeds were washed with sterile water. The seeds were put on wet cheese cloth in stainless steel tray which contained 700 mL sterile water and cover with another wet cheese cloth. Then, the soaked seeds were incubated at 35±2°C for 24 h. After germination, the germinated seeds were dried at 45°C until the moisture content was lower than 10%. The dried germinated seeds were kept at -40°C until analysis.

3. Effect of roasting time on physical and chemical properties

The effect of roasting time was determined in this study and the method was modified from Noosing et al. (2014). The germinated rice was roasted for 0, 5, 10 and 15 min at 100°C using hot-air roasting machine (Tefal, Model FZ 7072, Franch). In each sample, physical and chemical properties were investigated. The color values (L*, a*, b*, Chroma (C*) and hue (h°)) were determined as physical properties. The a_w value, proximate analysis, total phenolics, total anthocyanins, GABA and DPPH scavenging capacity were investigated for chemical properties.

4. Effect of brewing time on physical and chemical properties

The effect of brewing process was investigated by using different time. The method was modified from Noosing et al. (2014) and Burillo et al. (2018). The

roasted GBGR of 10 g were put into a sachet and sealed tightly (Fig. 1). For brewing, the roasted GBGR in the sachet was dipped into 150 mL water with temperature of 98°C at 3, 5, 7, 10 and 15 min. The sample solution was kept at room temperature until the temperature reached at 25°C and then kept at -40°C until analysis physical and chemical properties. For the physical properties, the values of L* a* b* C* and h° values were analyzed. The total soluble solids, pH, total phenolics, total anthocyanins, GABA and DPPH scavenging capacity) were investigated for the chemical properties.

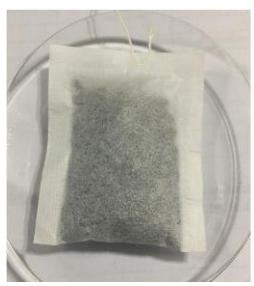

Fig. 1 Rice drink made from germinated glutinous black rice in sachet

5. Sample extraction

Roasted GBGR were extracted using the method of Kim & Lee (2002) and Wrolstad et al. (2005) with some modifications. The powder samples were prepared by grinding and sieving with 80 mesh sieve (Endecotts, England). A gram of roasted GBGR powder was mixed with 12 mL HCl in methanol (1% v/v), homogenized on ice for 2 min and sonicated at 4°C for 15 min using homogenizer (T10 basic Uitra-turrax, IKA® Staufen, Germany) and ultra-sonicator (CP 360T, CREST Ultrasonic, Malaysia), respectively. After that, the mixture was centrifuged at 11,000 rpm for 30 min at 4°C using refrigerated centrifuge (Sorvall RC 6-Plus, Thermo fisher, Thermo Scientific, Germany). The supernatant was collected and the residue was re-extracted with 13 mL HCl in methanol (1% v/v). After the second extraction, the volume of extract was adjusted to 25 mL in volumetric flask. The extract was stored at -40°C until analysis. The extracts were used in determination of total phenolics, total anthocyanins, DPPH scavenging capacity and GABA content of roasted GBGR.

6. Color measurement

Color parameters were quantified as the physical properties. The roasted GBGR and the drink from roasted GBGR after brewing were sampled as 100 g and 100 mL, respectively. Then, each sample was put into clear plastic tray before color measurement. The values of L*, a*and b* (CIE system) were generated by using colorimeter (Model WF30, U.S.A). The L* represents color lightness (0 = black and 100 = white), the a* represents the red (positive values) or green (negative values) and the b* represents yellow (positive values) and blue (negative values). The C* value represents color intensity. The h° value was expressed in 0-360 degrees with red color at 0°; yellow color at 90°; green color at 180°; blue color at 270° and magenta color at 360°. The C*and h° values were calculated using the values of a* and b* according to the equations; $C^* = (a^{*2} + b^{*2})^{1/2}$ and $h^\circ = \tan^{-1}(b^*/a^*)$, where $a^* > 0$ & $b^* \geq 0$; $h^\circ = \tan^{-1}(b^*/a^*)+360$, where $a^* > 0$ & $b^* > 0$ (McGuire, 1992). The colorimeter took 10 readings and generated an average for each sample. The experiment was done in replication with three measurements.

7. Determination of chemical properties

7.1 Water activity (a_w)

Water activity (a_w) were determined using a_w meter (Sprint Novasina, Model TH-500, Switzerland). The powdered samples were applied for a_w measurement. The experiment was done in duplication with triplicate measurements

7.2 Proximate analysis

Proximate analysis was performed using standard methods of Association of Official Analytical Chemists (AOAC, 2000). The contents of moisture, crude fat, crude protein, crude fiber and ash were determined. Carbohydrate content in sample was obtained by calculation. The experiment was done in duplication with triplicate measurements

7.3 Total phenolics

Total phenolic contents using Folin-Ciocalteu's reagent was modified from Maizura et al. (2011). The sample or standard (0.4 mL) were mixed with 2 mL of 10% (v/v) Folin-Ciocalteu's reagent and incubated at room temperature for 4 min. Then, 1.6 mL of 5% (w/v) sodium carbonate ($NaCO_3$) was added and stood for 30 min at ambient temperature. The mixture was centrifuged at 5,000 rpm for 10 min before absorbance measurement at 765 nm using UV-visible Spectrophotometer (T60, PG instrument, England). Gallic acid (0-100 µg/mL) was used as standard. The experiment was done in duplication with triplicate measurements

7.4 Total anthocyanins

The total anthocyanin contents were determined using a pH-differential method which was modified from

Giusti & Wrolstad (2005). Each 0.5 mL extract was separately mixed with 4.5 mL of 0.4 M hydrochloric acid–sodium acetate buffer (pH = 4.5) and 4.5 mL of 0.025 M hydrochloric acid–potassium chloride buffer (pH = 1). Absorbances at 510 and 700 nm were measured using a spectrophotometer (T10 basic, Uitra-turrax, IKA® Staufen, Germany). Total anthocyanins were calculated using the below equation.

$$\text{Monomeric anthocyanin (mg/liter)} = (A \times MW \times DF \times 1000)/(\varepsilon \times 1)$$

Where A is $(A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5}$; MW is the molecular weight of cyanidin-3-glucoside (449.2 g/mol); DF is the dilution factor; l is the cell length (1 cm.); ε is the molar extinction coefficient (26,900 L × $cm^{-1} \times mol^{-1}$).

The total anthocyanin contents were expressed as cyaniding-3-glucoside equivalents. The experiment was done in duplication with triplicate measurements

7.5 Determination of GABA

GABA contents in samples were determined using HPLC (Agilent 1100 series, Agilent Technologies, Santa Clara, CA, U.S.A). The analysis was modified from In-house method based on Thai agricultural standard TAS 4003-2012 (2012). A reverse phase HPLC system consisted of 5 mm Symmetry® (4.6x250 mm) column (Waters, Milford, MA, U.S.A) and fluorescence detector were used in this study. The column was maintained at 25°C. The mobile phase used was 0.05% trifluoroacetic acid in water (A), acetronitrile (B) and methanol (C). The solvent isocratic elution was performed as 55% (A), 25% (B) and 20% (C). The flow rate was 1 mL/min. The injection volume of extract was 5 µL. GABA was detected using excitation and emission wavelengths at 270 and 315 nm, respectively. The GABA content of sample was calculated by comparing the standard curve of GABA standard. The GABA content of each was determined in duplicate.

8. DPPH scavenging capacity determination

DPPH is a highly stable synthetic free radical. The solution of DPPH was freshly prepared using 50% ethanol. DPPH scavenging capacity was investigated using the method of Du et al. (2009). Briefly, the extract 1 mL was mixed with 1 mL of 200 µM DPPH solution. After incubation at room temperature for 30 min, the absorbance was measured at wavelength of 515 nm. Distilled water was used as blank and Trolox (0-50 µM) was used as standard. The experiment was done in duplication with triplicate measurements.

9. Consumer acceptability test

A consumer test consisting of 100 participants, untrained panelists, was administered in the sensory facilities. The prototype of rice drink made from GBGR was prepared and presented in random order with a three-digit random number coding on a plastic cup to minimize any bias. For sample preparation, roasted GBGR in a sachet was soaked in 150 mL of water at 98°C for 3 min before presenting to the panelists. The sample was placed inside plastic cup on a plastic tray along with a glass of water and a paper ballot sheet to evaluate five attributions: color, appearance, flavor, taste and overall. Each untrained panelist tasted and rated the sample based on the degree of liking on a 9-point hedonic scale (where 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like or dislike, 6 = liking slightly, 7 = like moderately, 8 = like very much and 9 = like extremely).

10. Shelf life study

Roasted GBGR is a dried product which was recommended to keep at room temperature. In this study, the rice drink from roasted GBGR in a sachet was packed in aluminum foil bag and stored at 35°C (average room temperature in Thailand) for 98 days. The product prototype was investigated its physical, chemical and microbial properties for every 7-day interval. Physical and chemical properties were analyzed as color and a_w, respectively. Microbial quality of product, total plate count, yeast and mold, and *Bacillus cereus* (*B. Cereus*) were tested following U.S. Food and Drug Administration, Bacteriological Analytical Manual (2001a; 2001b; 2001c).

11. Statistical analysis

Completely randomized design (CRD) was performed to study the effects of roasting and brewing process of rice drink in a sachet from roasted GBGR. The randomized completely block design (RCBD) was used to study the consumer acceptability test. The results were expressed as average ± standard deviation (S.D.). The difference between treatments was analyzed using analysis of variance (ANOVA) with Duncan's new multiple range. The difference was considered to be statistically significant at $p \leq 0.05$.

Results and discussion

1. Effect of roasting time on physical and chemical properties

Roasting process is an important step for improving

taste, color, texture, appearance and flavor of products, especially edible seeds including rice (Bagheri et al., 2019). In this study, GBGR was roasted at 100°C for 0, 5, 10 and 15 min. Color (L*, a*, b*, C* and h° values) and chemical compositions including a_w, moisture, ash, crude fiber, crude protein, crude fat and carbohydrate, total phenolics, total anthocyanins and GABA contents were investigated in the dif ferent 4 samples. The results showed that color of GBGR was significantly affected by roasting time as showed in Table 1 ($p \leq 0.05$).

Table 1 Color values of germinated black glutinous rice after roasting at different times

Color	Roasting time (min)			
	0	5	10	15
L*	$21.07^{a} \pm 0.39$	$15.49^{b} \pm 0.21$	$20.73^{a} \pm 0.75$	$21.11^{a} \pm 0.87$
a*	$2.77^{b} \pm 0.06$	$3.27^{a} \pm 0.34$	$1.63^{c} \pm 0.10$	$1.21^{c} \pm 0.05$
b*	$3.51^{b} \pm 0.15$	$4.30^{a} \pm 0.06$	$4.72^{a} \pm 0.52$	$4.92^{a} \pm 0.01$
C*	$4.60^{d} \pm 0.08$	$5.41^{c} \pm 0.17$	$7.00^{b} \pm 0.13$	$8.25^{a} \pm 0.13$
h°	$51.47^{d} \pm 1.92$	$67.93^{c} \pm 0.86$	$71.62^{b} \pm 0.69$	$78.61^{a} \pm 0.79$

Remark: The results were expressed as average ± standard deviation. The difference letters among different roasting times represented significant difference at $p \leq 0.05$.

The L*values of the 10 and 15 min-roasted samples were higher than those of the 5 min-roasted sample. However, the L* value of the 10 and 15 min-roasted samples were not significantly different from control (no roasting). The 5-min roasted sample possessed the highest positive value of a* which represented intense red color. The yellowness of samples was expressed as positive b* values and these values increased when the roasting time increased, similarly to C* and h° values of samples. The results interpreted that lighter and more yellowness induced by longer period of roasting, similar to the results of Bagheri et al. (2019) and Zeng et al. (2017). It might be due to thermal oxidation and degradation of polyphenols, especially anthocyanin. Furthermore, brown pigment from Maillard reaction might also be generated. In the other hand, the sample with 5 min-roasting time exposed darker and more redness. Possibly, the color of the sample was darker due to color of anthocyanin pigment in pericarp of GBGR. However, degradation of anthocyanin and generation of brown pigment from Maillard reaction might also slightly occur.

Table 2 showed changes of chemical compositions and DPPH scavenging capacity of GBGR after roasting. Roasting time significantly affected on a_w, moisture, crude protein, ash and carbohydrate contents in samples

Table 2 Chemical compositions of germinated black glutinous rice after roasting at different times

Chemical composition	Roasting time (min)			
	0	5	10	15
a_w	$0.66^{a} \pm 0.04$	$0.23^{b} \pm 0.02$	$0.14^{c} \pm 0.03$	$0.08^{d} \pm 0.00$
Moisture content (%)	$12.49^{a} \pm 0.18$	$4.59^{b} \pm 0.20$	$3.63^{c} \pm 0.04$	$2.06^{d} \pm 0.05$
Crude fat^{ns} (%)	3.31 ± 0.03	3.33 ± 0.26	3.66 ± 0.24	3.51 ± 0.12
Crude protein (%)	$17.84^{a} \pm 0.13$	$16.55^{b} \pm 0.09$	$16.54^{b} \pm 0.02$	$16.47^{b} \pm 0.05$
Ash (%)	$1.07b \pm 0.01$	$1.11b \pm 0.01$	$1.12b \pm 0.04$	$1.19^{a} \pm 0.01$
Crude fiber^{ns} (%)	2.34 ± 0.22	2.20 ± 0.10	2.40 ± 0.13	2.47 ± 0.02
Carbohydrate (%, by difference)	$65.29^{c} \pm 0.34$	$74.42^{b} \pm 0.28$	$75.05^{b} \pm 0.05$	$76.06^{a} \pm 0.11$
Total phenolics^{ns} (mg gallic acid equivalent/g dry weight)	1.48 ± 0.10	1.39 ± 0.04	1.48 ± 0.01	1.38 ± 0.04
Total anthocyanins^{ns} (mg cyanidin-3-glucoside equivalent/g dry weight)	0.04 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
GABA (mg/kg)	$99.01^{a} \pm 2.05$	$61.04^{b} \pm 2.87$	$60.76^{b} \pm 2.04$	$40.42^{c} \pm 0.28$
DPPH scavenging capacity^{ns} (mg Trolox equivalent/g dry weight)	124.65 ± 0.78	127.59 ± 1.63	126.31 ± 0.27	124.31 ± 1.32

Remark: The results were expressed as average ± standard deviation. The difference letters among roasting times represented significant difference at $p \leq 0.05$. ns: not significant at $p > 0.05$.

($p \leq 0.05$). As roasting time increased, a_w values and moisture content decreased. The reduction of aw value and moisture content in roasted GBGR was caused by evaporation. When heat is transferred to food with enough temperature and time, a difference of vapor pressures between surface and inside of food sample was induced, resulting evaporation of water (Yuenyongputtakal et al., 2017). Protein content in GBGR also declined when roasting time increased because protein denaturation and oxidation of some amino acids occurred during roasting (Yuenyongputtakal et al., 2017). Similar result was also found by Yenrina et al. (2019), who reported that roasting process (100 – 120°C for 30 min) diminished protein content in black glutinous rice. However, ash and carbohydrate contents enhanced. Increased ash with roasting time could be caused by decreasing in moisture and some antioxidants, as also found in Robusta coffee (Saloko et al., 2019). Similar results also found in cocoa (Djikeng et al., 2018) but that was not found in asparagus bean flour (Shah et al., 2015). Possibly, retained content of ash in food after processing might be associated with type and amount of mineral present in Oboh et al. (2010) and Nzewi & Egbuonu (2011). For carbohydrate content, the content was obtained by calculation and this value depended on fat, protein, moisture and ash contents in sample. In the other hand, fat and crude fiber contents did not depend on roasting time ($p > 0.05$). Roasting temperature and time used in this study was not significantly affected on TPC and TAC in roasted GBGR. The results were similarly to Ferreira et al. (2016), who

reported that the TPC of peanut with black testa were steady after roasting process using temperature of 175°C for 60 min. Yan et al. (2014) also found that some phenolics showed very minor decomposition at 200°C for 40 min. The degradation of anthocyanins in potato was induced by the rupture of glycosidic moiety and the formation of chalcones, when was subjected to temperature higher than 100°C (Nayak et al., 2011). However, temperature and time were not only caused by the change of anthocyanin molecules during processing, food matrix also associated. DPPH scavenging capacity of all samples was also not influenced by roasting time. GABA was also determined in the roasted GBGR and the representative chromatogram of GABA was showed in Fig. 2. As showed in Table 2, higher content of GABA remained in samples with lower duration time of roasting, similar to the results of Suppavorasatit et al. (2015) and Prommakool et al. (2014). They also found that reduction of GABA contents was induced by heat processes with long time duration. Reduction of GABA content in sample could be destroyed by heat in food processing (Tumpanuvatr et al., 2018). Therefore, the 5 min-roasted GBGR was chosen for further study due to highest GABA content and short processing time.

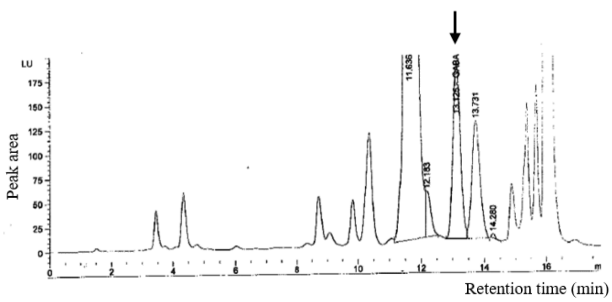

Fig. 2 Representative chromatogram of GABA in germinated black sticky rice. The GABA peak was marked with an arrow at retention time at 13.1 min.

2. Effect of brewing time on physical and chemical properties

For sample preparation in this study, GBGR was roasted at 100°C for 5 min. Ten grams of roasted sample was packed into a sachet (Fig. 1) before brewing at different times (3-15 min). During brewing, the temperature of water used was 98°C. After cooling, the color of the obtained rice drink was determined and reported as L*, a*, b*, C* and h° values. The results showed that brewing time significantly affected on L* and h° values ($p \leq 0.05$), whereas the a*, b* and C* values were not significantly different among samples ($p > 0.05$) (Fig. 3 and Table 3).

The GBGR drink possessed the highest value of L* when the sample was brewed at 7 and 10 min whereas, the GBGR drink with 15 min-brewing time showed darker color (the lowest L* value). The h° values were within the range of 270-360°, representing color shade of blue and red. This product color was possibly due to anthocyanin. Table 4 showed changes of chemical compositions of rice drink from GBGR at different brewing times (3-15 min). The pH values of rice drink were higher when brewing time increased ($p \leq 0.05$).

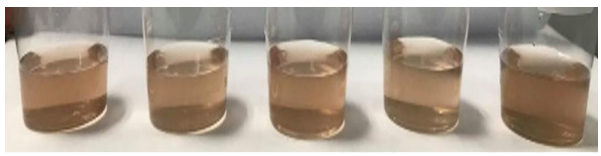

Fig. 3 Rice drink from germinated glutinous black rice after roasting at 100 ºC, 5 min and brewing at 3 min (a), 5 min (b), 7 min (c), 10 min (d) and 15 min (e)

Table 3 Color values of rice drink from germinated black glutinous rice (5 min roasting) at different brewing times

Color value	Brewing time (min)				
	3	5	7	10	15
L*	$42.17^{b} \pm 0.09$	$42.35^{b} \pm 0.50$	$44.79^{a} \pm 0.37$	$44.87^{a} \pm 0.49$	$40.29^{c} \pm 0.60$
a* ns	0.67 ± 0.02	0.83 ± 0.14	0.82 ± 0.16	0.87 ± 0.09	0.86 ± 0.01
b* ns	-1.45 ± 0.28	-1.33 ± 0.03	-1.36 ± 0.09	-1.33 ± 0.07	-2.22 ± 0.30
C* ns	1.77 ± 0.07	1.58 ± 0.04	1.58 ± 0.25	2.29 ± 0.30	1.34 ± 0.11
hº	$292.72^{bc} \pm 3.70$	$299.32^{b} \pm 3.70$	$306.07^{ab} \pm 1.21$	$314.41^{a} \pm 11.21$	$281.43^{c} \pm 2.21$

Remark: The results were expressed as average ± standard deviation. The difference letters among roasting times represented significant difference at $p \leq 0.05$. ns: not significant at $p > 0.05$

Table 4 Chemical compositions and antioxidant capacity of rice drink from germinated black glutinous rice (5 min-roasting time) at different brewing times

Chemical composition	Brewing time (min)				
	3	5	7	10	15
Total soluble solids (°Brix)	<1±0.00	<1±0.00	<1±0.45	<1±0.00	<1±0.00
pH	$6.28^{c} \pm 0.01$	$6.38^{b} \pm 0.05$	$6.29^{c} \pm 0.02$	$6.45^{b} \pm 0.01$	$6.55^{a} \pm 0.05$
Total phenolics (mg gallic acid equivalent/150 mL)	$4.36^{a} \pm 0.31$	$3.64^{bc} \pm 0.11$	$3.29^{c} \pm 0.19$	$3.51^{c} \pm 0.06$	$4.07^{ab} \pm 0.12$
Total anthocyanins (mg cyaniding-3-glucoside equivalent/150 mL)	$0.07^{a} \pm 0.01$	$0.07^{a} \pm 0.01$	$0.04^{c} \pm 0.00$	$0.04^{bc} \pm 0.01$	$0.05^{b} \pm 0.01$
DPPH scavenging capacity (mg Trolox equivalent/150 mL)	$803.16^{a} \pm 9.54$	$780.88^{a} \pm 12.29$	$708.95^{b} \pm 20.4$	$715.79^{b} \pm 37.4$	$662.63^{b} \pm 7.45$

Remark: The results were expressed as average ± standard deviation. The difference letters among roasting times represented significant difference at $p \leq 0.05$.

The value of pH was in range of 6.28-6.55. The color of anthocyanin is depended on pH. In acidic condition (pH 4-5), anthocyanins appear as red but turn blue when the pH increases (Laleh et al., 2006; Khoo et al., 2017). In acidic condition, flavylium cation (red color) is formed and stable at low pH. At increasing pH condition, anthocyanin is transformed as carbinol base and chalcone structures, followed by formation of anionic quinonoidal species (blue-purple color). Therefore, for rice drink from GBGR, the carbinol base and charcones might increase, while flavylium decreased, resulting in a slightly reddish brown color (as shown in Fig. 3). Brewing time with 3-15 min did not change total soluble solids ($p>0.05$). TPC and TAC also significantly affected by brewing time ($p\leq0.05$). The 3 and 5 min-brewing rice drink contained the highest amount of TAC (0.07 mg cyaniding-3-glucoside equivalent/150 mL). TAC in the product was in the range of 0.04-0.07 mg cyanidin-3-glucoside equivalent/150 mL (0.004-0.007 mg/g) which was similar to the amounts found in boiled black rice (*Oryza sativa* L. *Japonica*). Noorlaila et al. (2018) reported that boiled rice (*Oryza sativa* L. *Japonica*) contained total anthocyanins as 0.006 - 0.008 mg/g, whereas the anthocyanin content in raw rice was 0.02 mg/g. Cooked black rice incurred 60-70% loss of total anthocyanin. Difference contents of anthocyanin in food samples depended on type of anthocyanin, food condition, temperature, oxygen and analysis method. The GBGR drink with 3-min brewing time also contained the highest TPC (4.36 mg gallic acid equivalent/150 mL).The results of this study were similar to Braud et al. (2015) who found that short duration time (5-7 min) of brewing contained more TPC and TAC than longer time (15-30 min). On the other hand, Burilllo et al. (2018) reported that tea phenolics (gallic acid, epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate) increased as brewing time increased, resulting in enhancement of tea astringent. DPPH scavenging capacity was investigated in this study in order to determine antioxidant capacity of the rice drink.The results revealed that antioxidant capacity decreased as brewing time increased (Table 4). The highest values of DPPH scavenging capacity was showed in the sample with 3 and 5 min-brewing time. The antioxidant capacity of the rice drink might be due to its total phenolics and total anthocyanins contained in. Total phenolic content and total anthocyanins were lower since the phenolic compounds were obviously decomposed at high temperature and longer time, responsible to lessen DPPH scavenging capacity. As the results, GBGR drink which brewed with 150 mL of water with 98°C for 3 min was suggested in this study because this condition induced the highest total phenolics, total anthocyanins and strongest capacity of DPPH scavenging. GABA contents was determined in the GBGR drink with 3 min-brewing time and the drink contained 0.17±0.01 mg/150 mL.

3. Consumer acceptance

Sensory evaluation of the product prototype obtained from 100 questionnaire respondents and shown in Table 5. The prototype was the rice drink from GBGR with 5-min roasting time and brewed with 98°C water for 3 min. Most of respondents were female, accounting for 80%. Half of them (50%) were 18-30 years old and almost 60% were students. The sensory evaluation of liking score was based on 9-point hedonic scale. The respondents rated all attributes, including appearance, color, odor, flavor and overall preference, were almost 7 which indicated that the finished product was moderately liked. If the rice drink made from GBGR will be available in the market, 77% of respondents will buy.

Table 5 Mean liking scores of rice drink made from germinated black glutinous rice

Sensory attribute	Mean±S.D.
Appearance	6.95±0.64
Color	6.83±0.67
Odor	6.95±0.63
Flavor	6.89±0.47
Overall preference	6.84±0.65

4. Shelf life of product prototype

The product prototypes of roasted rice drink in a sachet made from GBGR were tightly packed in aluminum foil bag and stored at 35°C for 98 days. In every 7-day interval, the product prototypes were sampled in order to determine physical, chemical and microbiological properties. The results of physical properties of the rice drink were showed in Table 6 as color values in CIE systems. Significant change of L* value was not detected along with storage time of 98 days when stored at 35°C ($p>0.05$), whereas a*, b*, C* and h° values were significantly different ($p\leq0.05$). The positive values of a* (redness) and the negative values of b* (blueness) increased, indicating that the sample showed more redness and blueness. The highest values were shown at day 63 for a* value and in between day 63 and day 70 for b* value. The h° values were in range of 270°-360°. The results indicated that the drink samples expressed

dark color during storage time of 98 days at 35°C. Attribution of darker color of anthocyanin-rich samples during storage was caused by oxidation of double bond in anthocyanin molecule, depending on anthocyanin type, temperature and oxygen content (Stintzing et al., 2002).

Table 6 Color (L*, a*, b*, C* and h° values) of rice drink in a sachet made from germinated black glutinous rice stored at 35°C for 98 days

Day	Color value				
	L*^{ns}	a*	b*	C*	h°
0	39.15 ± 0.51	0.61^{bc} ± 0.07	-3.04^{fg} ± 0.17	3.07^{a} ± 0.17	281.26^{de} ± 1.38
7	39.36 ± 0.37	0.42^{c} ± 0.11	-2.82^{e} ± 0.07	3.15^{a} ± 0.13	278.47^{ef} ± 2.28
14	39.23 ± 0.31	0.65^{b} ± 0.17	-2.56^{bc} ± 0.14	3.15^{a} ± 0.13	284.44^{c} ± 4.40
21	39.44 ± 0.70	0.49^{de} ± 0.11	-2.90^{ef} ± 016	2.86^{bc} ± 0.06	279.58^{e} ± 2.25
28	38.81 ± 0.24	0.41^{e} ± 0.07	-2.76^{de} ± 0.10	2.65^{de} ± 0.10	278.56^{ef} ± 1.54
35	38.71 ± 0.37	0.30^{f} ± 0.15	-2.74^{de} ± 0.14	2.92^{b} ± 0.12	276.33^{f} ± 3.06
42	39.03 ± 0.26	0.53^{cde} ± 0.08	-2.65^{cd} ± 0.12	2.77^{cd} ± 0.07	292.40^{a} ± 1.08
49	38.38 ± 0.29	0.52^{cde} ± 0.18	-3.10^{g} ± 0.14	2.77^{cd} ± 0.14	279.64^{e} ± 3.47
56	38.33 ± 0.40	0.60^{bcd} ± 0.13	-2.65^{cd} ± 0.12	2.70^{de} ± 0.11	282.82^{cd} ± 2.58
63	40.21 ± 0.98	1.08^{a} ± 0.09	-2.12^{a} ± 0.38	2.72^{de} ± 0.12	276.52^{f} ± 3.79
70	39.42 ± 0.83	0.67^{b} ± 0.06	-2.09^{a} ± 0.17	2.53^{f} ± 0.20	285.09^{bc} ± 1.66
77	39.11 ± 0.36	0.69^{b} ± 0.08	-2.49^{bc} ± 0.12	2.58^{ef} ± 0.11	276.42^{f} ± 3.68
84	39.04 ± 0.45	0.58^{bcd} ± 0.08	-2.62^{cd} ± 0.17	2.62^{ef} ± 0.10	283.47^{cd} ± 2.37
91	38.38 ± 0.29	0.52^{cde} ± 0.18	-2.45^{b} ± 0.18	2.52^{f} ± 0.17	279.70^{e} ± 3.56
98	38.88 ± 0.54	0.67^{b} ± 0.55	-3.10^{g} ± 0.14	2.30^{g} ± 0.25	287.57^{b} ± 3.24

Remark: The results were expressed as average ± S.D. (n=2)
The small letters (a-g) indicted significantly difference among samples at p≤0.05
ns: no significant difference (p>0.05)

Chemical changes of rice drink were shown in Table 7 and the results showed that moisture content and a_w value were significant enhanced when storage time was extended (p≤0.05). The increasing of moisture content and a_w during storage can either depended on moisture permeability between sample and surrounding environment or absorption or chemical or biological reactions in materials (Razak et al., 2018; Östbring et al., 2020). However, the changes of water in roasted GBGR drink in a sachet still in an acceptable range (within 12% moisture content; $a_w < 0.6$) (Cervenka et al., 2006). GBGR drink in a sachet is a dried product which possessed a_w and moisture content in the range of 0.28-0.40 and 5.54-7.92%, respectively. For dried and starchy foods, yeast and mold can grow well at temperatures in the range 25-37ºC and *Bacillus sp.* can grow in the temperature range of 10-42ºC (Rachtanapun & Tangnonthaphat, 2011; Pexara & Govaris, 2010). Therefore, yeast and mold as well as *B. Cereus* can be used as indicator for food safety of GBGR drink in a sachet when stored at 35°C. The microbial results showed that total plate count, yeast and mold and *B. cereus* in the sample stored at 35°C for 98 days were in the range of $2.0x10^5$–$6.0x10^5$ CFU/g, less than 10 CFU/g and in range of $1.0x10^2$-$3.4x10^2$ CFU/g, respectively (Table 8) which were under the Thai agricultural standard TAS 4404-2012 (2012) Good manufacturing practices for germinated brown rice standard. Regarding the standard, total microorganisms, yeasts and molds as well as *B. cereas* should not exceed $1x10^6$, 500 and $1x10^3$ colony/g sample, respectively. Thus, it could be interpreted that roasted GBGR drink in a sachet which was packed in aluminum foil bag and stored at 35°C for 98 days was safe for consumers.

Table 7 Moisture content and aw value of rice drink in a sachet made from germinated black glutinous rice stored at 35°C for 98 days

Day	Moisture content (%)	a_w
0	5.54^{e} ± 0.75	0.28^{g} ± 0.10
7	6.18^{de} ± 0.06	0.31^{f} ± 0.01
14	6.23^{de} ± 0.31	0.32^{ef} ± 0.01
21	6.40^{cde} ± 0.29	0.34^{def} ± 0.00
28	6.45^{cde} ± 0.12	0.34^{def} ± 0.00
35	6.48^{cde} ± 0.17	0.34^{def} ± 0.01
42	6.52^{cde} ± 0.07	0.35^{cd} ± 0.01
49	6.53^{cde} ± 0.06	0.35^{cd} ± 0.03
56	6.82^{bcd} ± 0.70	0.37^{abc} ± 0.02
63	6.95^{bcd} ± 0.53	0.37^{abc} ± 0.02
70	7.01^{abcd} ± 0.57	0.37^{abc} ± 0.01
77	7.30^{abc} ± 0.02	0.37^{abc} ± 0.02
84	7.30^{abc} ± 0.48	0.38^{ab} ± 0.02
91	7.67^{ab} ± 0.35	0.40^{a} ± 0.04
98	7.92^{a} ± 0.73	0.40^{a} ± 0.02

Remark: The results were expressed as average ± S.D. (n=2)
The small letters (a-g) indicted significantly difference among samples at p≤0.05

Table 8 Total plate count, yeast and mold and Bacillus Cereus found in rice drink in a sachet made from germinated black glutinous rice during stored at 35°C for 98 days

Day	Microbiological test		
	Total plate count (CFU/g)	Yeast and mold (CFU/g)	*Bacillus cereus* (CFU/g)
0	$2.0x10^5$	<10	$2.0x10^2$
7	$2.6x10^5$	<10	$3.0x10^2$
14	$2.7x10^5$	<10	$1.0x10^2$
21	$2.5x10^5$	<10	$2.2x10^2$
28	$2.8x10^5$	<10	$1.8x10^2$
35	$3.8x10^5$	<10	$1.5x10^2$
42	$3.2x10^5$	<10	$1.8x10^2$
49	$4.0x10^5$	<10	$2.4x10^2$
56	$2.9x10^5$	<10	$1.8x10^2$
63	$3.2x10^5$	<10	$1.2x10^2$
70	$4.5x10^5$	<10	$2.2x10^2$
77	$3.7x10^5$	<10	$3.0x10^2$
84	$6.0x10^5$	<10	$1.7x10^2$
91	$3.0x10^5$	<10	$1.5x10^2$
98	$4.2x10^5$	<10	$3.4x10^2$

Remark: CFU: Colony forming unit

Conclusion

Roasting and brewing times significantly affected on color, chemical compositions and antioxidant capacity of rice drink made from GBGR. From this study, roasting time of 5 min and brewing time of 3 min were suggested in GBGR drink production since this condition induced higher levels of proteins, TPC, TAC, GABA and DPPH scavenging capacity as well as the product was also preffered by consumers. Physical, chemical and microbial changes of the product prototype during storage time of 98 days were accepted following the Thai agricultural standard TAS 4404-2012 Good manufacturing practices for germinated brown rice standard.

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Effect of Horse Mango (*Mangifera foetida*) Pulp on Quality and Consumer Acceptance of Sherbet Ice Cream

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Abstract

This objective of this research was to develop the local fruit of Thailand, horse mango (*Mangifera foetida*) into sherbet ice cream. Horse mango sherbet ice cream with 3 difference level of horse mango pulp (20, 40 and 60%) were study with physical quality, chemical quality microbiological quality and consumer acceptance. The results showed, yellowness color increasing related to quantity of horse mango pulp; so the 60% horse mango pulp content sherbet ice cream is the brightest yellow. Overrun value of 20, 40 and 60% horse mango pulp sherbet ice cream were 20.19 ± 0.91, 20.18 ± 0.32, 19.91 ± 0.37 respectively, it's statistically not significant ($p > 0.05$). The texture in each of the 3 levels of pulp was different, and increasing the horse mango pulp meant the firmness decreased. The melting rate was increased when increasing horse mango pulp. Energy, carbohydrate, protein, ash and retinol variation all increased when the level of of horse mango pulp increased. Microbiological quality followed Ministry of Public Health standards. Additionally, a higher concentration of fruit pulp increased the value of panelist's sensory score for color, odor, texture and overall acceptance.

Introduction

Horse mango (*Mangifera foetida*) is a native plant of south of Thailand and commonly found in tropical area as Malaysia, Indonesia, Myanmar and Singapore. Common name are malmut, limus, bachang and machang. This fruit in the family Anacardiaceae is in the same genus as mango so the shape similar to mango but mature have a strong exotic odor. Horse mango tree produce fruits during January to April while immature the skin is green color and change to yellow or golden yellow when mature. Horse mango is reported to typically contain that edible portion of 100 g flesh, it contains 78.5 g water, 0.8 g protein, 17.9 g carbohydrates, 16 mg calcium, 19 mg phosphorus, 0.09 mg thiamine, 255 µg carotenes, and 47.4 mg vitamin C. (Tyug et al., 2010). The raw fruit, because of acidic taste is used to make spicy salad, yellow curry or replaces as lime acid ingredient in shrimp paste dip, while ripened fruit was eaten flesh and less commonly to make dessert.

Ice cream is a product maintained at uniform consistency and prepared by pasteurization,

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homogenization, aeration and freezing. The main ingredients of ice cream are usually fat, nonfat dry milk, sweeteners, stabilizers, emulsifiers, water and flavoring agents (Badem & Alpkent, 2018) and it is a favorite dessert in Thailand, recognized worldwide and enjoyed by consumers of all ages. According to Food Intelligent Center, Thailand (2017) statistics; 13,850 million baht of ice cream were sold in 2017 and estimated ice cream market in Thailand will grow in the following years.

Nowadays healthy eating is a worldwide trend, the consumer looking forward to healthier material, one way is to add healthy ingredients with natural functions, such as fruit and vegetables.

Sherbet ice cream is one type of ice cream, main ingredients are water, sugar, fruit and/or fruit extracts, cream and a stabilizer. Sherbet ice cream has become a good choice of people who lookout for health, athletics, and weight control because this product contains both the nutritional value of fruit and the refreshing taste of frozen dessert.

For this reason, in this study, horse mango sherbet ice cream was produced and assess physical, chemical, biological and consumer acceptance to investigate the possibility of development about functional dessert from horse mango.

Materials and methods

1. Horse mango preparation

Horse mango was collected from Trang, Nakhon Sri Thammarat and Phatthalung provinces, ripening at room temperature. Healthy and undamaged horse mango were chosen for the preparation of the pulp. Ripe specimens were selected when they had 20% yellow peel, yellow flesh, correct odour and having a diameter of 8-10 centimeter as shown in Fig.1. They were cleaned with water to remove dirt and insects, peeled and washed again to eliminate the mango sap. They were then cut into small enough pieces for a blender to work, and blended for 2 minutes until smooth and finally filtered by 60 mesh sieves to become horse mango pulp as shown in Fig.2. Total soluble solid of horse mango pulp was used hand refractometer (Optika Model : HR-130, Italy), TSS value was 4°Brix. The pH value was 4, it was used a digital pH meter (Mettler toledo Model: Seven Compact pH meter, S210-Bio, Switzerland).

2. Preparation Horse mango to sherbet ice cream

Study maximized Horse mango pulp quantity to horse mango sherbet ice cream with 3 different level are 20% 40% and 60%, as shown in Table 1. The process and details of horse mango pulp sherbet was presented in Fig. 3.

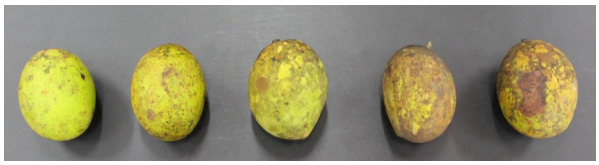

Fig. 1 Characteristic of horse mango from unripe to ripe

Fig. 2 preparation the horse mango to horse mango pulp

Table 1 Ingredient of sherbet ice cream from 3 formulas in different horse mango pulp

Ingredient	Quantity of horse mango pulp (%)		
	20	40	60
Horse mango pulp	20.0	40.0	60.0
Water	57.4	37.4	17.4
Sugar	15.7	15.7	15.7
Citric acid	0.1	0.1	0.1
Gelatin	0.1	0.1	0.1
Whip cream	6.7	6.7	6.7

Remark: Modify from Manfah & Nivet (2015)

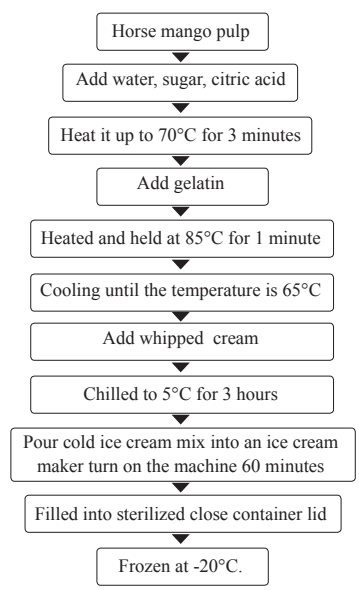

Fig. 3 The process of horse mango sherbet

3. Selection formula of horse mango sherbet ice cream

Horse mango sherbet ice cream 3 formulas was selected by sensory characteristic evaluation. The test used a 9 point hedonic scale (1 = disliked very much and 9 = liked very much) (Rawendra & Dwi, 2020). The sensory panel was composed of 50 untrained subjects whom were selected randomly from students, staffs and lecturers at Suan Dusit University, Trang Center. Horse mango sherbet ice cream was evaluated in the following aspects; color, odor, flavor, texture and overall acceptability. The judges evaluated the 3 formula of mango sherbet ice cream in the same session. The sample of horse mango sherbet ice cream were served at temperature -10°C in white cups coded with a 3 digit number. The panelists were evaluated individually in a random order, drinking to clean the mouth before and between evaluations of formula (Januario et al., 2018). All formula were statistically analyzed, and studied for their physical, chemical and microbiological characteristics. Then the formulas that received the highest scores were taken to a consumer acceptance test.

4. Quality of horse mango sherbet ice cream

4.1 Physical quality

4.1.1 Determined of color value using Hunter Lab Color Flex (Model A60-1012-312, Hunter Associates Laboratory Inc., USA). The measurements were show as L*(lightness), a*(redness) and b*(yellowness).

4.1.2 Determined of overrun of the ice cream were calculated using the equation by Muse & Hartel (2004)

$$\text{Overrun} = \frac{\text{Weight of mix} - \text{Weight of ice cream}}{\text{Weight of ice cream}} \times 100$$

The overrun was shown the increase volume caused the corporation of air into the ice cream mixture during ice cream making.

4.1.3 Texture analysis (firmness), the texture of ice cream was determined by texture analyzer (Model: TA-XTPlus). The firmness test using the 1 cm. diameter cylinder probe with target value 5.5 cm, speed test was 2 mm./s., load cell of 1 kN.

4.1.4 The melting test was carry out according to the procedure suggested by Garcia et al. (1995.) The samples were cooled to -20°C for 24 hours. 100 grams of each sample was placed individually on wire mesh attached to cylinder, with the cylinder, mesh, all equipment and the surrounding temperature all at 25°C. The ice cream that dripped through the cylinder was weighed.

4.2 Chemical quality

Proximate compound of mango sherbet ice cream including moisture, fat, protein, carbohydrate and ash were analyzed according to AOAC (2005) official method.

4.3 Microbiological Quality

Microbiological analysis of mango sherbet ice cream were total plate count and yeast and mold by FDA, BAM (Maturin & Peeler, 2001)

4.4 Consumer acceptance test

The horse mango sherbet ice cream which received high score from sensory test was tested for consumer acceptance test using the 5 points hedonic scale. The sensory test evaluated appearance, color, odor, flavor, texture and overall acceptability. The 100 untrained individual panelists were selected randomly at Huay Yod district, Trang province.

5. Statical analysis

The statical analysis was used SPSS software (SPSS Version 17; SPSS Inc., Chicago, USA). Analyzed data by ANOVA and using Duncan's New Multiple Range Test for identify difference of mango sherbet ice cream treatments at the 95% confidence level ($p<0.05$).

Results and discussion

1. Physical quality

1.1 Color

The color of horse mango sherbet ice cream with 3 different pulp at; 20%, 40%, and 60% showed that the sherbet ice cream are yellow because of the characteristics of the Horse mango pulp as shown in Fig.4. The color measurement data is present in Table 2 regarding color values, lightness (L*) of horse mango pulp 20% and 40% were significantly different at $p<0.05$ from 60%. The redness (a*) value of all 3 formulars decreased with a higher amount of mango horse pulp and were not significantly different at $p\geq0.05$. While yellowness (b*) value of all formulars increase and were significantly different at $p<0.05$. This result was similar to the result of Rattanathanalerk et al. (2005) study effect of thermal processing on the quality loss of pineapple juice, the resulted showed that pineapple juice changed color because of non-enzymatic browning and pigment destruction more than enzymatic browning since enzymes are damaged at 50°C. Hyoung & Gary (2003) studied effect of thermal pasteurization on Valencia orange juice color and pigments reported that total carotenoid pigment content loss was significant ($p<0.05$) after thermal

pasteurization at 90°C for 30 s. Color change after orange juice pasteurization, led to juice color becoming lighter. According to Petruzzi et al. (2017) suggested that heating processing led to a degradation of color.

Table 2 Color quality of difference level of horse mango pulp sherbet ice cream

Color	Horse mango sherbet ice cream		
	Horse mango pulp 20% (w/w)	Horse mango pulp 40% (w/w)	Horse mango pulp 60% (w/w)
L*	71.06 ± 1.69^{a}	71.04 ± 1.01^{a}	67.40 ± 0.60^{b}
a*^{ns}	-1.14 ± 0.26	-1.16 ± 0.38	-1.19 ± 0.28
b*	19.87 ± 1.45^{c}	27.51 ± 1.24^{b}	36.99 ± 1.03^{a}

Remark: a, b, c superscripts with different letters in the same row are significantly different ($p < 0.05$) and ns is not significantly different ($p \geq 0.05$), all value are mean ± S.D. for three samples.
L* : Brightness a* Redness b* Yellowness

Fig. 4 Characteristic of horse mango sherbet ice cream 20% 40% and 60% respectively

1.2 Overrun value, melting rate and texture

The calculated overrun was shown in Table 3. All of horse mango sherbet ice creams had overruns between 19.91-20.19%, it's shown that increase of horse mango pulp no effect to overrun so all formular of difference level of horse mango pulp in sherbet ice cream were not significantly different ($p>0.05$). In part of texture measurement, while increasing proportion of horse mango pulp the texture is softer as shown in Table 3. The force used to deform of ice cream with horse mango pulp 20% 40% 60% are 231.33 ± 6.77, 141.88 ± 15.47 and 71.55 ± 6.57 g force, respectively. All 3 levels of sherbet ice cream texture were significant ($p<0.05$).

Table 3 Overrun and texture quality of horse mango sherbet ice cream

Quality	Horse mango sherbet ice cream		
	Horse mango pulp 20%	Horse mango pulp 40%	Horse mango pulp 60%
Overrun^{ns}	20.19 ± 0.91	20.18 ± 0.32	19.91 ± 0.37
Texture: firmness (g Force)	231.33 ± 6.77^{a}	141.88 ± 15.47^{b}	71.55 ± 6.57^{c}

Remark: a, b, c superscripts with different letters in the same row are different ($p < 0.05$), all value are mean ± S.D. for three samples.
ns shown that there is no statistically significant differences ($p \geq 0.05$)

The melting rate, Melting rate is one of the most important characteristics of ice cream, this parameter was shown to be the amount of time it took ice cream to become a liquid of smooth consistency. In this case, the melting test was carry out at room temperature of 25 ± 2°C with the the humidity at 60%. The melting rate showed in Fig. 5. The result showed that first 10 minutes the ice cream of all 3 formulas no turned to liquid. They started to soft at 15-20 minutes, turned to liquid 10% after 25 minutes. and continue dissolve until complete dissolve at 60 minutes. And the horse mango sherbet ice cream with 60% pulp turned into liquid faster than other formula.

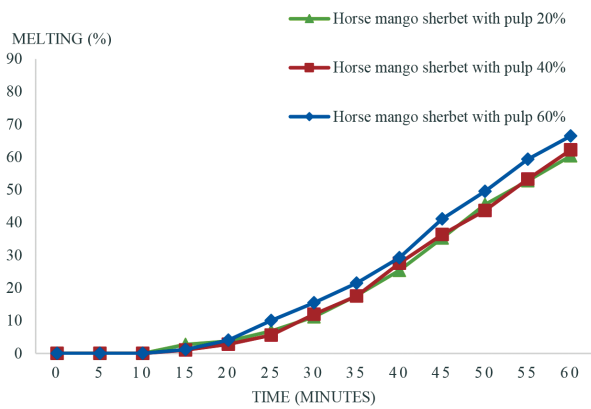

Fig. 5 Melting rate of the horse mango sherbet ice cream

This results were in agreement with Sofjan & Hartel (2004) who reported that the ice cream that has a low overrun value were melt faster, while ice cream with high overrun have a better resistance to melting properties. This is because the heat transfer rate is reduced with greater air volume. This is because higher viscose ingredient will limit the mobility of water molecules due to the space between the particles in the mixture is getting narrower. The space between the narrow particles will cause the air entering the mixture during the agitation process to be less, resulting in lower overrun value.

2. Chemical quality

In this study, the chemical analysis carried out was energy, carbohydrate, fat, protein, ash, retinal and water content are presented in table 4. All 3 levels of sherbet ice cream in part of energy, carbohydrate, retinol and water content were significant ($p<0.05$). Fat, protein and ash found that the horse mango pulp 40% and 60% were not significantly different at $p \geq 0.05$ but were significant ($p<0.05$) with horse mango pulp 20%. It can

be seen that increase of horse mango pulp was found to cause an increase in all chemical composition.

Table 4 Chemical composition of horse mango sherbet ice cream

Component	Horse mango sherbet ice cream (100 g)		
	horse mango pulp 20%	horse mango pulp 40%	horse mango pulp 60%
Energy (kcal)	109.02 ± 0.70^{c}	123.90 ± 0.16^{b}	128.04 ± 0.51^{a}
Carbohydrate (g)	19.90 ± 0.06^{c}	23.00 ± 0.12^{b}	23.80 ± 0.17^{a}
Fat (g)	3.10 ± 0.10^{b}	3.30 ± 0.06^{a}	3.40 ± 0.10^{a}
Protein (g)	0.38 ± 0.02^{b}	0.55 ± 0.03^{a}	0.56 ± 0.02^{a}
Ash (g)	0.16 ± 0.01^{b}	0.32 ± 0.02^{a}	0.52 ± 0.02^{a}
Retinol (mg)	0.02 ± 0.01^{b}	0.04 ± 0.01^{b}	0.06 ± 0.03^{a}
Water content (%)	76.50 ± 0.06^{a}	72.90 ± 0.01^{b}	71.70 ± 0.06^{c}

Remark: a, b, c superscripts with different letters in the same row are different ($p < 0.05$), all value are mean ± S.D. for three samples.

3. Microbial content

The microbial content of horse mango sherbet ice cream was show in Table 5. The result show that horse mango sherbet of all 3 formulars are in accordance with the Notification of the Ministry of Public Health No.354 B.E.2556 (Thailand Public Health, 2013) issue ice cream which determined total plate count does not exceed $6x10^5$ colony, not found yeast and mold per 1 cm^3 and the number of *E.coli* should be zero per 100 g of ice cream. The result of microbiological value was not exceeding the standard due to the process was sterilize and freeze.

Table 5 Microbial content of horse mango sherbet ice cream

Microbiological	Horse mango sherbet ice cream		
	horse mango pulp 20%	horse mango pulp 40%	horse mango pulp 60%
Total plate count (CFU/ml)	< 10	< 10	< 10
Yeast and mold (CFU/ml)	Not detected	Not detected	Not detected
Escherichia coli (100 ml)	Not detected	Not detected	Not detected

4. Sensory test

Sensory assessment of ice cream consists of sense of color, odor, horse mango flavor, texture and overall acceptance. The 50 panelists present the score show as Table 6, the horse mango sherbet ice cream with 60% pulp received highest score all attributes of ice cream except horse mango flavor got lowest score. Hence increasing the horse mango pulp effected to liking score of horse mango sherbet ice cream but decrease in flavor. Panelist's opinion that horse mango sherbet flavor is too strong at a high concentration, so the panelists prefer horse mango ice cream with a lower concentration.

Table 6 Sensory test of horse mango sherbet ice cream in difference level of mango pulp

Sensory attribute	Horse mango sherbet ice cream		
	horse mango pulp 20%	horse mango pulp 40%	horse mango pulp 60%
Color	6.32 ± 1.49^{b}	7.50 ± 1.11^{a}	7.88 ± 1.04^{a}
Odor	6.62 ± 1.18^{b}	7.18 ± 1.37^{a}	7.50 ± 1.23^{a}
Horse mango flavor	7.42 ± 1.34^{a}	7.14 ± 1.28^{ab}	6.86 ± 1.64^{b}
Texture	6.56 ± 1.54^{b}	7.36 ± 1.32^{a}	7.64 ± 1.24^{a}
Overall liking	6.76 ± 1.36^{b}	7.48 ± 1.07^{a}	7.50 ± 0.91^{a}

Remark: a, b superscripts with different letters in the same row are different ($p < 0.05$), all value are mean ± S.D. for three samples.

5. Consumer acceptance test

The horse mango sherbet ice cream for 60% pulp was sensory test for 100 untrained individuals (62 men and 38 women). Most of panelists were aged under 20 years old, most of them are students (59%), most of them had education levels below bachelor degree (60%) and average income per month less than or equal to 10,000 Baht (65%). Consumer behavior showed 80% of consumer decision the ice cream with flavor first, frequency of eating ice cream 1-2 times/week (59%) and buy ice cream product at convenient store (46%).

The consumer acceptance test on 4 aspects are color, odor, texture and overall acceptance as shown in Fig.6. The result showed the color scoring was 4.18, which was high; odor scoring was 4.00 which was high; textural scoring was 4.05, which was high and overall acceptance was 4.00, which was high. The consumer decision buys this ice cream if release to market (76%).

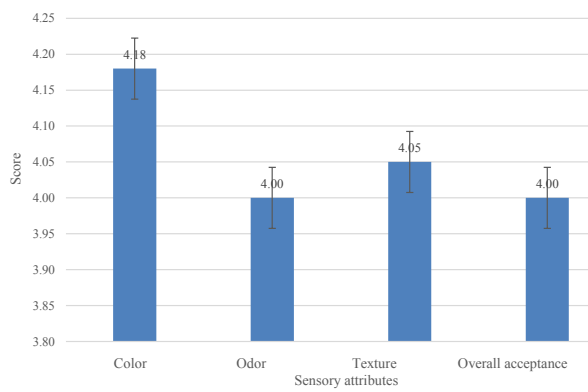

Fig. 6 Acceptance score of horse mango sherbet ice cream

Conclusion

This study showed that increasing horse mango pulp related to increase of yellowness, not difference for

overrun, decrease for hardness and high melting rate. Chemical quality, increase horse mango pulp show all composition (energy, carbohydrate, protein, ash and retinol) were increasing. Microbiological quality follow to notification of the ministry of public health, Thailand and the addition of fruit pulp with higher concentration increase the value of panelist's sensory score for color, odor, texture and overall acceptance. So, the 60% horse mango pulp suitable to be sherbet ice cream.

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Sun Protection of Rice, Gac Fruit and Wood Apple Powders for Developing Face Powder

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Abstract

Face powder, in different colors, is used to beautify the skin of the face and neck and make it appear more attractive than natural skin. General characteristics are that it should be fine, soft, easily applied to the skin, long-lasting, disperse well when moist and able to hide wrinkles or dark spots; further, in harmony with the skin color, it should reduce oiliness, have a pleasant smell or color, look natural and not harm the user. We studied the effect of gac fruit and wood apple on color and SPF in face powder containing rice. We examine the natural contents of rice, gac fruit and wood apple in face powder according to the percentage of each recipe. However, the natural extracts were able to absorb UV radiation and used in mixtures with synthetic extracts for sunscreen protection, thus reducing the use of synthetic products. Further, no heavy metals were found in the natural extracts.

Introduction

Thai people focus more than before on beauty and skin care, especially faces that clearly reflect the charm of women. Face powder is a popular cosmetic, for daily use, to beautify the face and help to conceal spots and smooth the skin. Skin powders come in many forms, including face powder, body powder, talcum powder for children, lotion, eyeshadow and powder used by teenagers to sparkle their skin. Face powder normally uses talcum, as the main ingredient, but talcum contains substances with asbestos-like properties, that cannot be digested and excreted from the body. Talcum aerosols contain tiny particles that may be inhaled and accumulate in the lungs. The cells in the lungs catch these particles, which adversely affect the respiratory system, especially those of infants (Chucham, 2012). In addition, studies have shown that using talcum powder in women's intimate areas increases the risk of ovarian cancer, from talcum penetrating into the vagina (Balsam & Sagarin, 1972; Kabthong et al., 2015). In some countries, many doctors recommend avoiding talcum powder, because of its unsafe properties. Other countries are following, even though the price of alternatives is higher and the quality is lower than talcum powder. As consumers pay more attention to health and beauty, rice became a choice for making cosmetic products. However, face powder made from rice powder is still not popular. For example, Chucham (2012) developed a face powder made from Thai jasmine rice. It was found that the particles of the jasmine rice powder were uneven and its texture was harsher than talcum. However, the powder offered a

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unique jasmine rice aroma, and the jasmine rice powder absorbed water better than talcum. The jasmine rice powder offered the best quality when it replaced 17% of talcum in the pressed face powder. It had a light yellow texture, was smooth and easily attached to the skin. The powder compressed well into the container and was not easily broken. Kabthong et al. (2015) studied the development of color from dragon fruit in combination with rice powder to achieve an optimum powder formula.

Health care, based on natural extracts, is gaining in popularity among people all over the world. This encourages Thais to increasingly use products made from herbs or natural extracts. In addition, natural extracts have developed a quality and performance comparable to those of synthetic substances. Therefore, health care can now avoid side effects and toxicity from some chemicals. Also, using natural substances is increasingly popular in cosmetic and beauty products (Chucham, 2012). Most cosmetic products are colored - either by natural or synthetic substances. It is thus important to consider the color in cosmetics: it must be safe and intrinsically non-toxic or in reaction with other substances mixed with it. The color should resist changes in light, heat or pH. Most natural colors were found to be non-toxic and inexpensive (Kabthong et al., 2015): they can be obtained from local plants, *e.g.* pandan, butterfly pea and gac fruit.

Gac Fruit (*Momordica cochinchinensis* Spreng) is a native plant in Vietnam, China, Myanmar, Thailand, Lao PDR, Bangladesh, Malaysia and the Philippines, and is commonly called spring bitter cucumber. The gac fruit contains 10 times more carotene than carrots and 12 times more lycopene than tomatoes. Lycopene is a group of carotenoids, found in some vegetables and fruits, which acts as a pigment to collect light for plants and prevent oxidation, generating free radicals from excess sun light. Therefore, it reduces oxidation and cancer. It also helps alleviate cell deterioration by protecting skin from sunlight, when mixed into cosmetics (Chuyen et al., 2015).

Wood apple (*Limonia Acidissima* L.) has several medicinal as well as cosmetic properties, for example, the fruit is used as a substitute for bael (*Aegle marmelos* (L.) Corrêa) in diarrhea and dysentery (Vishakha et al., 2019; Pratima & Rekha, 2014). Thus, today, herbs play vital role in every industry due to their wide variety of properties. Here, we show the importance of wood apple in cosmetics and illustrate its pharmacological activities and medicinal uses. Different parts of wood apple show different useful properties, *e.g.* essential oil obtained from the leaves has anti-bacterial activity, due to its carvacrol and cyclodecandine constituents; shells show anti-fungal activity against gram positive and gram negative bacteria, because of psoralene found in them; the pulp benefits the skin, because of its higher moisture content. The other key constituents are saponins, flavonoids, amino acids, beta carotene, tannins, carbohydrates, vitamin B and triterpene. These constituents are responsible for some cosmetic properties, so that wood apple has cosmetic applications (Vishakha et al., 2019).

Sunscreen cosmetics are very popular for protecting the skin from the sun. Sunscreen either reflects or absorbs UV. The active substances consist of aromatic rings, bound to carbonyl groups in the chromophore, which can absorb UV and then release energy in the form of heat. Chemicals commonly used in sunscreens, include benzoate, 2-phenyl-benzimidazole-5-sulfonic acid (PBSA), methyl anthranilate, homosalate, octocrylene (2-ethylhexyl-2-cyano-3,3-diphenyl-2-propenoate), octyl salicylate and octylmethoxycinnamate (Gasparro et al., 1998). Currently, several plant extracts are used as sunscreens, *e.g.* extracts (with sun protection factor (SPF)) from cucumber (3.19), tomato, (14.7), papaya (16.0), carrot (1.34), aloe vera (1.28) and coconut (7.38) (Kumar et al., 2016; Madhu et al., 2018; Shenekar et al., 2014; Malsawmtluangi et al., 2013). In addition, *Feronia limonia* (L.) Swingle, also commonly called wood apple or Thanaka in Thailand, similar to *Licodia acidissima* Limonia acidissima L. in the same family Rutaceae, shows various skin benefits, *e.g.* it is anti-inflammatory, does not thin facial skin, brightens it and reduces blemishes and dark spots, tightening pores, smooths facial skin and contains antioxidants as well as components which naturally block sunlight and contribute to SPF (Namchot et al., 2012; Tidchai, 2019).

Accordingly, we focused on using broken Pathum Thani and Riceberry rices, as the main material to develop face powder, replacing talcum. Other natural substances, gac fruit and wood apple, were used as coloring agents. We also assessed the SPF as an alternative for consumers choosing natural face powder instead of talcum. Our results may help increase the value of broken Pathum Thani and Riceberry rices, as well as the local plant varieties, by encouraging the use of face powder, made from natural sources, for future commercial potential.

Materials and methods

1. Sample preparation and face powder formulae

Pathum Thani and Riceberry rices were obtained from community enterprise of Thai Suan Pepper subdistrict, Pathum Thani province. Both rices were ground, until they passed a 200 mesh filter by peeling off the rice shells. Gac fruit provided from the plantation garden in Prasat district in Surin province, was made from the flesh covering its seeds. Wood apple powder was derived from wood apple tree branches that were collected from In Buri district in Sing Buri province. Face powder formulaes were prepared using the mixtures listed in Table 1, ground, mixed together and packed in containers (Senajuk et al., 2020). Powder formulaes were coded as Fgf_xwa_y where x denotes the fraction of gac fruit and y the fraction of wood apple, so that $Fgf_{18}wa_5$, had 18% gac fruit and 5% wood apple.

Table 1 Face powder formulae

Component (%w/w)	Fgf_0wa_{10}	Fgf_2wa_{10}	Fgf_5wa_{10}	$Fgf_{10}wa_{10}$	$Fgf_{10}wa_5$	$Fgf_{18}wa_5$	$Fgf_{25}wa_5$	$Fgf_{31}wa_4$
Pathum Thani rice	40	40	40	40	40	32	29	27
Riceberry rice	10	10	10	10	10	9	8	8
Wood apple	10	10	10	10	5	5	5	4
Gac fruit	-	2	5	10	10	18	25	31
Zinc oxide	5	5	5	5	5	5	4	4
Zinc stearate	10	8	5	5	5	4	4	4
Magnesium carbonate	10	10	10	5	5	5	4	4
Calcium carbonate	10	10	10	10	15	19	17	16
Kaolin	5	5	5	5	5	5	5	4
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

Remark: Samples were coded as described in the text.

2. Color measurement

Color was measured with a ColorFlex EZ Spectrophotometer, model D65. Colors perceived by human eyes derive from the light reflected from the object and delivered to the brain for translation into the color perception. The Commission Internationale de I'Eclairage (CIE) defined perception color standards described in the L*a*b* system which describes points in a 3D space. L* indicates how light (or dark) the sample is; higher values define lighter samples. The a* indicates a point on the red (+a*) – green (-a*) axis and b* indicates a position on the blue (-b*) – yellow (+b*) axis (Mir et al., 2013). A color difference between a reference and a sample, ΔE, can be derived from:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

where ΔL = L* - L, Δa = a* - a , and Δb = b* - b and L* , a* , b* refer to colors of the reference object and L, a , b refer to colors of the sample.

3. Sun protection factor measurement

Measurement of sun protection factors followed Dutra et al. (2004), with slight modification. A 100 g sample was weighed and transferred to a 100 ml volumetric flask, diluted to volume with ethanol then shaken for 5 minutes, then filtered through a Whatman No.1 filter paper. The filtrate was collected after rejecting the first 10 ml of filtrate. Then a 5.0 ml aliquot was transferred to 50 ml volumetric flask and diluted to volume with ethanol. Subsequently a 5.0 ml aliquot was transferred to a 25 ml volumetric flask and the volume completed with ethanol. The filtered solution was put in a quartz cell and the absorbance was measured from 290 to 320 nm at 5 nm steps by a UV-spectrophotometer. The measurement was repeated three times. The SPF was calculated following Mansur et al. (1986).

$$SPF = CF \times \sum_{290}^{320} EE\,(\lambda) x\, I(\lambda) x\, Abs(\lambda)$$

where *EE(λ)* is an erythemal effect spectrum, I*(λ)* mean solar intensity spectrum, *Abs(λ)* means absorbance of sunscreen products and *CF* means correction factor (=10).

4. Morphology and elemental composition

Sample morphologies were observed by scanning electron microscopy (SEM) using a model LEO 1455 VP, Germany, coupled with energy-dispersive X-ray (EDX Oxford, ISIS 300, England) analyzer that measured the elemental composition.

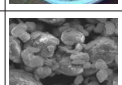
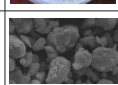
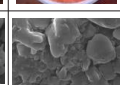
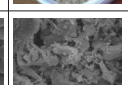
Results and discussion

1. Raw materials qualifications

In previous work, it was found that Riceberry extract samples contained phenolic and flavonoids with the highest level at 26.5 mg GAE/g of extract and 102 mg Rutin/g of extract. Free radical scavenging activity of the Riceberry extract sample indicated the EC_{50} at 108 µg/mL (Senajuk et al., 2020). Table 2 shows the SPF values, color, heavy metals, and morphology of the materials in making up the face powders. SPF was calculated by applying Mansur's equation (2). SPF

numbers of raw materials ranged from 1.12 (Pathum Thani rice) to 6.78 (wood apple). Almost all other samples are having the same or almost similar SPF values as calculated from Mansur equation. All the color values are noted that the whitest of all raw materials was measured to be Pathum Thani rice with L* 81.03, followed by gac fruit with wood apple the darkest, at L* ~56. The highest a* value was shown by gac fruit, the reddest at 42.77. Gac fruit was also the most yellow, with b*= 20.78. Checking for heavy metal contamination indicated that no raw materials contained heavy metals. The physical characteristics of the raw materials were similar. They were fine powders with different colors. The examination using SEM micrographs showed that physical characteristics were similar in clusters of spherical particles.

Table 2 SPF, Color, element content and morphology of raw materials

Properties	Riceberry rice	Pathum Thani rice	Gac fruit	Wood apple
SPF values	1.83±0.08	1.12±0.16	1.58±0.15	6.78±0.10
Color				
L*	63.92±0.01	81.03±0.07	56.70±0.02	56.73±0.26
a*	4.31±0.01	1.02±0.01	42.77±0.02	4.71±0.00
b*	12.67±0.02	16.20±0.04	40.94±0.05	20.78±0.02
%Element value				
Lead	nd	nd	nd	nd
Arsenic	nd	nd	nd	nd
Mercury	nd	nd	nd	nd
Barium	nd	nd	nd	nd
Image				
Morphology	 magnification x 10,000	 magnification x 10,000	 magnification x 10,000	 magnification x 10,000

Remark: nd means not detected

2. Influence of raw materials on SPF

Fig. 1 shows the effects of raw materials on SPF for the different face powder formulae. Fgf_0wa_{10}, with no added gac fruit had SPF = 0.69, but the SPF increase to 1.20 as gac fruit up to 10% was added. Reduction of wood apple content, $Fgf_{10}wa_{10}$ to $Fgf_{10}wa_5$, decreased SPF by about 10%. At lower wood apple content (5%), varying gac fruit led to little change of SPF values, close to 1. Commercial loose powder has SPF ~40, significantly higher than with natural ingredients. These commercial products contain synthetic chemicals, that provide high levels of sun protection. Our research suggests that natural substances can also provide sun protection and we are working in the lab on further development, which will require further advanced tools. Jarupinthusophon & Anurukvorakun (2021) developed compact powder by using Jasmine rice flour replaced 100% talcum, compared with commercial product and found that the developed compact powder does not provide much different sun protection effectiveness compared the commercial compact powder. However, increasing gac fruit content affected the color, as shown in Table 3. It is therefore concluded that the amount of wood apple affected SPF.

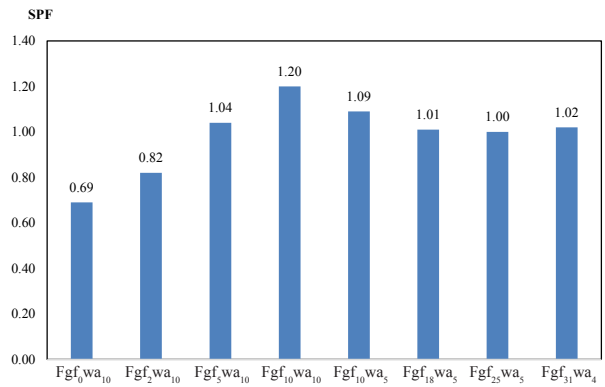


Fig. 1 Effect of raw materials on SPF

3. Influence of raw materials on Color

Colors were measured in the CIE L*a* b* space-see Table 3. The 'whitest' of all samples, Fgf_2wa_{10}, had L* = 79.63, followed by Fgf_0wa_{10}. The 'darkest' was $Fgf_{10}wa_{10}$ with L* = 76.00. As might be expected, the highest a* (i.e. reddest) was $Fgf_{31}wa_4$ with 31% gac fruit, a* = 5.12, but this value was still small, and the powder appeared 'white'. Yellowness, b*, varied very little in all the rice powder samples: 10% gac fruit and 10% wood apple, $Fgf_{10}wa_{10}$, showed the highest b* = 13.41. In contrast, the commercial face powder No.1 showed much higher b* = 22.90, i.e. it was significantly more 'yellow' than the rice derived powders.

Table 3 Color characteristics (CIF space) of face powder formulae

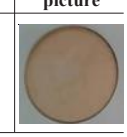
Formula	L*	a*	b*	ΔE	Physical picture
Commercial face powder No.1	73.23±0.00	10.92±0.00	22.90±0.00	-	

Table 3 (Continude)

Formula	L*	a*	b*	ΔE	Physical picture
Fgf_0wa_{10}	79.40±0.01	0.98±0.00	10.97±0.01	16.71±0.00	
Fgf_2wa_{10}	79.63±0.00	1.16±0.00	11.06±0.00	16.63±0.00	
Fgf_5wa_{10}	77.53±0.14	1.72±0.02	12.22±0.01	14.74±0.04	
$Fgf_{10}wa_{10}$	76.00±0.16	2.33±0.06	13.41±0.06	13.10±0.12	
$Fgf_{10}wa_5$	77.43±0.01	2.54±0.01	12.42±0.01	14.06±0.01	
$Fgf_{18}wa_5$	79.10±0.01	2.70±0.01	11.55±0.02	15.19±0.01	
$Fgf_{25}wa_5$	78.15±0.03	3.94±0.01	11.97±0.01	13.87±0.01	
$Fgf_{31}wa_4$	77.79±0.04	5.12±0.03	12.39±0.03	12.84±0.02	

Conclusion

We developed sunscreen face powder, using natural sources, with Riceberry and Pathum Thani rice replacing talcum and as gac fruit and wood apple, which are safe for skin use, providing color. They are suitable for use as ingredients in face powder, that offer a unique product, that strengthens local identify and builds pride, while generating income and creating careers for people in the community. The SPF values showed that natural sources can protect skin from sun damage and alleviate cell aging and reduce the use of synthetic sunscreens. Up to 10% of wood apple and gac fruit increased SPF, while excessive amounts of both, on the other hand, did not increase SPF, but increased color intensity. All natural samples had some UV protection capabilities. Along with their several other beneficial effects and safety, these natural products could become a good, inexpensive and readily available ingredients in sunscreen products.

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Effects of Dual Tasks Training on Balance Performance and Cognitive Functions in Older Adults with Mild Cognitive Impairments: A Randomized Controlled Study

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Abstract

Mild cognitive impairment (MCI) is a condition of older adult who are at the transitional state between the cognitive changes of normal aging and very early dementia. Numerous studies have established that mild exercise or cognitive training has positive effects on physical and cognitive functions in healthy older adults. Newly, the possibility of combined of physical exercise and cognitive training get noticed in the older adults with MCI. The objective of this study is to investigate the effect of mild exercise with cognitive training in older adults with MCI on measures of balance performance and cognitive functions. Ninety older adults with MCI were randomized into three groups. Mild exercise (ME) group (n = 30) underwent chair-based exercise, cognitive training (CT) group (n = 30) received Stroop color and word task training, while the mild exercise with cognitive training (ME+CT) group (n = 30) received both of them in the same time. In terms of demographic characteristics, there was no significant differences among three groups in baseline data. All groups underwent the training for 45 minutes per sessions, 12 training sessions within 30 days. Participants were evaluated for balance performance (via Mini-Balance Evaluation Systems Test (Mini-BESTest)) and cognitive functions (via Mini-Mental State Examination (MMSE)) prior to the training and then we immediately re-assessed after training. Significant main effects of group in Mini-BESTest ($p=0.003$) and MMSE ($p = 0.037$), and main effect of time in Mini-BESTest ($p = 0.033$) and MMSE ($p = 0.012$) (ME+CT > CT > ME group) were found. The ME+CT group also demonstrated improved balance performance and cognitive functions compared to the ME or CT group (post-training > baseline). A combination of mild exercise and cognitive training can improve balance ability and cognitive functions in older adults with MCI as well as mild exercise or cognitive training. It might be beneficial effect for delaying the declining functional capacity in older adults with MCI.

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Introduction

Population ageing is a global phenomenon. The number of older people is increasing. In 2019, the United Nations reported that the number of older people worldwide stood at 1 billion worldwide. Over the next three decades, the number of older people is projected to increase to more than 1.5 billion persons in 2050. The older adults have been defined by World Health Organization (WHO) as the person who has the chronological age of 65 years old or older (World Health Organization, 1999; Orimo et al., 2006).

Thailand is the second highest age population in the Southeast Asia region. It is estimated that the number of older people will increase by 12 million to 17 million by 2030. There is numerous evidences that has reported that the older people with increasing age have declining balance ability, functional capacity, and cognitive functions (Jaul & Barron, 2017; Maneeprom, et al., 2018; Thaweewannakij et al., 2013; Tiraphat & Aekplakorn, 2018). In addition, the older adults with declining cognitive functions were at increased risk of dementia (Sacuiu et al., 2018). The physical and cognitive decline could be occurred in the older adults, and may present as decreased muscle strength, the impaired balance control, and slower walking speed (Deandrea et al., 2010; Laughton et al., 2003).

Mild cognitive impairment (MCI) is a condition of older adult who are at the transitional state between the cognitive changes of normal aging and very early dementia (Petersen & Negash, 2008). MCI is characterized by: preserved general cognitive function, objective memory impairment following increasing age, lack of dementia, and little or no impairment of activities of daily living (ADL) (Petersen, 2004; Dubois & Albert, 2004; Portet et al., 2006). A previous review international study found that the prevalence of MCI varies widely from around 3% to 42% (Ward et al., 2012). Previous studies of the prevalence of MCI in Thailand reported MCI ranged from 16.7 %to 71.4%. These evidences were different depending on the methodology of study that included age range of older adults, education level, outcome measures and the areas of study (Griffith et al., 2020; Deetong-on et al. 2013; Kengsakul et al., 2015; Sangsirilak, 2016).

Consequences of older adults with MCI demonstrated that they have lower quality of life that the elderly individuals that are healthy (Hussenoeder et al., 2020). Specifically, the MCI has significant impact on postural balance in older adults. A recent previous study found that MCI was associated with balance deficits which related impaired central processing of visual information that is critical for balance control (Bahureksa et al., 2017; Liu et al., 2020). Moreover, balance performance was associated with cognitive function in individuals with MCI. Tangen and co-workers investigated the relationships between balance and cognition in individuals with MCI. They found that cognitive function as the executive function was associated with balance performance in individuals with MCI as shown in Balance Evaluation Systems test (BESTest) (Tangen et al., 2014).

Mild exercise or low-intensity exercise is useful to enhance health benefits for older adults and better exercise adherence that related to the moderate-and high-intensity exercise (Sanders et al., 2020; Tse et al., 2015; Brown et al., 2000). The low-intensity exercise might offer both physical and cognitive improvements in older adults. The common types of mild exercise include chair-based exercise, Tai Chi, walking, or stretching exercises. (Tse et al., 2015). There were previous studies demonstrated that the effects of low-intensity exercise or mild exercise would be effective in improving physical and cognitive function in healthy older adults (Tse et al., 2015; Butcher et al., 2008). One previous study in the United States of America investigated the effect of mild exercise in older adults with MCI.The previous study showed that the older adults with MCI had improvement in executive function as represented by Mini-Mental State Examination (MMSE) scores and increased performance in Stroop test (Baker et al., 2010). Moreover, the chair-based exercise is one of the types of the mild exercise which promotes functional mobility and balance performance in the older adults. The chair-based exercise is performed primarily in the seated position and contain the components of strengthening exercise, cardiovascular fitness training or endurance training. The previous studies found that the effects of chair-based exercise might improve lower limb strength, balance performance and activities of daily living in the older adults as represented in the timed up and go test and the Barthel indexoutcomes (Cancela Carral et al., 2017, Robinson et al., 2018). Therefore, the mild exercise or the low-intensity exercise in the older adults with MCI is needed.

Cognitive training is a non-pharmacological intervention for delaying progression of MCI-to-Alzheimer's disease (Hakun et al., 2015; Lampit et al.,

2014). Previous studies demonstrated that elderly who received the cognitive training as Stroop task training improve the cognitive function. Stroop task is a model for studying cognitive performance including the executive function, selective attention to specific information during decision-making tasks and choosing appropriate responses (Stroop, 1935). The mechanism of neural plasticity that involved Stroop task is the "uses it and improve it" theory (Kleim & Jones, 2008). Moreover, the cognitive training may promote neurogenesis, ability of brain structure and/or function change, or plasticity in individuals with elderly (Aldwin & Gilmer, 2004). Also, previous study demonstrated that cognitive training as Stroop task training lead to a significant reduction in reaction time, error number and increase in data processing speed test in elderly women with MCI. It may reflect the plasticity of old brain making it challenging to learn the new tasks (Fatemeh et al., 2016).

Up-to-date, dual tasks training appear to provide more consistent cognitive or motor function benefits in older adults. There are different types of dual tasks training, such as the combination of cognitive training and motor training, which appear to produce an improvement in memory, balance and mobility in older people (Norouzi et al., 2019; Brustio et al., 2018). Moreover, dual tasks training as combination of mild exercise and cognitive training demonstrated the potential to increase balance ability (Pichierri et al., 2011) and/or improve cognitive functions (Law et al., 2014; Lauenroth et al., 2016) in older adults with MCI. However, some studies investigated effect of dual tasks training on balance performance or cognitive function alone (Pichierri et al., 2011; Law et al., 2014; Lauenroth et al., 2016). Nonetheless, no studies that investigated the effect of dual tasks training on both outcomes that consisted of balance performance and cognitive functions in older adults with MCI (Pichierri et al., 2011; Law et al., 2014; Lauenroth et al., 2016).

As was mentioned, the older adults with MCI had the balance deficits and the balance problem related with the cognitive impairment above. The dual tasks training such as the combination mild exercise and cognitive training might produce the improvement of balance performance and the cognitive functions enhancement. Moreover, it could help to prevent the older adults who had suffering falls due to the cognitive impairment. Therefore, the purpose of this study was to investigate the effects of dual tasks training as combination of mild exercise and cognitive training (ME+CT) on balance performance and cognition functions in older adults with MCI. We hypothesized dual-task training (ME+CT) would improve the balance performance and the cognition function in older adults with MCI, compared with the mild exercise (ME) group or the cognitive training (CT) group.

Materials and methods

1. Participants

Participants were recruited by Watsanawet Social Welfare Development Center Elderly, Phra Nakhon Sri Ayutthaya Province, and local communities in Mueang District, Ubon Ratchathani Province and Dan Khun Thot District, Nakhon Ratchasima Province. They were screened according to the inclusion and exclusion criteria.

The inclusion criteria were: i) Female and male elderly aged 65-89 years with mild cognitive impairment (MMSE scores; uneducated < 14 scores, educated with primary school ≤ 17 scores, educated higher level of primary school ≤ 22 scores) (Institute of Geriatric Medicine, 1999), ii) able to read, iii) able to stand without devices, iv) able to walk with or without devices, v) independent activity daily living, and vi) normal vision and hearing or corrected by medical procedures.

The exclusion criteria were: i) neurological conditions (i.e. cardiovascular disease, Parkinson's disease or psychiatric disease affecting balance performance and communication), ii) cardiovascular disease with no medical treatment (i.e. heart disease, congestive heart failure), iii) high blood pressure > 160/100 mmHg or having the symptoms which were dizziness, faint, nausea, high pulse rate, a lot of sweat at the day of data collecting, and iv) severe pain from musculoskeletal problem (i.e. severe osteoarthritis of knee).

2. Study design and procedure

The present study was approved by the Human Research Protection Committee at Rangsit University, Thailand (number RSEC 48/2560) and registered at the Thai Clinical Trials Registry (TCTR20180605002).

The study design was the randomized controlled trial (RCT) with single blinded by assessors. Participants were randomly allocated into 3 groups by simple random sampling; mild exercise (ME) group, cognitive training

(CT) group, or mild exercise with cognitive training group (ME+CT). Random assignment was performed by an unbiased observer not related with the present study by the traditional random picking sealed names of participants and intervention groups from separate containers, to blindly allocate each participant to one of the three groups.

After the participants signed the consent forms, they completed demographic and clinical information including; age, gender, status, education level, occupational, underlying disease. Then, they were asked to evaluate pre-training assessments. Each participant was asked to complete the training program for 45 minutes per session, 12 training sessions within 30 days of the pre-training assessment. The participants performed the post-training assessments once again after the training. The CONSORT flow diagram for the present study is illustrated in Fig. 1.

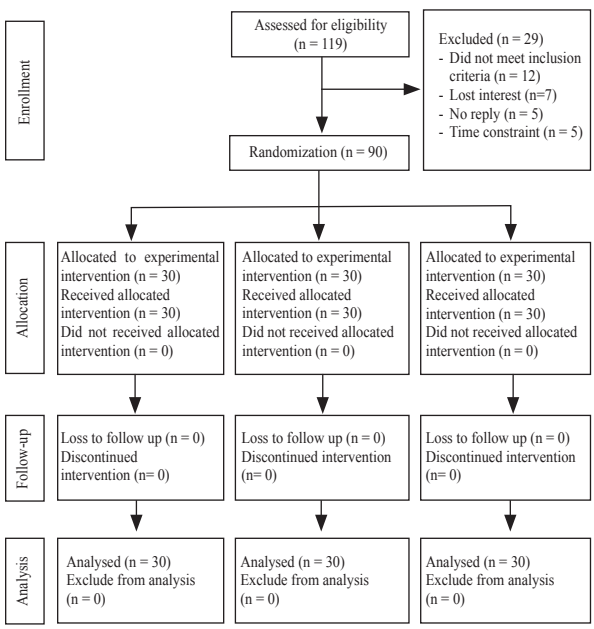

Fig. 1 CONSORT (Consolidated Standards of Reporting Trials) diagram showing the flow of participants through each stage of study

3. Intervention

The three interventions with a maximum of 30 participants per group had a frequency of three sessions per week and a duration of 4 weeks. Each of the 12 sessions lasted 45 minutes (Cancela Carral et al., 2017). Training amount was comparable between the three groups. All interventions were described as follows.

3.1 Mild exercise (ME group)

Participants of the ME group received chair-based exercise which was low-intensity or mild aerobic exercise (intensity = 20-39% of maximal heart rate). The ME consisted of three phases; warm-up 5 minutes, exercise 35 minutes with the rest time as the participant needed, cool down 5 minutes. The warm-up and cool down phases included breathing exercise and passive stretching exercise of upper limbs and lower limbs. The participants were asked to perform exercise in sitting position on the chair with backrest and both feet placed on the floor. The details of exercise phase included chair-based exercise by doing active exercise and progression in each week which were described in Table 1. The exercises were adapted and progressed (the number or sets, the number of repetitions) by the physical therapist to meet the needs of each individual participant to account for the differences between participants (Cancela Carral et al., 2017; Robinson et al., 2018). In the present study, the exercise prescription and the progression of each exercise pose; 10-30 repetitions/set, 3-5 sets per session, 3 days per week, 4 weeks. The progression of exercise is the increased in the number of repetitions per set and the sets per session.

3.2 Cognitive training (CT group)

Participants of the CT group received the Stroop color and word training which based on the Stroop test. The Stroop color and word training in the present study consisted of three tasks as follows; task 1: verbally reading a list of color words (blue, red, green) printed in black ink, task 2: verbally stating the color of a series of Xs printed in blue, red or green ink, and task 3: verbally reading a list of color words that were printed in colored ink (i.e. RED printed in blue ink or RED printed in red ink). The participants underwent the repetitive practice and progressed the training by performing the tasks as quickly as possible that the participants read the corrected color words within shorten time. Each Stroop color and word training task was 15-minutes per task and the rest time between task session for 2-3 minutes.

3.3 Combined mild exercise and cognitive training (ME+CT group)

Participants of the ME+CT group underwent the combined mild exercise and cognitive training at the same time.

All three groups were supervised by three physical therapists who had clinical experience of physical therapy at least two years. They underwent one week of pre-study training to standardize the implementation of the exercise programs. The participants underwent the individually intervention and

Table 1 Mild exercise and progression based on chair-based exercise

Exercise pose	Week 1	Week 2	Week 3	Week 4
1	Unilateral shoulder flexion	Bilateral shoulder flexion	Alternating shoulder flexion between left side and right side	Combine unilateral full shoulder flexion and elbow extension
2	Unilateral shoulder abduction	Bilateral shoulder abduction	Bilateral shoulder abduction in oblique axis	Combine bilateral full shoulder flexion and elbow extension
3	Unilateral elbow flexion-extension	Bilateral elbow flexion-extension	Alternating elbow flex-ion-extension between left side and right side	Combine unilateral shoulder flexion 90 degree and elbow flexion
4	Unilateral wrist flexion-extension	Bilateral wrist flexion-extension	Alternating wrist flexion-extension between left side and right side	Combine bilateral shoulder flexion 90 degree and elbow flexion
5	Unilateral finger flexion-extension (opened-closed hand)	Bilateral finger flexion-extension (opened-closed hand)	Alternating finger flexion-extension (opened-closed hand) between left side and right side	Combine unilateral shoulder flexion 90 degree, elbow extension and wrist extension
6	Unilateral hip flexion (leg elevation with flexed knee)	Bilateral hip flexion (leg elevation with flexed knee)	Alternating hip flexion between left side and right side	Combine bilateral shoulder flexion 90 degree, elbow extension and wrist extension
7	Unilateral hip abduction (separate one leg, opening and closing in each leg)	Bilateral hip abduction (separate knees, opening and closing legs)	Alternating hip adduction in oblique axis	Combine unilateral knee extension with ankle dorsiflexion
8	Unilateral knee extension	Bilateral knee extension	Alternating knee extension between left and right legs	Combine bilateral knee extension with ankle dorsiflexion
9	Unilateral ankle dorsiflexion and plantar flexion	Bilateral ankle dorsiflexion and plantar flexion	Alternating ankle dorsiflexion and plantar flexion between left and right feet	Alternating combined knee extension with ankle dorsiflexion

Remark: Exercise prescription and progression: each exercise pose 10-30 repetitions/set, 3-5 sets per session, 3 days per week, 4 weeks. The progression of exercise is the increased in the numbers of repetition per set and the sets per session.

the progression of training was individually based. The interventions were delivered in three different inclosed sites in three separate local communities in Lak Hok, Mueang Pathum Thani, Pathum Thani province to minimize or prevent experimental contamination effects.

3.4 Outcome measurements

Outcome of measurements in the present study consisted of Mini-Mental State Examination (MMSE) and Mini-Balance Evaluation Systems Test (Mini-BESTest).

The MMSE Thai-2002 version was developed from the MMSE that developed by Marshal F. Folstein and colleague in 1975. It was used to measure thinking ability or cognitive impairment. The MMSE is a 30-point test that measures 5 domains which include: the time and place orientation, short-term memory, attention and solving problems, language, and comprehension and motor skills (Folstein et al., 1975). Three different levels of cognitive functions were classified as followed: severe (score 0-10); moderate (11-20); mild (21-25) (Pernecaky et al., 2006). The Thai version of MMSE was used to measure the cognitive functions in the present study. The content validity, reliability, and specificity of Thai-MMSE were high (Institute of Geriatric Medicine, 1999).

The Thai version of Mini-BESTest examination was used to measure the balance performance in the present study which was developed and translated from original version by Rattanavichit and co-workers in 2020 (Rattanavichit et al., 2020; Franchignoni, et al., 2010). It consisted of four domains; i) anticipatory postural adjustments, ii) reactive Postural Responses, iii) sensory orientation, iv) dynamic balance during gait and cognitive effects. The Mini-BESTest has scored out of 28 points to include 14 items that are scored from 0 to 2 (0: not able to perform, 2: able to perform well). The five different levels of balance performance were determined as followed: very severe deficit (score 0-5); severe deficit (6-11); moderately severe deficit (12-17); moderate deficit (18-23); mild deficit to normal (24-28) (Franchignoni et al., 2015). The cut-off score of the Mini-BESTest was 16 (out of 28) (Yingyongyudha, et al., 2016). Also, the Thai version of Mini-BESTest showed excellent intra-rater reliability (ICC = 0.97-0.98) and inter-rater reliability (ICC = 0.941) (Rattanavichit et al., 2020).

The assessors in the present study were physical therapy interns who underwent measurement training from the primary investigator for 60 hours. The inter-rater reliability in all outcome measures of them was high to excellent (intraclass correlation coefficient (ICC) range=0.88- 0.99).

4. Data analysis

Sample size calculation by G*power version 3.1.9.2 was based on a power of 0.80, alpha level of 0.05, and effect size was 0.50. The sample size was calculated based on previously reported the total of 90 person and 30 persons per group (Lipardo & Tsang, 2020).

$$\frac{n}{g} = \frac{\left\{Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}\right\}^2 \sigma^2}{\delta^2}$$

$$\frac{n}{g} = \frac{\{1.96 + 0.84\}^2 0.61^2}{0.53^2}$$

Statistical analysis was performed using IBM SPSS Statistics 23 for Windows. Data were tested for normal distribution with Kolmogorov–Smirnov tests and homogeneity of variances for between-group comparisons with Levene's tests. For all statistical comparisons, the significance level was set at $\alpha = 0.05$. The groups were analyzed for differences in the baseline demographic and clinical information variables using ANOVAs. The use of multivariate repeated-measures analysis of variance (ANOVA) was initially planned to analyze the group, time, and group–time interaction effects. Post hoc analysis to determine differences in pair- wise group comparisons was performed using Fisher's least significant difference (LSD) test. To compare within-group changes across time for data with normal distribution, the repeated-measures ANOVA with between-group analysis was utilized. For non-normal data, Friedman's ANOVA was applied. Post hoc analysis for time effects was conducted using Fisher's least significant difference (LSD). An intention-to-treat analysis, with the last observation carried forward, might be used for missing data due to dropouts. However, the present study was no drop-out rate, the intention-to-treat was not be analyzed. The *p*-value of <0.05 was considered significant for all computations. Utilizing a derived effect size (Cohen's $d = .39$) (Lipardo & Tsang, 2020)

Results and discussion

One-hundred and nineteen individuals with older adults were screened for enrollment in the present study. Ninety older adults with MCI met the inclusion criteria, and were randomly allocated to the ME, CT and ME+CT groups. No participants reported any adverse side effects after the training, and all 90 participants (100% of compliance) in three groups completed the study and no drop-out rate. The demographic data, clinical characteristics and baseline data for all participants are presented in Table 2. The demographic variables based on gender, age, status, education level and undelying disease were stratified among the ME, CT and ME+CT groups. Thirty participants were allocated in each group. The average age was 69.26 (4.68), 73.43 (7.02), and 70.46 (4.17) years for the ME, CT and ME+CT group respectively. Most of them were female (70% in ME group, 53.33% in CT group and 76.66% in ME+CT group), marriage status (96.67% in ME group, 96.67% in CT group and 93.33% in ME+CT group), graduation from a primary school (73.34% in ME group, 83.33% in CT group and 66.67% in ME+CT group), and having the hypertension as the underlying disease in ME group (33.33%) and CT group (43.34%), but no having underlying disease in ME+CT group (50%). There were no significant differences between three groups in baseline data of the balance performance as represented by Mini-BESTest scores and the cognitive functions as represented by MMSE scores (Table 1, all $p > 0.05$).

Table 2 Demographic data, clinical characteristics and baseline data (n= 90)

Variables	All (n = 90)	ME group (n = 30)	CT group (n = 30)	ME+CT group (n = 30)	*p*-value
Age (years), mean (SD)	71.05 (5.29)	69.26 (4.68)	73.43 (7.02)	70.46 (4.17)	0.090
Gender (female), n (%)	60 (66.67%)	21 (70.00%)	16 (53.33%)	23 (76.66%)	-
Status, n (%)					
Single	4 (4.44%)/	1 (3.33%)	1 (3.33%)	2 (6.67%)	-
Marriage	86 (95.56%)	29 (96.67%)	29 (96.67%)	28 (93.33%)	
Education level, n (%)					
Lower or primary 6	67 (74.45%)	22 (73.34%)	25 (83.33%)	20 (66.67%)	
Secondary 3	19 (21.11%)	6 (20.00%)	5 (16.67%)	8 (26.66%)	
Secondary 6	2 (2.22%)	-	-	2 (6.67%)	-
Diploma or high vocational certificate	1 (1.11%)	1 (3.33%)	-	-	
Bachelor Degrees	1 (1.11%)	1 (3.33%)	-	-	
Underlying diseases, n (%)					
Hypertension	27 (30.00%)	10 (33.33%)	13 (43.34%)	4 (13.33%)	
Diabetes mellitus	5 (5.56%)	2 (6.67%)	1 (3.33%)	2 (6.67%)	
Dyslipidemia	1 (1.11%)	-	1 (3.33%)	-	-
> 2 underlying diseases	31(34.44%)	11 (36.67%)	11 (36.67%)	9 (30.00%)	
No Underlying disease	26 (28.89%)	7 (23.33%)	4 (13.33%)	15 (50.00%)	
Baseline Mini-BEST scores, mean (SD)	19.36 (3.62)	18.13 (3.24)	18.17 (2.26)	19.70 (1.54)	0.215
Baseline MMSE scores, mean (SD)	18.62 (2.92)	17.63 (2.37)	17.60 (1.96)	18.20 (1.65)	0.541

Remark: There was no significant difference in baseline characteristics of participants among three groups; the ME, CT and ME+CT groups.

Abbreviations: Mini-BESTest: Mini-Balance Evaluation Systems Test; MMSE: Mini-Mental State Examination; ME group: mild exercise; CT group: cognitive training group; ME+CT group: mild exercise with cognitive training group.

There were significant main effects of group in Mini-BESTest scores (p = 0.003) and MMSE scores (p = 0.037) (ME+CT > CT >ME group, Table 3b, Table 3b. represented in p-value[a]). There were also significant main effects of time in Mini-BESTest scores (p = 0.033) and MMSE scores (p = 0.012) (all groups increasing post-training, Table 3b, Table 3b. represented in p-value[b]). The mean of Mini-BESTest scores and MMSE scores were significantly increased from the baseline. The mean of Mini-BESTest scores, changed from baseline 18.13 (3.24) to 19.07 (4.11) in ME group, from 18.17 (2.26) to 19.53 (3.15) in CT group, and from 19.70 (1.54) to 21.53 (2.86) in ME+CT group. All mean of Mini-BESTest scores at post-training indicated the moderate level of balance performance. Furthermore, the mean of MMSE scores, by using Thai-2002 version MMSE questionnaire, changed from baseline 17.63 (2.37) to 18.63 (3.11) in ME group, from 17.60 (1.96) to 19.07 (2.97) in CT group, and from 18.20 (1.65) to 20.57 (2.65) in ME+CT group. All mean of MMSE scores after training indicated the moderate level of cognitive function. However, there were no significant interaction effect of time by group in in Mini-BESTest and MMSE scores (Table 3b represented in p-value[c]).

Table 3 Comparison of balance performance and cognitive functions at baseline and post-training in all randomization groups

(a) Comparison of balance performance (representing Mini-BESTest scores) and cognitive functions (representing MMSE scores) at baseline and post-training in all randomization groups

Outcome measures	ME group (n = 30)		CT group (n = 30)		ME+CT group (n = 30)	
	Pre	Post	Pre	Post	Pre	Post
Mini-BESTest scores, mean (SD)	18.13 (3.24)	19.07 (4.11)	18.17 (2.26)	19.53 (3.15)	19.70 (1.54)	21.53 (2.86)
MMSE scores, mean (SD)	17.63 (2.37)	18.63 (3.11)	17.60 (1.96)	19.07 (2.94)	18.20 (1.65)	20.57 (2.65)

In addition, post-hoc analysis of Mini-BESTest scores and MMSE scores revealed significant differences between CT and ME+CT group, and between ME and ME+CT group (Table 4a). The post-hoc analysis of Mini-BESTest scores and MMSE scores revealed significant differences between baseline and post-training (post-training > baseline, Table 4b)

Table 4 Significant values of multiple comparisons of Mini-BESTest and MMSE scores by post-hoc analysis

(a) between groups by pairwise comparisons (ME vs CT, CT vs ME+CT and ME vs ME+CT)

Pairwise	Mini-BESTest scores	MMSE scores
ME vs CT	0.696	0.697
CT vs ME+CT	0.006*	0.042*
ME vs ME+CT	0.002*	0.016*

(b) between timepoints by pairwise comparisons (baseline vs post-training)

Pairwise	Mini-BESTest scores	MMSE scores
baseline vs post-training	0.012*	0.033*

Remark: Computed by LSD multiple comparisons; *p-value <0.05; **p-value <0.01

Abbreviations: Mini-BESTest: Mini-Balance Evaluation Systems Test; MMSE: Mini-Mental State Examination; ME group: mild exercise; CT group: cognitive training group; ME+CT group: mild exercise with cognitive training group

We investigated the effect of dual tasks training (ME+CT) on balance performance and cognitive functions. To the best of our knowledge, this is the first study to compare the effects of training on both balance performance and cognitive functions as represented by Mini-BESTest scores and MMSE scores among three groups (ME+CT vs. ME vs. CT groups). Overall, all three groups improved in balance performance and cognitive functions after training. Moreover, there were significant differences in balance performance and cognitive functions between ME+CT group vs. CT group, and ME+CT group vs. ME group. Consequently, the dual

(b) Significance values based on Analysis of Variance for balance performance (representing Mini-BESTest scores) and cognitive functions (representing MMSE scores)

Outcome measures	p-value[a]	95%CI[a]	Effect size[d]	p-value[b]	95%CI[b]	Effect size[d]	p-value[c]	95%CI[c]	Effect size[d]
Mini-BESTest scores	0.003**	-4.25 to-0.68	0.066	0.033*	-3.62 to-0.50	0.026	0.647	-1.62 to-3.42	0.005
MMSE scores	0.037*	-3.37 to -0.50	0.037	0.012*	-4.25 to -0.68	0.036	0.067	-0.66 to-3.39	0.031

Remark: [a] p-value : between-groups main effect from two-way mixed ANOVA; [b] p -value : main effect of time from two-way mixed ANOVA; [c] p -value : interaction time by group from two-way mixed ANOVA; * p<0.05, ** p<0.01, 95% CI: 95% confidence interval; [d] Partial χ^2

Abbreviations: Mini-BESTest: Mini-Balance Evaluation Systems Test; MMSE: Mini-Mental State Examination; ME group: mild exercise; CT group: cognitive training group; ME+CT group: mild exercise with cognitive training group

tasks training as combination of mild exercise with cognitive training induced the improvements in balance performance and cognitive functions in the elderly with MCI.

1. Effects of mild exercise

We found that there were significant differences in Mini-BESTest scores between pre- and post-training in ME group.Chair-based exercise may enhance the lower limb strength that is the important component which is the biomechanical constraint in balance control process in the older adults (Cruz et al., 2010). The findings of the present study corresponding with the previous study that determine the effects of low-intensity group exercise on muscle strength and balance of elderly people in the communities (Chompoopan et al.,2019). After participating in the low-intensity exercise, the experimental group had significantly improved in their dynamic balance as represented by performance time in timed up and go test when compared to the control group (Chompoopan et al., 2019). However, we measured the balance performance by using the Mini-BESTest which consisted of static and dynamic balance. The Mini-BESTest include the tasks that divided into four subcomponents: anticipatory postural adjustments, postural responses, sensory orientation, and dynamic gait (Franchignoni et al., 2010). Therefore, the Mini-BESTest scores representing balance performance in the present study involved both static and dynamic postural balance in elderly with MCI.

Furthermore, we found that there were significant differences in MMSE scores between pre- and post-training in ME group. The results of the present study are consistent with the previous study of Baker and colleagues in 2010. The previous study conducted in the elderly with MCI who had similar cut-off of MMSE scores to the present study. They found that there were improvements in cognitive functions as measured by MMSE and Stroop test in the elderly with MCI (Baker et al., 2010). Moreover, the upregulation level of BDNF after mild exercise was found in the previous study (Baker et al., 2010). One possible mechanism could describe that the exercise might be mediated by exercise-induced upregulation level of BDNF (Rasmussen et al., 2009; Whiteman et al., 2014; Leckie et al., 2014). In addition, the mild exercise which were described by the animal studies, activated hippocampal neurons through the glutamatergic pathway and contributed to exercise-induced hippocampal neurogenesis through the androgen receptor (Okamoto et al., 2012; Burns et al., 2010). Thereby, the mild exercise may be relevant for the prevention and slowing down of neurodegenerative diseases, such as Alzheimer's disease (Burns et al., 2010).

2. Effects of cognitive training

Also, we found that older adults with MCI in CT group performed increase in Mini-BESTest and MMSE scores after cognitive training as Stroop task training. Stroop tasks involve cognitive performance, especially, the executive function and decision-making tasks (Stroop, 1935). The principle of brain plasticity that support the Stroop task is the "uses it and improve it". The training could induce the specific function of the brain and lead to an enhancement of that specific function (Kleim & Jones, 2008). In addition, the cognitive training may enhance the neural plasticity changes in elderly by promoting the neurogenesis process (Aldwin & Gilmer, 2004). The findings of the present study are corresponding with previous study of Fatemeh and co-workers in 2016. It demonstrated that a significant reduction in reaction time, error number and increase in data processing speed test in elderly women with MCI after Stroop task training (Fatemeh et al., 2016). Even though, the previous study showed only the improvement in term of cognition outcome. Thereby, the cognitive training is one of brain exercise for benefit of slowing down in MCI progression (Hakun et al., 2015).

3. Effects of the dual tasks training (combination of mild exercise with cognitive training: ME+CT)

In addition, we discovered that the effects of dual tasks training (ME+CT) could improve cognitive functions and balance performance in the elderly with MCI. The results of the present study are consistency with three previous studies. They demonstrated that the improvements in balance performance (Pichierri et al., 2011) or improving cognitive functions (Law et al., 2014; Lauenroth et al., 2016) after dual tasks training as combination of mild exercise and cognitive training were found in the older adults with MCI. Although, the previous studies measured the effects of dual tasks training on balance performance or cognitive function alone, the present study we investigated the effects of dual tasks training on both balance performance and cognitive functions. Therefore, the dual tasks training could reflect the outcome measure of balance ability and cognitive functions in the older adults with MCI. The dual tasks training as cognitive-motor task training was more beneficial than single-task training alone in improving executive function domain of cognitive functions of elderly persons, and the improvement was

not directly due to modulating Aβ metabolism (Yokoyama et al., 2015). It is well known that Aβ peptides are metabolized by the insulin-degrading enzyme that also metabolize insulin (Malito et al., 2008) and that hyperinsulinemia due to peripheral insulin resistance and conditions associated with impaired glucose metabolism, such as obesity, or type 2 diabetes, are linked to cognitive dysfunction and Alzheimer's disease (Smith et al., 2010). Therefore, the dual tasks training as combination of mild exercise and cognitive training can improve the balance ability and cognitive functions in the older adults with MCI.

As mentioned above, the effect of the mild exercise alone, the cognitive training alone, and the combined mild exercise and cognitive training could improve balance performance and cognitive functions. However, the minimally clinically importance differences (MCID) value of MMSE scores > 2 points that indicated the significant improvement in general cognitive outcomes (Huntley et al., 2015). In the present study, the MCID in MMSE scores in the ME+CT group was 2.37 points, while the MCID in MMSE scores in two other groups were less than 2 points. Moreover, the MCID value of Mini-BESTest scores > 3.5 points in patients with balance disorders (Godi et al., 2013). The MCID values Mini-BESTest scores in ME, CT and ME+CT groups were 0.94, 1.36, and 1.83, respectively. The reason that why the findings demonstrated that the MCID values Mini-BESTest scores less than 3.5 points is the Mini-BESTest is the testing which the participants performed static and dynamic balance in standing position. Nonetheless, the participants in the present study underwent the mild exercise as the chair-based exercise in sitting position, and the Stroop color and word training in sitting on the chair with back-rest. Therefore, the evaluations of balance performance in the elderly with MCI may employ more difficulty task conditions like a standing position. It might not reflect the transference of motor learning in the elderly with MCI after undergoing 12-session of training.

There are a few of the limitations of the present study. Firstly, the cognitive training as Stroop task in the present study was used material by the paper within 45 minutes. The participants might not be interesting during research data collections. Further studies would be developed the Stroop task by using applications from computer-based training for being remarkable and attention of participants. Second, the BDNF level that represented neurotrophic factor involving the mild exercise would not measure in the present study. Therefore, the further studies that measured the BDNF level for detecting effect of mild exercise in the elderly with MCI would help to clarify this issue. Finally, the training sessions would not measure the long-term effects of training. It would be interesting to investigate an extended study with follow-up periods to determine whether the immediate effects persisted.

Conclusion

The researcher suggests that a combination of mild exercise and cognitive training could improve balance performance and cognitive functions in the older adults with MCI as well as the mild exercise alone and the cognitive training alone. Improving functional ability in the elderly with MCI has great potential to benefit not only the elderly with MCI, but also their family or caregivers and spread to ageing health and care systems. In addition, the mild exercise, cognitive training, and a combination of mild exercise and cognitive training as the dual tasks training might help to be delay the progression of MCI to Alzheimer's disease in the older adults. Further research into the role of exercise as an intervention for older adults with dementia is now required, with larger trials over a longer time period which also assess the impact on caregivers, health and well- being of elderly people.

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Anthocyanin Content, Physicochemical, Nutritional and Sensory Properties of Purple Sweet Potato and Riceberry Biscuits with Rice Bran Organogel

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Abstract

Consumers' awareness on healthful and functional foods has been increasing worldwide. This research aimed to evaluate the anthocyanin content, physicochemical, nutritional, and sensory properties of biscuits prepared from purple sweet potato and riceberry flours. The biscuits were evaluated for their physicochemical properties including color, water activity, moisture contents, and texture profiles. The results showed that the lightness values (L*) ranged from 21.05 to 21.52 with red (a* = 4.97−5.23) and yellow (b* = 0.74−0.84) pigments. The biscuits had the moisture content ranging from 21.72 to 23.70% and the water activity (a_w) was within the range that most microorganisms cannot survive (a_w = 0.845 to 0.883). Increasing the purple sweet potato flour content in the biscuit formulation resulted in decreased hardness, cohesiveness, springiness, gumminess, and chewiness. All biscuit formulations were analyzed for the nutritional values using the nutritional analysis program. The energies of biscuits ranged from 306.55 to 309.48 kcal. Therefore, the recommended packaging for biscuits was two servings per container. Anthocyanin contents were evaluated using the pH-differential method. The result showed that the formulation with the highest anthocyanin content was formulation P5 (90 purple sweet potato flour: 10 riceberry flour, 66.50±0.99 mg/100g of biscuit). Moreover, formulation P5 also received the highest score in the consumer sensory acceptability test (6.64±1.33). These results suggested that biscuit formulation P5 could be used as a prototype for sweet potato and riceberry flour-based products to promote healthier diet.

Introduction

Functional foods and ingredients have received a considerable attention from the food industry because of consumers' demand for more healthful foods (Childs, 2015). Among bakery products, biscuits are the most popular and versatile snack foods due to a variety of taste, availability, long shelf life, and inexpensive price (Murugkar et al., 2014). Main ingredients of biscuits generally include wheat flour and fat (Klunklin & Savage,

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2018). Gluten in wheat flour may not be suitable for consumers with celiac disease (Emami et al., 2018). Alternatives to wheat flour are flours from corn, purple sweet potato, and rice. Purple sweet potatoes (*Ipomoea batatas* (L.) Lam.) are starchy tubers that are beneficial to health. They are excellent sources of dietary fiber, minerals, vitamins, and phytochemical compounds (Li et al., 2019) and exhibit an intense purple color because of the accumulation of acylated anthocyanins as peonidin-based and cyanidin-based anthocyanins (Phomkaivon et al., 2018). Riceberry (*Oryza sativa* L.), a new valuable rice variety in Thailand, is a popularly consumed by the locals (Settapramote et al., 2018). It has become well-known for its taste and lower commodity price compared to wheat flour. In addition to containing higher levels of dietary fiber than wheat, riceberry also contains cyanidin-3-O-glucoside and peonidin-3-O-glucoside (Klunklin & Savage, 2018). These anthocyanin compounds possess various biological properties such as free radical scavenging, anticarcinogenic, and antihypertensive activities (Montilla et al., 2011).

Fats in biscuits are mainly composed of triglycerides, including monounsaturated, polyunsaturated, and saturated fatty acids. Saturated fatty acids could unfavorably affect health (Puscas et al., 2020). Recent studies have shown that trans and saturated fats are associated with cardiovascular diseases (Zhu et al., 2019). Alleviating adverse effects from oil consumption could be accomplished through modifying the structure of liquid oils into gels, hydrogel, or organogel, reducing saturated fat intake (Hwang et al., 2013; Yilmaz & Ogutcu, 2014). Several types of organogels have been developed from edible oils including rice bran oil. Rice bran oil is one of the popular edible oils because it contains several phytochemical compounds such as tocotrienols, tocopherols, phytosterols, polyphenols, squalene, and gamma-oryzanol, that are beneficial to human health (Li et al., 2017).

Products with higher phytonutrient content are preferred by consumers with diabetic and obese conditions. This study aimed to develop biscuits with higher phytonutrient. Flours from sweet potato and riceberry were particularly selected for the study because they were inexpensive raw materials containing various bioactive compounds. Furthermore, the use of rice bran oil in the form organogel could reduce the presence of trans fat in the biscuits. Consumer acceptability, anthocyanin content, physicochemical, nutritional and sensory properties of the biscuits were also evaluated.

Materials and methods

1. Raw materials

The purple sweet potato flour (Doi Ang Khang, Banpangsak community), riceberry flour (Fair D, Grace Bio Co., Ltd), baking powder (Imperial, KCG corporation), eggs (Betagro, Betagro Public Co.,Ltd), brown sugar (Mitr Phol, Mitr Phol Group), and iodized salt (Prung Thip, Thai Refined Salt Co.,Ltd), were purchased from a local market in Thailand. All ingredients were food grade.

The margarine from rice-bran-oil organogel was provided by Mahidol University, Bangkok, Thailand. The method for preparing the organogel was modified from Hwang et al. (2013). Briefly, the oil phase was prepared by dissolving organogelator (4.0% (w/w)) in rice bran oil at 75°C. The water phase was prepared by mixing water with 1% (w/w) salt, 0.5% (w/w) butter flavor, 0.05% (w/w) β-carotene, and 0.03% (w/w) citric acid, and subsequently poured into the oil phase while homogenizing (T-25 basic Ultra Turrax®, Janke and Kunkel IKA, Germany) for 5 minutes. The water-in-oil emulsion was then cooled to 4°C for 1 hour. The organogels were formed by placing the emulsion at −18°C for 4 hours. Once the gelation was complete, the organogels were stored in a refrigerator at 4°C until use.

2. Biscuit preparation

The biscuit formulations were developed by varying the ratio between purple sweet potato and riceberry flours (Table 1). The two types of flour were blended with 0.7 g of baking powder and 0.1 g of salt. Then, 25.8 g of egg white and 4.2 g of brown sugar were added and blended using an electric hand mixer (Electrolux, EHM3407, China) for 2 min. After the mixture attained a spongy consistency, 14.0 g of egg yolk and 8.4 g of rice-bran-oil organogel were slowly poured into it. Then, the mixture was threshed until homogeneous. The dough was rolled out with 0.3 cm of thickness and cut to 0.8 cm of width x 2.5 cm of length. The biscuits were put on a baking tray covered with a baking paper. After applying margarine on top, the biscuits were baked in an oven (Electrolux, EOT2805K, China) at 100°C for 15 min.

Table 1 Biscuit formulations

Ingredients (per 100 grams)	Formulations (purple sweet potato flour : riceberry flour)				
	P1 (50:50)	P2 (60:40)	P3 (70:30)	P4 (80:20)	P5 (90:10)
Purple sweet potato flour	23.4	28.1	32.8	37.4	42.1
Riceberry flour	23.4	18.7	14.0	9.4	4.7

3. Physicochemical characterizations of biscuits

3.1 Color analysis

The color of the biscuits was analyzed using the Chroma Meter CR-400 colorimeter (Konica Minolta, Japan). The color meter was calibrated using a white plate CR-A43 (y = 85.70, x = 0.3177 and y = 24 0.3340) and assessed using the DP mode. The analyzed color parameters lightness (L*) (0 = black to 100 = white), a* (greenness (-) to redness (+)), and b* (blueness (-) to yellowness (+)). The measurement was performed twice.

3.2 Moisture content analysis

The moisture content measurement was performed using a moisture analyzer MA37 (Sartorius, Germany). The condition was set at 105°C using the fully automatic mode. Ten grams of ground sample was approximately weighed on the moisture analyzer. The measurement was duplicately carried out and reported as % moisture.

3.3 Water activity analysis

Water activity was measured by the auto start mode using water activity meter LabSwift-aw (Novasina, Switzerland). The ground sample was added to a sample dish and then put into the water activity meter. The measurement was performed twice.

3.4 Texture profile analysis

Texture analysis of biscuits with a diameter of 30 mm was performed using a texture analyzer CT3 (Ametek Brookfield, USA) equipped with the TA-AACC36 AACC spec probe. The program was set to the test speed at 1 mm/s. Texture characteristics including hardness, adhesiveness, resilience, cohesiveness, springiness, gumminess, and chewiness were obtained. Duplicate measurements were carried out.

4. Nutritional values of biscuits

Nutritional values including energy, carbohydrate, protein, and fat of the biscuits were acquired using the nutritional analysis program (INMUCAL-Nutritions version 4.0, Institute of Nutrition, Mahidol University). The obtained data sets were reported as per 100 g of biscuit sample.

5. Anthocyanin content determination

The total anthocyanin content was determined by the pH-differential method (Sutharut & Sudarat, 2012). Anthocyanin pigments undergo reversible structural transformations with a change in pH, resulting in different absorption spectra. The colored oxonium form predominates at pH 1.0 and the colorless hemiketal form prevails at pH 4.5. A ground biscuit sample with a mass of 5 g was mixed with 10 mL of distilled water. After centrifugation, each 0.5 mL of sample solution was mixed with 0.5 mL of potassium chloride (0.025 M of KCl; Ajax Finechem Pty.Ltd, Autralia) buffer, pH 1.0 and 0.5 mL of sodium acetate (0.4 M of CH_3 COONa; Loba Chemie Pvt.Ltd, India) buffer, pH 4.5, respectively. The absorbance of each dilution was measured at 520 and 700 nm using a microplate reader (Synergy HTX BioTek instruments, USA). The absorbance of the diluted sample (A) was calculated as follows:

$$A = (A_{520} - A_{700})\text{pH } 1.0 - (A_{520} - A_{700})\text{pH } 4.5$$

The monomeric anthocyanin pigment concentration (cyanidin-3-glucoside) in each original sample was calculated using the following formula:

$$\text{Monomeric anthocyanin pigment (mg/L)} = (A \times MW \times DF \times 1000)/(\varepsilon x1)$$

where MW is the molecular weight of cyanidin-3-glucoside (449.2), DF is the dilution factor, ε is the molar absorptivity (26,900 $M^{-1}cm^{-1}$). The value was converted to mg of total anthocyanin content /100 g sample.

6. Sensory characteristics of biscuits

The sensory scores of five formulations were evaluated by 50 volunteers that did not possess sweet potato and rice allergy. Volunteers were students of Valaya Alongkorn Rajabhat University under the Royal Patronage who consented to join this study. The served biscuit samples were labeled with a randomized three letters-digit. After each sample tasting, participants were required to rinse the mouth with water. The appearance, color, texture, flavor, taste, and overall preference of biscuit samples were assessed using the 9-point hedonic scale (1: extremely dislike to 9: extremely like). The study was approved by the research ethics committee (Ref. no. 0002/62).

7. Data analysis

Statistical data were analyzed using ANOVA. The LSD's test was applied to detect the statistical differences among the biscuit samples ($p \leq 0.05$). A completely

randomized design (CRD) was used in physicochemical properties of biscuits and a randomized complete block design (RCBD) was applied in the sensory evaluation.

Results and discussion

1. Physicochemical properties

The color, water activity, and moisture contents of five biscuit formulations are shown in Table 2. There was no significant difference in lightness (L*), where the values ranged from 21.05 to 21.52. The a* (4.97 - 5.23) and b* (0.74 - 0.84) values showed redness and yellowness pigments, respectively. The red pigment of biscuits was possibly caused by thermally degraded anthocyanin pigments in purple sweet potato and riceberry (Wiriyawattana et al., 2018). The yellow pigment was likely caused by anthocyanins in the high chalcone (yellow) form (Xiu-li et al., 2015). The moisture content and the water activity of biscuit samples were also evaluated. These values displayed a direct association with the shelf life of baked products, stability, and susceptibility to microbial contamination (Mahloko et al., 2019). Even though the biscuits had a relatively high moisture content ranging from 21.72 to 23.70%, the water activity (a_w) exhibited a rather low value ranging from 0.845 to 0.883. Most microorganisms cannot propagate within the reported water activity, including *Staphylococcus aureus*, many yeasts (*Candida, Torulopsis, Hansenula, Micrococcus*), most molds (*mycotoxigenic penicillia*), most *Saccharomyces* (bailii) spp., and *Debaryomyces* (Tapia et al., 2020).

Table 2 The color, water activity, and moisture contents of biscuits

Biscuit formulations	Color			Water activity (a_w)	Moisture (%)
	L*[ns]	a*	b*		
P1	21.41±0.11	5.14±0.02[b]	0.82±0.01[a]	0.882±0.00[a]	21.72±1.03[c]
P2	21.20±0.06	5.23±0.01[a]	0.74±0.02[b]	0.879±0.00[ab]	22.13±0.19[bc]
P3	21.05±0.11	4.97±0.02[c]	0.83±0.01[a]	0.868±0.02[ab]	23.70±0.23[a]
P4	21.33±0.49	5.22±0.04[a]	0.84±0.01[ac]	0.883±0.01[a]	23.61±0.04[a]
P5	21.52±0.09	5.10±0.01[b]	0.80±0.02[ad]	0.845±0.02[b]	23.20±0.20[ab]

Remark: [a-d] different letters in the same column indicate values that are significantly different ($p \leq 0.05$).
[ns] in the same column indicate values that are not significantly different ($p > 0.05$).

The texture profile analysis of biscuit samples was evaluated. Hardness, cohesiveness, springiness, gumminess, and chewiness of the samples are shown in Table 3. The P1 formulation had the highest hardness (1175.00 ± 1.41 g). Increasing purple sweet potato flour content resulted in decreased hardness. Addition of purple sweet potato flour also contributed to decreasing cohesiveness (ranging from 0.86-0.57), springiness (ranging from 0.91-0.76 mm), gumminess (ranging from 1065-55.00 g), and chewiness (ranging from 9.69-0.42 mJ). This was possibly because of the high dietary fiber in purple sweet potato, which has the ability to swell and resulted in soft biscuit (Dhingra et al., 2012). Texture profiles could be employed for predicting consumer acceptability. Softer biscuits are preferred by consumers (Klunklin & Savage, 2018). This suggested that the addition of purple sweet potato flour might increase consumer acceptance.

Table 3 Texture profiles of biscuits

Characteristics	Biscuit formulations				
	P1	P2	P3	P4	P5
Hardness (g)	1175.00± 1.41[a]	936.00±2.83[b]	918.00±1.41[c]	414.00±2.83[d]	98.00±141[e]
Cohesiveness	0.85±0.01[a]	0.83±0.01[a]	0.73±0.02[b]	0.68±0.01[c]	0.57±0.02[d]
Springiness (mm)	0.91±0.02[a]	0.88±0.01[a]	0.88±0.01[a]	0.82±0.01[b]	0.76±0.01[c]
Gumminess (g)	1065.50±2.12[a]	805.00±2.83[b]	661.50±2.12[c]	404.00±1.41[d]	55.00±1.41[e]
Chewiness (mJ)	9.69±0.10[a]	7.08±0.09[b]	5.70±0.03[c]	3.29±0.04[d]	0.42±0.02[e]

Remark: [a-e] different letters in the same row indicate values that are significantly different ($p \leq 0.05$).

2. Nutritional properties of biscuits

The nutritional properties of biscuits are presented in Table 4. Most of the energy of biscuits came from carbohydrate (42.86–44.83 g). Five formulations of biscuits did not show a statistically significant difference in energy distribution from carbohydrate, which was in the range of 55.39–58.49%. The energy from these formulations was in accordance with the recommendation of acceptable macronutrient distribution ranges (AMDRs), which provided the guideline for energy intake from carbohydrate to be approximately 35–70% (Lee et al., 2015). The sugar content was in the range of 4.27 to 4.90 g. The values did not exceed the recommended guideline daily amounts (GDA) of 90 g per a day. The increased purple sweet potato in the biscuit resulted in increased carbohydrates and decreased sugar content, indicating that carbohydrates could be in the form of dietary fiber. The fatty acid energy distribution was in the range from 35.07 to 36.50% (11.95–12.55 g). Moreover, the biscuits contained low saturated fatty acid (1.48–1.49 g) and cholesterol (0.18 g). The World Health Organization (WHO) recommended the amount of saturated fatty acid to be less than 10% of the total energy intake. The lower saturated fatty acid and cholesterol is more preferred by consumers who are more conscious of the risk of degenerative disease such as

cardiovascular diseases and obesity-related type 2 diabetes (Gershuni, 2018). The lower level of saturated fatty acid of the biscuits might be attributed to the utilization of rice-bran-oil organogel in the formulation. Furthermore, the biscuits were high in protein (5.33-6.27 g, 6.63–8.11% of energy distribution). Purple sweet potatoes were reported to be lower in protein and fat than riceberry (Kurnianingsih et al., 2020; Settapramote et al., 2018). Therefore, higher ratio of purple sweet potato in the biscuit decreased fat and protein. The energy of biscuits was in the range of 306.55 to 309.48 kcal. According to the recommendation by the Thai dietary reference intakes (Thai DRIs), Ministry of Public Health, the biscuits was suitable for 2 servings per container.

Table 4 Nutritional properties of biscuits (100 g)

Nutritional properties	Biscuit formulation				
	P1	P2	P3	P4	P5
Energy (kcal)	309.48	308.74	310.57	307.29	306.55
Carbohydrate (g)	42.86	43.35	44.38	44.33	44.83
Energy distribution from carbohydrate (%)	55.39	56.16	57.16	57.71	58.49
Protein (g)	6.27	5.97	5.72	5.38	5.33
Energy distribution from protein (%)	8.11	7.74	7.34	7.00	6.63
Fat (g)	12.55	12.40	12.30	12.10	11.95
Energy distribution from fat (%)	36.50	36.14	35.60	35.43	35.07
Saturated fat (g)	1.48	1.48	1.49	1.49	1.49
Cholesterol (g)	0.18	0.18	0.18	0.18	0.18
Sugar (g)	4.90	4.74	4.61	4.43	4.27

3. Anthocyanin content of biscuits

Anthocyanin contents of the biscuits are shown in Table 5. The flours of purple sweet potato and riceberry at a ratio of 50:50 had the lowest anthocyanin content. The P5 formulation displayed the highest anthocyanin content (66.50 ± 0.99 mg/100g of biscuit), but it did not significantly differ from the P4 formulation (62.93±0.29 mg/100g of biscuit). The result indicated that the addition of purple sweet potato increased the level of anthocyanins, which include peonidin and cyanidin with 3-sophoroside-5-glucoside acylated with p-hydroxybenzoic acid, ferulic acid and caffeic acid (Li et al., 2019). The anthocyanins of purple sweet potato have been reported for their stability after heating and ultraviolet irradiation because of their complex chemical structures (Kano et al., 2005). In addition, anthocyanins of purple sweet potato could contribute to the anti-arteriosclerosis activity (Kano et al., 2005), suggesting that the biscuit formulation with higher ratio of purple sweet potato flour is likely to be healthier.

Table 5 Anthocyanin content of biscuits

Formulations	Total anthocyanin content (mg/100g of biscuit)
P1	33.81 ± 1.92^{d}
P2	41.18 ± 1.31^{c}
P3	49.89 ± 1.22^{b}
P4	63.93 ± 1.29^{a}
P5	66.50 ± 0.99^{a}

Remark: $^{a-d}$ different letters in the same column indicate values that are significantly different ($p\leq0.05$).

4. Sensory properties of the biscuits

The sensory characteristics of the biscuits are shown in Table 6. Sensory evaluation is considered a valuable tool in determining consumer acceptability of the developed products. The results showed that the appearance (5.11-5.57) and color (5.13-5.55) of all biscuits were not significantly different ($p\leq0.5$). This was possibly due to the similar dark purple color of both purple sweet potato and riceberry flours. The biscuit formulation P5 had the highest score in the texture property, i.e. 6.55±1.52, followed by formulations P4, P2, P3, and P1. This result suggested that consumers preferred softer biscuits. Moreover, the formulations with higher purple sweet potato flour were more popular in flavor possibly due to the presence of sweet-scented volatile oil in the sweet potato flour. The main components of volatile oil from purple sweet potato were hexadecanoic acid, phenylacetaldehyde, guaiacol, and *p*-vinylguaiacol (Nakamura et al., 2013). Regarding the taste, the P5 formulation exhibited the highest score. Nevertheless, it was not significantly different from the P4 formulation. The overall acceptance of the P5 formulation 5 displayed the highest values among all formulations. Therefore, the P5 formulation was a potential candidate for a healthy biscuit.

Table 6 Sensory properties of biscuits

Sensory properties	Biscuit formulation				
	P1	P2	P3	P4	P5
Appearance^{ns}	5.11±1.17	5.32±1.20	5.36±1.19	5.43±1.35	5.57±1.21
Color^{ns}	5.13±1.21	5.23±1.31	5.32±1.19	5.45±1.32	5.55±1.44
Texture	4.86 ± 1.81^{d}	5.77 ± 1.48^{c}	5.75 ± 1.51^{b}	6.21 ± 1.41^{abc}	6.55 ± 1.52^{a}
Flavor	5.45 ± 1.33^{bc}	5.40 ± 1.38^{abc}	5.61 ± 1.45^{abc}	5.68 ± 1.37^{ab}	6.00 ± 1.55^{a}
Taste	4.79 ± 1.33^{d}	5.53 ± 1.61^{c}	5.75 ± 1.48^{bc}	6.13 ± 1.61^{abc}	6.62 ± 1.54^{a}
Overall acceptance	5.19 ± 1.35^{d}	5.77 ± 1.24^{c}	5.83 ± 1.40^{bc}	6.28 ± 1.39^{abc}	6.64 ± 1.33^{a}

Remark: $^{a-d}$ different letters in the same row indicate values that are significantly different ($p\leq0.05$).
^{ns} in the same row indicate values that are not significantly different ($p>0.05$).

Conclusion

Biscuit formulations based on purple sweet potato and riceberry flours were successfully developed. The texture of the biscuits could be modified by varying the sweet potato flour content. The biscuit formulations with the highest sweet potato flour content exhibited good nutritional characteristics, low water activity, desirable sensory properties, and favorable texture characteristics. The nutritional profiles the biscuit formulation suggested that the biscuits were suitable for 2 servings per container. The results from our studies set forth a utilization of anthocyanin-rich ingredients for producing a healthier and nutritionally-rich daily snacks.

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Effects of Dietary Lasia (*Lasia spinosa* (L.) Thwaites) Extract on Growth Performance and Physiological Indices of Common Lowland Frog (*Rana rugulosa*)

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Abstract

A wide range of biological and pharmacological activities of lasia (*Lasia spinosa* (L.) Thwaites) are well established. However, little attention has been paid to the growth-promoting effects of lasia on aquatic animals. This study was aimed at elucidating the effects of dietary lasia extract (LE) on growth performance and physiological indices of common lowland frog (*Rana rugulosa*). Lasia leaf extract was prepared and phytochemical screening showed the presence of flavonoids, terpenoids, phenolic compounds, tannins, steroids, glycosides, and anthraquinones. Four treatments of male frog (initial weights of 14.00±1.00 g) were fed for 8 weeks with diets supplemented with 0 (control), 1, 3, and 5 g LE/kg diets. The results revealed that growth performance parameters were significantly improved in frog fed with LE-supplemented diets compared with the control ($P < 0.05$). The survival rate, hepatosomatic index, intestinosomatic index, splenosomatic index, cardiosomatic index, and renosomatic index did not change among the treatments ($P > 0.05$). An increase in intraperitoneal fat weight was noticed in frog fed with LE-supplemented diets compared with the control ($P < 0.05$). Dietary LE significantly improved villi height, villi width, and the absorptive surface area of the frog intestine compared to control ($P < 0.05$). Hemoglobin was markedly increased in frog fed with 5 g LE/kg diet compared with the other treatments ($P < 0.05$). There were no significant differences in hematocrit, white blood cells, and red blood cells among all treatments ($P > 0.05$). Dietary LE did not affect the levels of serum total protein, albumin, alkaline phosphatase, aspartate transaminase, triglyceride, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol ($P > 0.05$). Decreased cholesterol levels were detected in frog fed with LE-supplemented diets compared with the control ($P < 0.05$). Glucose value was significantly enhanced in frog fed with 5 g LE/kg diet compared with the other treatments ($P < 0.05$). Taken together, these findings support the beneficial effects of dietary LE on the growth and physiological indices of the frog. The suitable level of LE observed by using the second-order polynomial regression analysis was 3.60 g LE/kg diet.

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Introduction

The role and importance of aquaculture and fisheries are to enhance world food and nutrition security for all (Awad & Awaad, 2017). Additionally, products from aquaculture provide income and livelihoods for many locations around the globe (Citarasu, 2010; Rico et al., 2013). An increase in consumption and demand for food sources has led to improved cultivation of several species of aquatic animals (FAO, 2020). Thailand is well-known as one of the producers and exporters of aquaculture products in the world. The main internationally traded products from Thailand are shrimp, tilapia, catfish, and carp. Interestingly, common lowland frog (*Rana rugulosa*) is also one export aquatic species with a high tendency in the global market because of the presence of the high nutritional value of its meat (Somsueb & Boonyaratpalin, 2001). Department of Fishery of Thailand reported that frog production rose from 1537 tonnes in 2007 to 3898 tonnes in 2017 (Department of Fishery, 2020). Muanmueangsong et al. (2014) reported the data from Taksin (2005) that he said the markets of frog products of Thailand are Hong Kong, China, Singapore, the European Union, and USA. Due to a decrease in frog population in nature, intensive and semi-intensive farming systems have been developed to enhance production output per unit (Pariyanonth & Daorerk, 1994). However, these production procedures are now faced with many problems including farmer expertise and lack of knowledge regarding the frog culture, low water quality, several diseases and pathogens, malnutrition, low growth rate, and cannibalism (Kamatit et al., 2016; Saman & Thiammueang, 2021). Therefore, good management and husbandry practices are required.

Nowadays, the application of drugs and chemicals in aquaculture operations has dramatically increased worldwide and plays a key role in the world food supply chain (Citarasu, 2010; Okocha et al., 2018). The use of these drugs is aimed to improve production performance, disease resistance, and the general well-being of animals (Rico et al., 2013; Van Hai, 2015). However, the public has been concerned about the benefits and risks associated with the use of synthetic drugs in animal feeds for a long time (Bulfon et al., 2015; Reverter et al., 2014). Residual drugs detected in aquaculture products may harm people who consume them (Awad & Awaad, 2017). Additionally, the uncontrolled administration of antimicrobials in cultivation practices may result in the emergence of antimicrobial-resistant strains (Bilen et al., 2018; Hoseinifar et al., 2020; Van Doan et al., 2019). To reduce the potential risks associated with the application of chemicals, medicinal plants and their novel compounds are continually developed and verified (Amin et al., 2019; Chakraborty & Hancz, 2011; Dawood et al., 2020; Jankham et al., 2020). In raniculture, many plant species such as *Butea superba, Curcuma longa, Pueraria mirifica, Nelumbo nucifera* Gaertn., and *Limnophila aromatica* have been evaluated in the laboratory conditions and shown as potential substitutes for the use of antibiotics to enhance growth indices and health status in heterogeneous farm conditions (Kaewtapee et al., 2011; Thainum & Chitmanat, 2019; Srinuansom et al., 2019; Thummek et al., 2016; Wongtha et al., 2019).

Lasia (*Lasia spinosa* (L.) Thwaites) is a perennial herb that belongs to the family Araceae. It is a native plant of Southeast Asia, where it occurs in shaded areas along the river, wet forests, and wetlands. Phytochemical analysis indicated the presence of flavonoids, terpenoids, phenolic compounds, steroids, saponins, coumarins, glycosides, and anthraquinones (Hong Van et al., 2006; Napiroon et al., 2013). Phytoandrogens and phytoestrogens were also detected in rhizomes, leaves, and roots of this plant (Suthikrai et al., 2007). Interestingly, lasia has been reported to possess a variety of pharmacological effects including antioxidant, antinociceptive, anti-inflammatory, anti-diarrheal, antimicrobial, and cytotoxic properties (Alam et al., 2011; Deb et al., 2010; Goshwami et al., 2012; Nanasombat & Teckchuen, 2009). Recently, Kaewamatawong et al. (2013) evaluated acute and subacute toxicity and reproductive effects of lasia extract in male rats and they found the extract of lasia did not show any signs of acute and subacute toxicity in rats. Blood biochemical values and the weight of the body and internal organs of the experimental groups were similar to the control group. However, testicular weight and sperm count significantly increased in the treated rats. These findings indicate the safe use of lasia in traditional medicines and home remedies. As mentioned above, there are very few reports indicated the application of lasia as a natural feed additive in animal diets (Munglue et al., 2019; Suthikrai et al., 2005; Suthikrai et al., 2007). Available evidence indicated that dietary supplementation with lasia improved growth and nutrient utilization efficiency in large ruminants (Suthikrai et al., 2007). Additionally, hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*) fed with the diet supplemented with 3% lasia

extract for 8 weeks showed a significant improvement of growth rate and healthy intestinal villi (Munglue et al., 2019). Based on these scientific reports, lasia may be effectively used to replace the synthetic feed additives in aquaculture industries (Chakraborty & Hancz, 2011; Reverter et al., 2014; Suthikrai et al., 2007). To our knowledge, there is no information regarding the beneficial effects of lasia in the frog culture. The objective of this study was to evaluate the effects of lasia extract (LE) on growth performance parameters and physiological indices of common lowland frog.

Materials and methods

1. Animal ethics and regulation

This research was performed in the Ubon Ratchathani Rajabhat University Fishery Farm. The experimental protocol of this research was approved by the Institutional Animal Care and Use Committee, Ubon Ratchathani Rajabhat University, Thailand. The approval number is AN63006.

2. Lasia preparation and extraction

Lasia extract was performed according to the report of Munglue et al. (2019). Arial parts of lasia were harvested from Sirindhorn District, Ubon Ratchathani, Thailand during August and October. The plant specimen (Munglue 006) was kept at the Program of Biology, Faculty of Science, Ubon Ratchathani Rajabhat University, for further reference. The plant samples were cleaned using tap water, cut into small pieces approximately 1 to 2 cm, and dried in a hot air oven at 45°C for 1 week. Dried lasia samples (100 g) were macerated with 75% ethanol (300 mL) (1:3 w/v) for 1 week and then it was filtered through Whatman paper No.1. The resultant mixture was evaporated to remove the solvent using rotary evaporator (Buchi, R-200, Switzerland) under reduced pressure and low temperature. The solution was subsequently transferred to lyophilizer (Labconco Corporation, Missouri, USA). The crude extract (10.15 g) was collected and kept in a refrigerator (-20°C) until use.

3. Phytochemical screening

Phytochemical constituents including alkaloids, flavonoids, terpenoids, phenolic compounds, tannins, steroids, saponins, coumarins, glycosides, and anthraquinones were determined using colorimetric methods of Evans et al. (2002) and Kar (2007). The results were reported as (+) presence or (-) absence to indicate the active compounds in the plant extract.

4. Experimental diet preparation

A commercial diet containing 42% crude protein, 5% crude fat, and 5% fiber (Charoen Pokphan Food PCL, Samutsakorn, Thailand) was obtained and used as a basal diet. The dietary protein level in this study was in the range optimal for frog rearing (Coppo et al., 2001; Marschall, 1978). In this research, the levels of LE (0 (control), 1, 3, and 5 g/kg diets) were estimated according to the previous study (Munglue et al., 2019). The diets were mixed with LE using cassava starch as a binder and 200 mL distilled water added. The mixture was pelleted by using a meat mincer (2 mm). Pellets were then dried in a hot air oven at 45°C for 24 h and kept in sealed plastic bags at 4°C in a refrigerator until use.

5. Experimental frog and frog culture

Male frogs with the mean initial weight of 14.00 ± 1.00 g were obtained from Ubon Ratchathani Fishery Cooperative, Ubon Ratchathani, Thailand. A total of 240 frogs were transferred to the Ubon Ratchathani Rajabhat University fishery farm. They were randomly distributed into 12 circular cement tanks (0.70 m in diameter and 0.45 m in height) filled with cleaned water and covered with black shade nets. Water quality parameters were checked daily using ExStik® EC500 (Extech Instrument Corporation, U.S.A.) and maintained in standard levels for frog farming (temperature 29.0 ± 1.0°C, pH 7.5 ± 0.3, alkalinity 100 - 200 mg/L, and dissolved oxygen 6.00 ± 0.10 g/L). After a 2-week acclimatization period, three cement tanks of frogs were randomly determined as one of four experimental treatments containing different levels of LE (0 (control), 1, 3, and 5 g LE/kg diets). Frogs were fed with the experimental diets on Styrofoam *ad libitum* two times a day (08.00 and 16.00 h) for 8 weeks.

6. Growth parameters

At the end of the feeding trial, four frogs from each tank were individually weighed. Additionally, all frogs in each tank were counted to calculate the survival rate (SR). Growth parameters including weight gain (WG), specific growth rate (SGR), average daily gain (ADG), and feed conversion ratio (FCR) were evaluated as follows:

WG (g) = final weight (g) – initial weight (g);
SGR (%/day) = 100 × [(*In* final weight (g) – *In* initial weight (g))/number of days in the experimental period];
ADG (g/day) = weight gain (g)/ number of days in the experimental period;
FCR = feed intake (g)/weight gain (g);
SR (%) = 100 × (final number of frog/initial number of frog).

7. Relative organ weights

Four frogs from each replicate were randomly collected and double-pithed. Liver, intestine, spleen, heart, kidney, and intraperitoneal fat samples were collected, cleared from the blood and connective tissues, and weighed to calculate the relative organ weight using the following equation:

$$\text{Relative organ weight (\%)} = 100 \times (\text{organ weight (g)}/\text{frog body weight (g)}).$$

8. Histological analysis

The intestines were divided into 3 parts including the anterior, middle, and posterior and cut transversally. The contents in the intestinal lumen were cleared using 0.90% sodium chloride solution and fixed in phosphate-buffered formalin 10% (v/v), pH 7.4, for 24 h. After dehydration, the samples were embedded in paraffin wax, sectioned at 5 µm thick, and mounted on glass slides. The tissues were then stained routinely with Hematoxylin & Eosin (H & E) as the method reported by Escaffre et al. (2007). Observations on intestinal histology were performed using a light microscope connected with a computer using Dino Capture 2.0 software. The six longest intact villi were selected for the measurement of villi height and villi width according to the report of Jankham et al. (2020). The absorptive surface area of the intestine was also estimated using the following equation (Abdel-Tawwab et al., 2018):

$$\text{The absorptive surface area of the intestine } (\mu m^2) = \text{villi height } (\mu m) \times \text{villi width } (\mu m).$$

9. Blood collection

At the end of the feeding period, the frog fasted for 24 h. Four frogs from each tank were randomly collected, weighed, and double-pithed with a needle. The abdominal wall was opened and frog blood was carefully collected by cardiac puncture using a sterile syringe and divided into 2 parts. One part was transferred to 3 ml tube containing 10% ethylenediaminetetraacetic acid (EDTA), which was used as an anticoagulant, for hematological analysis. Another part was gently transferred to an Eppendorf tube, left to clot at room temperature for 3 h, and centrifuged at 3000 rpm for 10 min. The collected serums were kept in microtubes and stored at -20°C for biochemical analysis (Campbell & Ellis, 2007).

10. Hematological analysis

Red blood cells (RBC) and white blood cells (WBC) were measured by using a Neubauer hemocytometer (Campbell & Ellis, 2007). Hematocrit (Ht) was evaluated using the microhematocrit method (Ramezanzadeh et al., 2020). The level of hemoglobin (Hb) was measured using the cyanomethemoglobin method (Abdel-Tawwab et al., 2018).

11. Biochemical analysis

Aspartate transaminase (AST) was determined by the method reported by Schumann et al. (2002). Serum alkaline phosphatase (ALP) was evaluated according to Tietz et al. (1983). Serum glucose was analyzed using Trinder's method (Barham & Trinder, 1972). Cholesterol level was tested using the cholesterol oxidase-phenol+aminophenazone (CHOD-PAP) method (Flegg, 1973). Triglyceride was measured by following Cole et al. (1997). Commercial reagent kits obtained from Erba Lachema s.r.o. (Czech Republic) were used to detect high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) based on a modified polyvinyl sulfonic acid (PVS) and polyethylene-glycol ether (PEGME) coupled classic precipitation method (PVS/PEGME).

12. Data analysis

All data were tested for the normal distribution and the homogeneity of variances by using Kolmogorov-Sminov test and Levene's test, respectively. If data were non-normal distribution, the arcsin square-root transformation was used. All data were subsequently subjected to one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test to test significant differences among the treatments when $P < 0.05$. The optimal level of LE for the frog culture was estimated using the second-order polynomial regression analysis. Results were represented as mean ± standard error of the mean (SEM).

Results and discussion

1. Phytochemical screening

Phytochemical screening indicated that LE contains flavonoids, terpenoids, phenolic compounds, tannins, steroids, glycosides, and anthraquinone. However, the plant extract showed the absence of alkaloids, saponins, and coumarins (Table 1). These findings are similar to the report of Napiroon et al. (2013) who showed that lasia consists of organic compounds, terpenoids, phenolic compounds, coumarins, and alkaloids. Moreover, lasia leaf extract has been reported to possess flavonoids, terpenoids, phenolic compounds, steroids, saponins, coumarins, glycosides, and anthraquinones

(Munglue et al., 2019). When compared with the other reports, a difference in chemical compounds found in lasia in this present study may be due to extraction methods, geographical sources, soil compositions, harvesting seasons, and weather conditions (Evan et al., 2002; Kar, 2007).

Table 1 Phytochemical screening of LE

Phytochemicals	Tests performed	Results
Alkaloids	Dragendorff's Tests	-
	Mayer's Test	-
Flavonoids	NaOH Tests	+
Terpenoids	Salkowski Test	+
	Liebermann-Burchard Test	+
Phenolic compounds	Ellagic Acid Test	+
	Phenol Tests	+
Tannins	Gelatin Test	+
	Lead acetate Test	+
Steroids	Salkowski Test	+
Saponins	Foam Test	-
Coumarins	Test for coumarins	-
Glycosides	Liebermann's Test	+
	Molisch's Test	+
	Keller-Kiliani Test	+
Anthraquinones	Borntrager's Test	+
	Modified Borntrager's Test	+

Remark: + = presence; - = absence; LE = lasia extract

2. Growth parameters

Growth parameters, feed utilization, and the rate of survival of frog fed dietary LE are presented in Table 2. At the end of the feeding trial, it was found that FW, WG, SGR, and ADG of frog fed with 3 and 5 g LE/kg diets were significantly higher than those of frog fed with 1 g LE/kg diet and the basal diet ($P < 0.05$). Additionally, FCR values significantly decreased in frog fed with LE-supplemented diets compared with the control diet ($P < 0.05$). No significant difference in the SR was noted among the treatments ($P > 0.05$). By using the second-order polynomial regression analysis on the FW ($y = -2.824x^2 + 20.355x + 64.348$, $R^2 = 0.876$, $p = 0.000$), the optimal level of LE was found to be 3.60 g/kg diet (Fig. 1).

Medicinal plants and their chemical compounds have long been used in aquaculture industries as growth enhancers, appetizers, anti-stressor, and immune stimulants (Awad & Awaad, 2017; Citarasu, 2010). However, more research is required to clarify their diverse beneficial physiological effects on various species of aquatic animals. It was postulated that lasia has long been used in folk medicine to treat various diseases (Deb et al., 2010; Goshwami et al., 2012). Besides, it has been

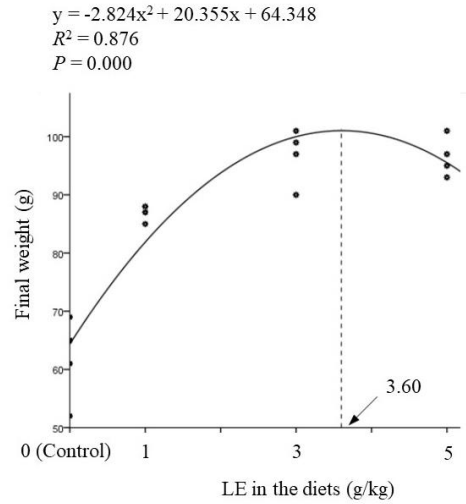

Fig. 1 The second-order polynomial regression analysis on the final weight of frog fed LE-supplemented diets for 8 weeks

reported to have beneficial effects on the growth of fish and terrestrial animals such as buffalos (Munglue et al., 2019; Suthikrai et al., 2005; Suthikrai et al., 2007). However, there is no information up to now on the effects of dietary LE on raniculture. This work has aimed to use lasia as a growth promoter in frog diets.

Table 2 Growth parameters and survival rate of common lowland frog fed LE-supplemented diets for 8 weeks

Parameters	LE in the diets (g/kg)			
	0 (Control)	1	3	5
IW (g)^{ns}	14.50 ± 0.28	15.50 ± 0.28	14.00 ± 0.70	14.75 ± 0.25
FW (g)	61.75 ± 3.63^{c}	68.75 ± 0.62^{b}	96.75 ± 2.39^{a}	95.50 ± 1.70^{a}
WG (g)	46.75 ± 1.22^{c}	72.25 ± 0.47^{b}	82.75 ± 2.49^{a}	81.75 ± 1.54^{a}
SGR (%/day)	2.51 ± 0.02^{c}	2.66 ± 0.03^{b}	3.33 ± 0.00^{a}	3.37 ± 0.01^{a}
ADG (g/d)	0.83 ± 0.06^{c}	1.29 ± 0.03^{b}	1.47 ± 0.04^{a}	1.46 ±0.02^{a}
FCR	3.78 ± 0.32^{a}	2.40 ± 0.01^{b}	2.10 ± 0.06^{b}	2.12 ± 0.03^{b}
SR (%)^{ns}	95.00 ± 2.88	95.00 ± 2.84	96.66 ± 1.62	96.66 ± 1.63

Remark: Data are presented as mean ± SEM. Different superscripts in the same row are significantly different ($P < 0.05$). Superscript ^{ns} indicates no statistical difference ($P > 0.05$); LE = lasia extract; IW = initial weight (g); FW = final weight (g); WG = weight gain (g); SGR = specific growth rate (%/day); ADG = average daily gain (g/day); FCR = feed conversion ratio; SR = survival rate (%)

This present study demonstrated that growth parameters were significantly improved in frog fed with LE containing diets. No significant difference was observed in the SR among the treatments. These results suggested growth-stimulatory activity of LE in frogs without a negative effect. Similar results have been reported by Munglue et al. (2019) who found that hybrid catfish fed with LE-supplemented diets for 8 weeks showed a significant increase in growth performance

parameters compared with the basal diet. A study by Wongtha et al. (2019) reported that incorporating *L. aromatica* in frog diets significantly improved feed utilization and growth performance. The underlying mechanism of LE on the growth of frogs remains unknown. It is established that herbal plants could act as growth promoters by improving feed palatability, feed intake, nutrient metabolism, digestive enzyme activity, and the molecular pathways of protein synthesis in various cell types (Awad & Awaad, 2017; Citarasu, 2010). In addition, a variety of micronutrients from plants such as steroids, alkaloids, flavonoids, glycosides, phenolics, saponins, terpenoids, anthraquinones, and coumarins have been reported to have a marked enhancement in the growth of frog (Wongtha et al., 2019). Taken together, an increase of growth parameters of frog fed with LE-supplemented diets may be due to phytonutrients that found in *L. spinosa* such as flavonoids, terpenoids, and phenolic compounds (Bulfon et al., 2015; Chakraborty et al., 2014; Reverter et al., 2014).

3. Relative organ weights

The data on the relative weights of internal organs are summarized in Table 3. There were no significant differences in the values of HSI, ISI, SSI, CSI, and RSI ($P > 0.05$). However, a marked increase of IPF values was noted in frog fed with LE-supplemented diets compared with the control ($P < 0.05$).

Table 3 Relative organ weights of common lowland frog fed LE-supplemented diets for 8 weeks

Parameters	LE in the diets (g/kg)			
	0 (Control)	1	3	5
HSI (%)^{ns}	5.00±0.15	4.62±0.27	5.32±0.14	4.75±0.42
ISI (%)^{ns}	1.61±0.07	1.57±0.06	1.68±0.07	1.65±0.07
SSI (%)^{ns}	0.04±0.01	0.05±0.01	0.04±0.01	0.04±0.01
CSI (%)^{ns}	0.63±0.04	0.56±0.02	0.52±0.02	0.55±0.02
RSI (%)^{ns}	0.53±0.03	0.47±0.04	0.46±0.02	0.54±0.03
IPF (%)	4.79±0.27^{c}	5.47±0.16^{ab}	5.27±0.21^{bc}	6.15±0.26^{a}

Remark: Data are presented as mean ± SEM. Different superscripts in the same row are significantly different ($P < 0.05$). Superscript ^{ns} indicates no statistical difference ($P > 0.05$); LE = lasia extract; HSI = hepatosomatic index (%); ISI = intestinosomatic index (%); SSI = spleen somatic index (%); CSI = cardiosomatic index (%); RSI = renosomatic index (%); IPF = intraperitoneal fat (%)

The relative organ weight is an important index of physiological and pathological status in experimental animals (Fisher & Myers, 2000; Gupta et al., 2017). The previous studies have proved that changes in the weight of internal organs may influence by a variety of biological, chemical, and physical factors (Brodeur et al., 2020; Dekić et al., 2016; Penn et al., 2011). Therefore, studies on the relative weights of organs may provide information on dosage regimens, the mode of action, and the toxic effects of natural products in aquatic organisms (Porwal et al., 2017; Smith et al., 2017). In this research, relative organ weights of the liver, intestine, spleen, heart, and kidney in the experimental groups did not differ significantly when compared with the control group. Thus, this finding suggested that lasia can be submitted as a supplement in frog feed without negligible side effects. Interestingly, a significant increase of the fat body mass was recorded in frog fed with LE-supplemented diets. It is well known that the physiological significance of the fat body is related to the modulation of reproductive processes, metamorphosis, hibernation, and energy homeostasis in amphibians (Zancanaro et al., 1996; Zhu et al., 2019). The results of this work were consistent with those of Klahan & Pimpimol (2018) who found that frogs fed with the diets supplemented with crude papain extract for 90 days displayed high levels of protein and lipid in the edible flesh compared with the control diet. Additionally, hybrid catfish that were fed diets containing *Euphorbia hirta* plant leaf extract for 90 days showed a significant increase in IPF values compared with the control (Panase et al., 2018). To date, there is no information available on the effect of dietary LE on a fat depot in frogs. However, it may speculate that some phytochemicals could improve feed utilization and enhance protein and lipid accumulation in frogs that were fed with LE-supplemented diets (Panase et al., 2018; Turan & Akyurt, 2005; Sun et al., 2018). Therefore, further researches are necessary to elucidate the mechanism of the effect of dietary LE on the fat storage in frogs.

4. Histological analysis

The intestinal histology of frog fed with LE-supplemented diets is given in Table 4 and Fig. 2. In the anterior intestine (2A – 2D), significantly increased villi height and absorptive area were observed in frog fed with LE-supplemented diets compared with the control ($P < 0.05$). Enhanced villi width was detected in frog fed with 5 g LE/kg diet compared with the other treatments ($P < 0.05$). In the middle intestine (2E – 2H), the frog that were fed with 1 g LE/kg diet showed a significant increase in villi height compared with the other treatments ($P < 0.05$). Significantly improved villi width and the absorptive surface area were found in frog fed with LE-supplemented diets compared with the control ($P < 0.05$). In the posterior intestine (2I – 2L), a significant increase in villi height and the absorptive area

Table 4 Histological analysis of common lowland frog fed LE-supplemented diets for 8 weeks

Parameters	LE in the diets (g/kg)			
	0 (Control)	1	3	5
Anterior intestine				
Villi height (μm)	2039.18±202.07^{b}	3968.29±232.84^{a}	3251.88±132.27^{a}	3852.22±578.73^{a}
Villi width (μm)	299.71±9.62^{b}	307.86±9.30^{b}	296.84±10.42^{b}	351.13±12.48^{a}
Absorptive area (μm^2)	604073.37±48625.07^{c}	1232175.87±95392.01^{a}	967684.72±42739.13^{b}	1377547.73±218692.28^{a}
Middle intestine				
Villi height (μm)	2654.30±192.16^{b}	4255.35±380.50^{a}	2963.25±222.35^{b}	2940.27±254.33^{b}
Villi width (μm)	237.94±10.13^{b}	273.12±16.06^{a}	279.18±10.58^{a}	275.96±10.87^{a}
Absorptive area (μm^2)	561775.07±25979.12^{b}	1186668.01±145735.58^{a}	828634.73±67594.08^{a}	838348.29±97817.84^{a}
Posterior intestine				
Villi height (μm)	1361.37±95.70^{b}	1224.38±23.63^{b}	1774.38±99.79^{a}	1269.66±78.20^{b}
Villi width (μm)	274.09±22.58^{a}	232.31±8.81^{b}	276.33±5.72^{a}	275.81±9.88^{a}
Absorptive area (μm^2)	290711.72±22541.60^{b}	285714.44±14582.06^{b}	489893.62±29026.25^{a}	351780.93±25718.30^{b}

Remark: Data are presented as mean ± SEM. Different superscripts in the same row are significantly different ($P < 0.05$). LE = lasia extract

was observed in frog fed with 3 g LE/kg diet compared with the other treatments ($P < 0.05$). Additionally, decreased intestinal villi width was noticed in frog fed with 1 g LE/kg diet compared with the other treatments ($P < 0.05$).

Study on intestinal histology is an important indicator of digestion and absorption processes in animals (Adeshina et al., 2019; Escaffre et al., 2007). Results of this study indicated that frog fed with LE-supplemented diets showed a significant increase in villus height, villi width, and absorptive area in all parts of the intestine. These findings are in agreement with the report of Munglue et al. (2019) who demonstrated that hybrid catfish fed with dietary LE for 8 weeks showed a significant increase in villus height, villi width, and goblet cell number compared with the control diet. Similarly, Thummek et al. (2016) recorded a significant enhancement of villi height, villi width, and muscular thickness of frog fed with diets containing 1, 3, and 5% *N. nucifera* stamen extract for 11 weeks. Also, dietary supplementation of *N. pubescens* stamen extract improved villi height, villi width, and muscular thickness of frog (Kamatit et al., 2016). It was noted that nutrient digestibility and metabolic activity are related to intestinal histomorphology (Amin et al., 2019; Escaffre et al., 2007; Wilson & Castro, 2011). Dietary

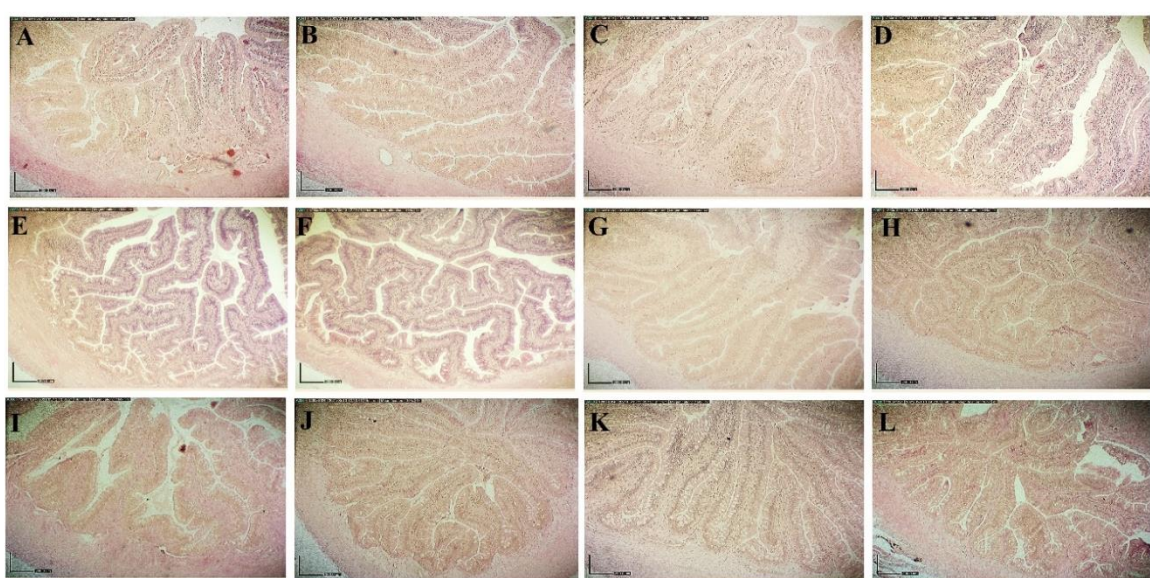

Fig. 2 Light microscopy of the anterior (A-D), middle (E-H), and posterior (I-L) intestine of frog fed the diets supplemented with 0 (A, E, and I), 1 (B, F, and J), 3 (C, G, and K), and 5 (D, H, and L) g LE/kg diets for 8 weeks. Scale bar = 200 μm.

supplementation with medicinal plants produced an increase of villus height, villi width, and the absorptive surface area of the intestine by the modulation of the renewal of the intestinal epithelial cells, resulting in the improvement in nutrient absorption capability, and subsequently the enhancement in feed efficiency and weight gain (Abdel-Tawwab et al., 2018; Antushevich et al., 2014; Crosnier et al., 2006). Moreover, antimicrobial and antioxidant properties of medicinal plants could reduce pathogenic microorganisms in the gut and enhance efficient nutrient digestion and performance (Chakraborty & Hancz, 2011; Nanasombat & Teckchuen, 2009; Tatli Seven et al., 2008). There are limited research studies on the effect of dietary LE on the intestinal histomorphology of frogs. It is hypothesized that the improvement of villi height in frog has been achieved due to such phytochemicals found in LE, which would modulate cell division, cell proliferation, and cell apoptosis in the tract and these could positively affect the growth of frog (Antushevich et al., 2014; Crosnier et al., 2006; Wlodarska et al., 2015).

5. Hematological analysis

As shown in Table 5, it was demonstrated that the highest level of Hb was found in frogs fed with 5 g LE/kg diet ($P < 0.05$). However, dietary LE did not affect the values of Ht, WBC, and RBC compared with the control ($P > 0.05$).

Hematological indices have long been used to assess the health status and the immune system of aquatic animals (Abdel-Tawwab et al., 2018; Mohammadi et al., 2018; Ramezanzadeh et al., 2020). The levels of RBC, Hb, and Ht are important indicators of erythrocyte status and oxygen transport efficiency (Coppo et al., 2005a). Moreover, WBC level would demonstrate the ability of innate immune response to defend frog from the external environment (Gabriel et al., 2015; Ramezanzadeh et al., 2020). In this report, frog fed with a diet containing 5 g LE/kg showed a significant increase of Hb compared with the other groups. However, no significant differences in WBC, RBC, and Ht were noticed in frog fed with LE-supplemented diets. Hematological values observed in this research were similar to those published on common lowland frog (Wongtha et al., 2019), bullfrog (*Rana catesbeiana*) (Coppo et al., 2005b), and the African clawed frog (*Xenopus laevis*) (Chang et al., 2015). It is speculated that changes in Hb are related to environmental and physiological conditions (Coppo et al., 2005a, 2005b). Previous reports suggested that medicinal plants like Aloe (*Aloe vera*) (Gabriel et al., 2015), purple coneflower (*Echinacea purpurea*) (Oskoii et al., 2012), and barberry (*Berberis vulgaris*) (Ramezanzadeh et al., 2020) had a positive impact on Hb levels by increasing erythropoiesis or hemosynthesis in hematopoietic organs (Gabriel et al., 2015; Iji et al., 2010). Therefore, dietary LE could have erythropoietin effects on hematopoietic stem cells or could increase intestinal absorption and utilization of thiamine, riboflavin, vitamin E, niacin, and folic acid in frogs (Gabriel et al., 2015; Talmadge et al., 2004).

Table 5 Hematological analysis of common lowland frog fed LE-supplemented diets for 8 weeks

Parameters	LE in the diets (g/kg)			
	0 (Control)	1	3	5
Hb (g/dL)	9.90 ± 92^{b}	9.80 ± 0.39^{b}	9.75 ± 0.67^{b}	11.76 ± 0.43^{a}
Ht (%)^{ns}	27.58 ± 2.27	22.66 ± 2.07	30.16 ± 4.08	25.75 ± 1.65
WBC ($\times 10^4$ cell/mm^3)^{ns}	8.44 ± 0.41	8.81 ± 0.25	8.55 ± 0.23	8.73 ± 0.17
RBC ($\times 10^{11}$ cell/L)^{ns}	1.51 ± 0.13	1.20 ± 0.20	1.52 ± 0.12	1.31 ± 0.11

Remark: Data are presented as mean ± SEM. Different superscripts in the same row are significantly different ($P < 0.05$). Superscript ^{ns} indicates no statistical difference ($P > 0.05$); LE = lasia extract; Hb = hemoglobin (g/dL); Ht = hematocrit (%); WBC = white blood cells ($\times 10^4$ cell/mm^3); RBC = red blood cells ($\times 10^{11}$ cell/L)

6. Biochemical analysis

It was found that glucose level was markedly increased in frog fed with 5 g LE/kg diet compared with the other treatments ($P < 0.05$). The reductions of serum cholesterol levels were observed in frog fed with LE-supplemented diets compared with the control diet ($P < 0.05$). However, dietary LE supplementation had no detectable effects on serum total protein, albumin, AST, ALP, triglyceride, HDL-C, and LDL-C values compared with the control ($P > 0.05$) (Table 6).

Serum biochemical parameters have been used to demonstrate the health condition of aquatic animals (Cathers et al., 1997; Naiel et al., 2020; Wilson et al., 2011). Changes in blood chemical values of frogs may be attributed to both environmental and physiological aspects (Adeshina et al., 2019; Coppo et al., 2001; Coppo et al., 2005a). The present results revealed 5 g LE/kg diet significantly enhanced serum glucose level in the cultured frog. It is well known that changes in serum glucose levels are generally related to carbohydrate metabolism, energy utilization, O_2 consumption, stress, and several clinical illnesses (Adham et al., 2002; Coppo et al., 2005b). The highest glucose content observed in frog fed with 5 g LE/kg diet would indicate physiological

Table 6 Biochemical analysis of common lowland frog fed LE-supplemented diets for 8 weeks

Parameters	LE in the diets (g/kg)			
	0 (Control)	1	3	5
Total protein (g/dL)^{ns}	4.18±0.59	5.76±0.63	4.86±0.58	5.00±0.26
Albumin (g/dL)^{ns}	1.08±0.30	1.06±0.12	1.81±0.22	1.22±0.52
Glucose (mg/dL)	169.68±9.19^{b}	165.15±7.63^{b}	168.95±5.18^{b}	224.24±6.55^{a}
Cholesterol (mg/dL)	90.05±1.19^{a}	24.66±4.93^{b}	23.24±2.33^{b}	22.83±7.31^{b}
Triglycerides (mg/dL)^{ns}	12.58±0.84	19.47±2.93	19.55±1.04	19.28±9.74
AST (U/L)^{ns}	38.49±6.76	31.65±5.54	39.69±6.24	37.72±3.28
ALP (U/L)^{ns}	128.78±5.20	193.24±4.33	148.76±8.00	175.12±7.54
HDL-C (mg/dL)^{ns}	42.70±4.81	42.85±5.66	50.96±7.53	40.54±5.93
LDL-C (mg/dL)^{ns}	35.03±8.43	33.26±5.64	39.80±3.71	32.35±4.04

Remark: Data are presented as mean ± SEM. Different superscripts in the same row are significantly different ($P < 0.05$). Superscript ^{ns} indicates no statistical difference ($P > 0.05$); LE = lasia extract; AST = aspartate transaminase (U/L); ALP = alkaline phosphatase (U/L); HDL-C = high-density lipoprotein cholesterol (mg/dL); LDL = low-density lipoprotein cholesterol (mg/dL).

responses to stress (Güllü et al., 2016). In the current experiment, serum cholesterol contents were significantly declined in frog fed with LE-supplemented diets. This is in agreement with the results presented by Wongtha et al. (2019) who found that frogs fed for 8 weeks with different doses of *L. aromatica* showed a significant decrease in cholesterol levels compared with the control. Decreased cholesterol contents in this research may clarify a hypocholesterolemic effect of dietary LE, which would contribute to an improvement in cholesterol metabolism by increasing bile acid secretion or decreasing cholesterol absorption in the intestine of the frog (Nguyen et al., 2001; Yang et al., 2013). Also, LE may enhance cholesterol deposit in the fat body of frogs (Kasinathan et al., 1978; Panase et al., 2018). The mechanism by which dietary LE improve cholesterol metabolism in the frog is still questioned. It is hypothesized that some phytochemicals such as flavonoids and phenolic compounds found in LE would encourage the hypocholesterolemic effect (Park et al., 2014; Zou et al., 2005). Further research studies related to this aspect are needed to evaluate the effects of LE on the metabolism of cholesterol in frog. Serum total protein and albumin are useful for the clinical assessment of protein metabolism and the functions of the liver and kidney (Eckersall, 2008). Decreased total protein and albumin contents are correlated with malnutrition, chronic liver and kidney diseases, immunological disorders, and inflammations (Kreutzer et al., 2008). There were no changes in total protein and albumin levels in frog fed with LE-supplemented diets, suggesting that dietary LE did not have harmful effects on protein metabolism in frog. The level of AST is consistent with normal physiological functions of the liver, skeletal muscle, and cardiac muscle (Hoffmann & Solter, 2008). Markedly increased serum AST could suggest cellular injury in the liver or muscle (Campbell, 2012; Kreutzer et al., 2008). Serum ALP is found in bone, liver, kidney, and intestine (Coppo et al., 2001; Sharma et al., 2014). Serum ALP is increased following osteopathies and intestinal diseases (Golub & Boesze-Battaglia, 2007; Sharma et al., 2014). Serum HDL-C and LDL-C levels are correlated with lipoprotein metabolism in the frog (Bruss, 2008). Increased HDL-C and LDL-C may be due to hepatic and renal disorders, malnutrition, and infections (Coppo et al., 2005a). The present work indicated serum total protein, albumin, AST, ALP, HDL-C, and LDL-C levels did not change significantly among the treatments and those levels were similar to the reports of Coppo et al. (2005a) and Coppo et al. (2005b). Therefore, these findings encourage the use of LE as a natural feed additive in frog diet without a detrimental effect.

Conclusion

In conclusion, this research indicated the improvement in growth performance, feed utilization efficiency, and intestinal histology of frog fed LE-supplemented diets without a negative effect on the SR. Dietary LE did not affect the values of HSI, ISI, SSI, CSI, and RSI. Increased IPF was found in frog fed with LE-supplemented diets. There were no changes in Ht, WBC, and RBC levels among the treatments. Serum total protein, albumin, AST, ALP, triglyceride, HDL-C, and LDL-C were not influenced by LE-supplemented diets. However, Hb and serum glucose increased significantly in frog fed with 5 g LE/kg diet compared with the other treatments. Frogs that were fed with LE-supplemented diets showed a significant decrease in cholesterol levels. Preliminary phytochemical evaluation of LE demonstrated the presence of flavonoids, terpenoids, phenolic compounds, tannins, steroids, glycosides, and anthraquinone. The optimal level of LE estimated by using the second-order polynomial regression analysis was found to be 3.60 g LE/kg diet. Taken together, these findings provide valuable scientific evidence supporting the beneficial effects of dietary LE in the culture of frog.

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UV-C Disinfection in the Environment of Pathogen Transmission

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Abstract

Coronavirus disease 2019 (COVID-19) is a respiratory infection caused by SAR-COV-2 (COVID-19 virus). COVID-19 typically rapidly spread from one person to another via respiratory droplets (>5-10 μm in diameter) during coughing and sneezing or touch. Thus, environmental surfaces should be frequently cleaned with water, detergent, and followed by application of disinfection as chemical disinfectants and physical disinfection. Ultraviolet germicidal irradiation (UVGI) is the use of ultraviolet energy to inactivate viral, bacterial, and fungal species because the UVC directly damages deoxyribonucleic acid (DNA), Ribonucleic acid (RNA), and also prevent the bacterium from being viable. The most effective wavelength is in 245 -285 nm. Conventional ultraviolet disinfection consists of a mercury amalgam, which is a hazardous material to the environment. In addition, excessive exposure causes sunburn skin, skin cancer and damage to the corneas of the eyes. Therefore, the risk management such as enclosure cabinets, indicator warning sign light, and personal protection equipment (PPE) should be considered.

Introduction

On 31 December 2019, Wuhan Municipality in Hubei Province, People's Republic of China reported a cluster of pneumonia cases with unknown etiology. On 30 January 2020, more than 9,700 confirmed cases in China and 106 confirmed cases in 19 other countries, the World Health Organization (WHO) Director General declared the outbreak a public health emergency of international concern (PHEIC). On 11 February, WHO named the disease, COVID-19, short for "coronavirus disease 2019." On the same day, the International Committee on Taxonomy of Viruses (ICTV) announced "severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)" as the name of the new virus which causes COVID-19 (Pan American Health Organization [PAHO] & World Health Organization [WHO], 2020; Ragan et al., 2020). Coronavirus disease 2019 (COVID-19) is a respiratory infection caused by SAR-COV-2 (COVID-19 virus). Common symptoms include fever, cough, sneezing, sore throat, breathlessness, fatigue, pneumonia, acute respiratory distress syndrome (ARDS) (Hafeez et al., 2020) and multi organ dysfunction (Singhal, 2020; Hafeez et al., 2020; Occupational Safety and Health Administration [OSHA], 2020). Some symptoms of SARS-CoV-2 induced COVID-19 are a bit similar to influenza and seasonal allergies (pollen allergies) therefore suspected patients may also exhibit

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temperature which can be detected by thermo-scanners, as well as be detected with an accurate and rapid diagnostic kit of SARS-CoV-2 (Shereen et al., 2020). Time from exposure and symptom onset is generally between two and 14 days, with an average of five days. The elderly and people with underlying diseases are susceptible to infection and prone to serious outcomes (Guo et al., 2020).

COVID-19 transmission

SARS-CoV-2 is an enveloped virus with an outer lipid envelope as shown in Fig.1 (Hafeez et al., 2020), which makes it more susceptible to disinfectants compared to nonenveloped viruses. The virus that causes COVID-19 is typically rapidly spread from one person to another via respiratory droplets (>5-10 μm in diameter) during coughing and sneezing (WHO, 2020a) or close contact (Salem et al., 2020). Droplet transmission of the COVID-19 virus can occur by direct contact with infected people and indirect contact with surfaces. Environmental infection control of surfaces and limiting person-to-person contact are important steps (Jones, 2020). Recommended preventive measures include washing your hands with soap, covering the mouth when coughing, and monitoring and self-isolation for fourteen days for people who suspect they are infected (Hafeez et al., 2020). The survival time of the coronavirus 2019-nCoV were differed at different environment as shown in Table 1 (Zhou, 2020). Regular housekeeping practices include routine cleaning of surfaces, equipment, and other elements of the work environment to reduce exposure to hazards (OSHA, 2020). In addition, healthy indoor air quality (IAQ) should be interpreted by cleaning and disinfection of environmental surfaces (Kamaruzzaman & Sabrani, 2011).

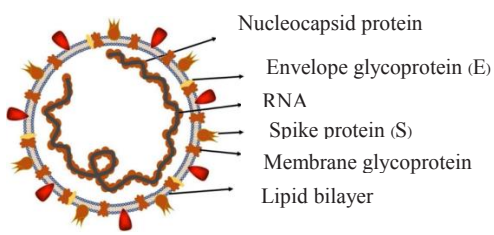

Fig. 1 A structure of SARS-CoV-2
Source: Hafeez et al. (2020)

Table 1 Survival time of the coronavirus 2019-nCoV on different surfaces and specific temperatures

Different environments	Temperature	Survival time
Air	50 – 59 F	4 hours
	77 F	2 – 3 minutes
Droplets	<77 F	24 hours
Nasal mucus	132.8 F	30 minutes
Liquid	167 F	15 minutes
Hands	68 – 86 F	< 5 minutes
Non-woven fabric	50-59 F	< 8 hours
Wood	50 – 59 F	48 hours
Stainless steel	50 – 59 F	24 hours
75 % alcohol	Any temperature	< 5 minutes
Bleach	Any temperature	< 5 minutes

Source: Zhou (2020)

Environment disinfection

Cleaning is the removal of gross contamination, organic material, and debris from the premises or respective structures, via mechanical means like sweeping (dry cleaning), mechanical action (brushing or scrubbing) and/or the use of water and soap or detergent (wet cleaning). Its goal is to minimize dirt, debris, and organic material such as blood, secretions and excretions but does not kill microorganisms (Foreign Animal Disease Preparedness and Response Plan [FAD PReP], 2018; WHO, 2020a; WHO, 2020b). Disinfection is the methods used on surfaces to destroy or eliminate a specific species of infectious microorganism through physical (e.g., heat) or chemical (e.g., disinfectant) means (FAD PReP, 2018). It is the primary mechanism for the inactivation of pathogenic organisms to prevent the spread of diseases to the environment (Environmental Protection Agency [EPA], 1999).

1. Chemical disinfection

Environmental surfaces should be frequently cleaned with water, detergent, and disinfectant, respectively. Chemical disinfectants inactivate a wide variety of microorganisms. In indoor spaces, routine application of disinfectants to material surfaces via spraying or fogging (also known as fumigation or misting) is not recommended (WHO, 2020a). Spraying individuals with disinfectants (such as in a tunnel, cabinet, or chamber) is not recommended under any circumstances owing to health effects on eyes, respiratory system, skin irritation, and gastrointestinal (WHO, 2020a; WHO, 2020b). The concentration and contact time of disinfectant are also critical for effective surface disinfection (WHO, 2020b) as shown in Table 2 (Rutala et al., 2019; Ogilvie et al., 2021). The most commonly used alcohol-based disinfectants are

ethyl alcohol (ethanol 70-90%) and isopropyl alcohol (isopropanol). The oxidizing agents peroxide-based disinfectants are hydrogen peroxide, which solutions of 5-20% are considered for bactericidal, enveloped virus, fungicidal, and sporicidal (FAD PReP et al., 2014). The chlorine-based product (e.g., hypochlorite) at 0.1% (1000 ppm) was recommended for general environmental disinfection (WHO, 2020a). Chlorhexidine (also known as chlorhexidine gluconate) also effectively inactivates the virus (Zhou, 2020). Owing to the length of time that COVID-19 virus can survive on inanimate surfaces varies depending on factors such as the amount of contaminated body fluid (e.g. respiratory droplets) and environmental temperature as well as humidity; routine environmental cleaning on touched surfaces e.g. door handles, tabletops, light switches by using detergent solution and disinfectant solution can reduce environmental contamination (WHO, 2020c).

Table 2 Concentration and contact time of different chemical disinfectants for surface disinfection

Chemical disinfectants	Concentration required to destroy	Contact time
Alcohol	Ethyl alcohol and isopropyl alcohol 60%–90% solutions in water (volume/volume)	10 seconds
Chlorine compounds	5.25%–6.15% Sodium hypochlorite	1 minute
Formaldehyde	Formalin, which is 37% formaldehyde by weight	2 minutes
Glutaraldehyde	≥2% Aqueous solutions of glutaraldehyde, buffered to pH 7.5–8.5 with sodium bicarbonate	<2 minutes
Hydrogen peroxide	0.5% Hydrogen peroxide	1 minute
Iodophors	Iodine 75–150 ppm	seconds to minutes
Ortho-phthalaldehyde (OPA)	0.55% 1,2-benzenedicarboxaldehyde (OPA).	5 minutes
Peracetic acid or peroxyacetic	1,500–2,250 ppm	15 minutes
Peracetic acid and hydrogen peroxide	0.08% Peracetic acid plus 1.0% hydrogen peroxide, 0.23% Peracetic acid plus 7.35% hydrogen peroxide	20 minutes
Benzalkonium chlorides	0.2% w/w in water	15 seconds

2. Physical disinfection

Physical disinfection can be used by heat and ultraviolet radiation. Heat is the thermal inactivation of infectious agents. Heat destroys microorganisms by causing deoxyribonucleic acid (DNA) disruption, protein denaturation, oxidative damage, and loss of membrane integrity. The time required is inversely related to the temperature and directly related to the number of microorganisms. Heat can be applied under moist (e.g., autoclave, steam) or dry (e.g., incineration, baking) conditions. Moist heat can be effectively applied through steam under pressure when dealing with thermally resistant bacterial spores. Dry heat applications can be useful for the disinfection of heat-resistant materials, such as glass or metals. Moist heat applications are generally more effective and require less time than dry heat. In addition, the coronavirus is sensitive to sustained heat at 132.8°F for 30 minutes (Zhou, 2020).

The Ultraviolet (UV) in sunlight is in the wavelength 300-400 nm and is not very effective to inactivate microorganisms. The most effective biocidal wavelength is in 245-285 nm. Inactivation of microorganisms results from destruction of nucleic acid through induction of thymine dimers. Bacterial spores will require 10 times the exposure time as the vegetative forms of the organisms. Its germicidal effectiveness is influenced by wavelength; UV intensity, type of suspension; and type of microorganism (Rutala et al., 2019). In addition, UV in 245–285 nm may be useful for the control of SAR-COV-2 airborne pathogens in enclosed areas because the coronavirus is sensitive to ultraviolet rays (Zhou, 2020).

Ultraviolet disinfection

UV emitted from the region of the solar spectrum between visible light (400-750 nm) and x-rays. The UV spectrum is commonly divided into UVA (wavelength of 400 nm to 315 nm), UVB (315 nm to 280 nm), UVC (280 nm to 200 nm) (Martin et al., 2008), and vacuum UV (VUV) at 100-200 nm (Vatansever et al., 2013). The atmospheric ozone layer in the stratosphere, about 10-50 kilometers above the Earth's surface absorbs and scatters UV light (UVB and UVC) from the Sun (Carlowicz, 2013). The absorption of radiation by atmospheric gases and scattering by atmospheric aerosols, and clouds, as well as the earth's surface prevent all UVC radiation from reaching the troposphere and the earth's surface (Kerr, 2005). The earth absorbs much radiation from the Sun to warm the atmosphere, the land, and the oceans.

UVA and UVB cause sun burning, photo aging skin (Vatansever et al., 2013), and also damaged eyes (Solomon, 2008). Prolonged human exposure to solar UV radiation may result in acute and chronic health effects on the skin, eye and immune system (WHO et al, 2002). UV radiation emitted by sunbeds, is a complete carcinogen, as it acts both as an initiator and a promoter.

An initiator causes a genetic mutation or epigenetic changes while a promoter causes rapid cell growth. There is no threshold level of UV–fluence and UV-dose for the induction of skin cancer. The beneficial effects of sunbed use, such as generation of vitamin D (Orazio et al., 2013), are outweighed by the adverse effects. There is no need to use sunbeds to induce vitamin D production because alternative sources of vitamin D are readily available. Therefore, there is no safe limit for exposure to UV radiation from sunbeds (Scientific Committee on Health, Environmental and Emerging Risks [SCHEER], 2017). In addition, solar UV radiation penetrates to ecological significant depths in aquatic systems and can affect in the food web (Häder et al., 2007). Exposure to solar UV radiation can reduce productivity of aquatic organisms. Corals live in an environment characterized by high ambient levels of UVC radiation resulting in the death of gastrodermal cells and injured as evidenced by pale coloration (Basti et al., 2009).

UVC has also been an important tool for wastewater treatment (Vatansever et al., 2013). The Photocatalytic of UVC radiation and the adsorption with nanoclay can remove an anionic dye (RR120) from wastewater (Siahpoosh & Soleimani, 2017). The application of UVC radiation caused COD removal in the Sulphate Radical-Based Advanced Oxidation Processes (SR-AOP) of winery wastewater treatment (Amor et al., 2019). The harmful anionic surfactants in aqueous ecosystems can be degraded by using H_2O_2/UVC system (Rios et al., 2017). The UVC/H_2O_2, VUV and VUV/H_2O_2 processes removal total organic carbon (TOC) from the Biologically-treated hospital wastewater (BHW) (Moussari et al., 2019). The combined ozone/UV treatments were able to achieve zero *E. coli* and *A. niger* count for cloudy and turbid medium, like flour slurry (Sangadkit et al., 2020).

UVC has also been an important tool for food processing industry (Vatansever et al., 2013). It over 60 seconds decreased the inoculated *Staphylococcus aureus* population in precooked shredded bullfrog meat (Silva et al., 2015). It reduced the Salmonella spp. in caiman meat (Canto et al., 2019). Its radiation improved the safety of fish fillets from fresh water and marine sources (Ahmed & Amin, 2019). The combination of its irradiation and lactic acid bacteria (LAB) inhibited the growth of *Salmonella enteritidis* during storage of fresh-cut apples (Chen et al., 2017). It treated strawberries inhibit microbial loads and delay ripening process of fruit products (Idzwana et al., 2019). Its treatment reduced the natural microflora growth of fresh rocket leaves (Rivera-Gutiérrez et al., 2015). The combination of it and aerosolized 2% malic acid can inhibit the growth of foodborne pathogens on fresh-cut lettuce (Seong et al., 2016). Its radiation removed larvae (Adams et al., 2018) and increased mortality of adult insects (Poushand et al., 2017).

Ultraviolet germicidal irradiation (UVGI) has been used in disinfection infectious agents. UVGI emits nonionizing electromagnetic radiation with UVC wavelength of 254 nm inactivate viral, bacterial, and fungal species (Martin et al., 2008) through the damage to DNA and ribonucleic acid (RNA). The UVC spectrum has been produced by mercury vapor arc lamps enclosed in a quartz tube (Martin et al., 2008; Vatansever et al., 2013). The dosage of UVC was proportional to distance from the UV device, exposure time, the amount of energy in watts per unit surface area (WHO, 2020b). Dose and is expressed in microwatt seconds per centimeter squared (μWsec/cm^2). Divide by 1000 to express the dose in the preferred notation millijoule per centimeter squared (mJ/cm^2) (Renzel, 2016). Furthermore, humic materials can shield embedded bacteria (EPA, 1999). Thus, UV-disinfection reactors should be designed to deliver the highest amount of radiation at direct radiation zone (Artichowicz et al., 2020). UVGI can reduce the concentrations of airborne bacteria in the indoor environment (Kujundzic et al., 2006; Makarapong et al., 2020; Szeto et al., 2020). The combination of UVC mainly 254 nm and VUV at 185 nm can inactivate airborne human pathogens for bacteria (Szeto et al., 2020). In addition, airborne bacteria were reduced between 79 and 91% while most surfaces also showed reductions in bacteria from 48 to 69% (Lee, 2017). The capacity of a portable ultraviolet-C equipment (UV Sanitizer Corvent® -UVSC-) in disinfection Pathogens: *Acinetobacter baumannii, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Candida albicans* was achieved with 99.99% at 120 seconds of exposure time (Guridi et al., 2019). UVGI was disinfection of air in health care facilities to meet indoor air quality (IAQ) requirements (Memarzadech et al., 2010; Warren et al., 2020). The advantage of UVC using in environmental disinfection were shown in Table 3 (Ontario Agency for Health Protection and Promotion, 2018; Elguijja et al., 2020).

Table 3 Advantage and disadvantage of UVC using in environmental disinfection

No.	Advantage	Disadvantage
1.	Broad-spectrum activity against pathogens involved in healthcare-associated infections	-
2.	Can be used for disinfecting both environmental surface as well as medical devices	Cannot be used as stand-alone disinfection
3.	Rapid contact time, for example, 15 minutes for vegetative bacteria	-
4.	Has a sporicidal activity after longer exposure for up to 50 minutes	-
5.	Does not require closing the HVAC (Heating-Ventilation-Air Conditioning) system, nor sealing the room	The room must be vacated for decontamination
6.	Eco-friendly, with no residue	-
7.	Low operating costs	High capital costs

Conventional ultraviolet (UV) disinfection consists of a mercury amalgam encapsulated within a quartz sleeve (Simons et al., 2018). Due to the Minamata conventional of The United Nations Environment Programme (UNEP) relating to storage of Mercury compounds (Article10) and mercury waste in Basel Convention on the Control of Transboundary Movements of Hazardous Wastes (Article 11), the mercury content of conventional was limited in order to ensure human and environmental health (Kim & Kang, 2018). Because of Mercury is a very toxic substance on both human health and the environment. Inhalation of mercury vapour can cause harmful effects on the nervous as insomnia, memory loss, digestive and immune systems. With the environment, mercury vapour can bio-accumulates and converted to methyl mercury by microorganisms. Therefore, the Ultraviolet Light-emitting diode (UVC LED), which does not contain mercury, and Xenon lamps have gained prominence. In addition, arrays of LED and pulsed xenon-based ultraviolet light (PX-UVC) can be efficient inactivation (Mori et al., 2007; Simons et al., 2018; Kim & Kang, 2018; Xiao et al., 2018; Casini et al., 2019). However, the UV-C Light-emitting diode (LED) at a wavelength of 240 nm produces ozone, which is a strong oxidant and toxic air pollutant (Vatansever et al., 2013; Global Lighting Association, 2020).

UVC Disinfection of COVID-19 virus

Owing to SARS-CoV-2 as the virus which causes COVID-19, the previous UVC methodology and results of other researchers concentrating on virus were concluded as shown in Table 4.

Table 4 Example of UVC exposure on virus

Description of virus	Methodology (wavelength, fluence, duration of exposure)	Results	References
H1N1 on Petri dishes at distance of 5 cm at 25°C	VUV 185 nm, 21 mW /cm^2, 0 to 15 minutes	Inactivate by 2.2-log10 within 5 minutes and more than 4-log10 within 20 minutes	Szeto et al., 2020
H3N2 on Petri dishes	VUV 185 nm, 21 mW /cm^2, 0 to 15 minutes	Inactivate by 3.0-log10 within 5 minutes and more than 4-log 10 within 20 minutes	Szeto et al., 2020
SARS-CoV-2	UVC 254 nm, 80 J/mL, 52 minutes	Reduction ≥ 4.79 ± 0.15 log10 in plasma and 3.30 ± 0.26 log10 in whole blood	Ragan et al., 2020
SARS-CoV in plasma	UVC 254 nm, 30 J/cm^2	Inactivate by ≥3.1-log10	Eickmann et al., 2020
Crimean–Congo haemorrhagic fever virus (CCHFV) in plasma	UVC 254 nm, 30 J/cm^2	Inactivate by ≥3.2-log10	Eickmann et al., 2020
Nipah virus (NiV) in plasma	UVC 254 nm, 30 J/cm^2	Inactivate by ≥2.7-log10	Eickmann et al., 2020
SARS-CoV-2 on Petri dishes	Deep ultraviolet light-emitting diode (DUV-LED) 280 ±5 nm, 225 mW/cm^2, 60 seconds	Inactivate >3.3-log10	Inagaki et al., 2020
MS2, Qβ, and ϕX174 viruses in a chamber-type air disinfection system	UVC LED 254 nm, 45 mJ/cm^2, 10 minutes	Inactivate 5-log10	Kim & Kang, 2018
Ebola on Petri dishes	UVC 254 nm, 4-17 J/m^2, 0 to 30 seconds	Survival 3%–4%	Sagripanti & Lytle, 2011
Lassa on Petri dishes	UVC 254 nm, 4-17 J/m^2, 0 to 30 seconds	Survival 9%–10%	Sagripanti & Lytle, 2011
Respiratory Adenovirus	UVC 254 nm, 2608 W s/cm^2, 30 minutes	Survival 32.9%	Walker & Ko, 2007
Coronavirus.	UVC 254 nm, 599 W s/cm^2, 30 minutes	Survival 12.2%	Walker & Ko, 2007
SARS-CoV	UVC 254 nm, 4016 μW/cm^2 (where μW = 10^{-6} J/s), 15 minutes	Inactivation 400-fold within 6 minutes	Darnell et al., 2004
SARS coronavirus strain CoV-p9 at distance of 80 cm	260 nm, > 90 w/cm^2, 15 to 150 minutes	CPE (cytopathic effect) dropped from +++CPE (51–75% cells) to +CPE (less than 25% cells) after 15 minutes	Duan et al., 2003

UVC technology is an important component of eliminating viral infections in environment (Paria et al., 2018) and also blood-borne pathogens (Ragan et al., 2020). The robotic UVC radiation was effective in inactivating 99.9% of *Pseudomonas aeruginosa,* which is more tolerate to UVC than the coronaviruses, on various surfaces including glass, plastic and stainless-steel within 10 minutes at the distance of 3 meters from the device (Vorapaluk et al, 2020). In addition, UVC 254-nanometer light robots of The San Antonio-based company Xenex Disinfection Services, which the robot named KENNEDY could eliminate corona virus 99.999% after two minutes of exposure at one-meter distance (Kalyani et al., 2020). Increasingly placed on the market with a greater range of UVC applications comes a greater risk of accidental exposure (Global Lighting Association, 2020). Excessive exposure causes sunburn skin, skin cancer and damage cornea of eyes. Risk management were engineering controls such as enclosure cabinets, screened area and administrative controls such as indicator warning sign light elimination of reflected UV from shiny surfaces, personal protection equipment e.g. latex gloves and eye protection. A UV-C warning symbol according IEC 61549-310-1 (Fig. 2), should be black on the yellow background with wording "Warning UV-C emitted from this product. Avoid eye and skin exposure to unshielded product. Follow installation instruction and user manual" (Global Lighting Association, 2020). In addition, humans and animals should not be exposed to high levels of UV light due to the potential for damage to the skin or eyes (FAD PReP et al., 2014). Notably, these technologies developed for use in health-care settings are used during terminal cleaning (cleaning a room after a patient has been discharged or transferred), when rooms are unoccupied for the safety of staff and patients (WHO, 2020b).

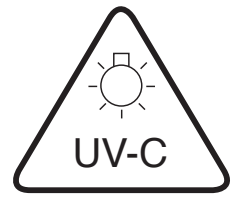

Fig.2 Warning sign light

Moreover, the environmental measures proposed according to the probability level of COVID-19 virus infection spreading and workplace risk level were shown in Table 5 (Cirrincione et al., 2020). It aims at limiting the survival of the virus in key environments of non-healthcare facilities such as schools, institutions, offices, daycare centers, businesses, and community centers that do not house persons overnight. Extraordinary cleaning and disinfection procedures must be adopted using the appropriate disinfectants in the very airy confined spaces, personal protection equipment (PPE) as well as proper disposal of regulated waste (Department of Public Health, 2020).

Conclusion

COVID-19 typically rapidly spread from one person to another via respiratory droplets (>5-10 μm in diameter). Thus, environmental surfaces should be frequently cleaned with water, detergent, and followed

Table 5 Environmental measures proposed according to the workplace risk level

Risk level.	Probability level of infection spreading	Environmental measures
Low	• Located in areas where there are no reported cases of disease contamination in the entire province • With a maximum of 10 employees • With mainly carry out office activities with a limited flow of customers	• Take extraordinary cleaning and sanitization • Considering a density of 1 person every 10 square meters • Keep a distance of 2 m between 2 or more people
Medium	• Located in areas where there are reported cases of disease contamination in the province • With a maximum number of 50 employees • With mainly carry out commercial activities; Which expose employees to sporadic contact with customers	• All measures indicated for the previous level controls • Indoor temperature >20 °C, indoor humidity >60% • Preparation of special bins for the collection of contaminated materials
High	• Located in areas in which in the neighboring cities or in the same city of the workplace, there are clear cases of disease contamination • With a maximum number of over 50 employees • With carry out front-office activities in continuous contact with customers	• All measures indicated for the previous level • Prepare a suitable room for the isolation of any suspicious cases
Very high	• Very-high-exposure-risk jobs include healthcare workers such as doctors, nurses, dentists, paramedics, emergency medical technicians	• All measures indicated for the previous level • Ensure appropriate air-handling systems • Negative pressure compared to the atmospheric one • Sanitization through the use of physical means such as ultraviolet irradiation (UV)

by application of disinfection as chemical disinfectants and physical disinfection. UVGI in the wavelength of 245–285 nm directly damages deoxyribonucleic acid, ribonucleic acid, and also prevent the bacterium from being viable. However, its risk is mercury amalgam, which is a hazardous material. In addition, excessive exposure causes sunburn skin, skin cancer and damage to the corneas of the eyes.

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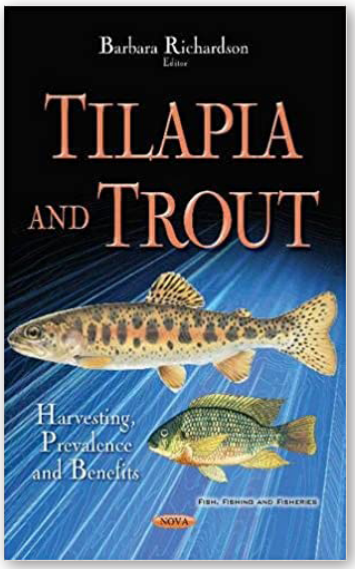
Book Review

Narin Charoenphun



Book name:	Tilapia and Trout: Harvesting, Prevalence and Benefits
Series:	Fish, Fishing and Fisheries
Editor:	Barbara Richardson
Published:	Nova Science Publishers, Inc., 2017
Paperback:	169 pages
Language:	English
ISBN:	978-1-53610-557-5

Tilapia and trout are delicious and highly nutritious fish. Currently, they are economically valued and have gained popularity in widespread consumption. This book discusses the harvesting, prevalence and benefits of tilapia and trout. There are six main chapters of a research and review articles.

Chapter 1: Risks and benefits of tilapia

Chapter 2: Human health risk assessment of heavy metals in the consumption of the fish

Chapter 3: Utilization of by-products and waste generated from the tilapia processing industry

Chapter 4: Thermal ecology of brown trout and the climate change challenge

Chapter 5: Reparative neurogenesis in the adult trout brain and peculiarity of development in the trout's brain cells in primary culture

Chapter 6: Effects of plant-based feeds on the immune responses of rainbow trout

This book is recommended for students, food scientist, fishery scientist, environmentalists, academics, and researchers who want to increase their knowledge. The book can also benefit the public to gain understanding of tilapia and trout in different aspects.

Reviewer

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