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

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295 Nakhon Ratchasima Road, Dusit, Bangkok, Thailand 10300
Phone: +662 244 5801-2 Fax: +662 668 7460

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295 Nakhon Ratchasima Road, Dusit, Bangkok, Thailand 10300
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Ethanol Production from Sago Palm Residue Pretreated with Two-Stage Chemical Process by Using Seed Sludge

Patcharee Intanoo* & Nunticha Thammo

Department of Industrial Chemistry and Textile Technology, Faculty of Science, Maejo University, Chiang Mai, 50290 Thailand

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Abstract

The objectives in this work are to study the pretreatment of sago palm residue via two-stage chemical pretreatment process and to investigate the optimum fermented temperature for ethanol production by using seed sludge from wastewater treatment plant. The two-stage chemical pretreatment process with 0.5 %w/v sodium hydroxide followed by 0.26 %w/v sulfuric acid of sago palm residue was correlated with the removal of lignin and the disruption of cellulose structure, respectively. The pretreated sago palm residue was hydrolyzed with cellulase enzyme (Cellusoft ® CR conc) in order to convert cellulose and hemicellulose of sago palm residue to reducing sugar. The reduction of sugar was further fermented to produce ethanol by seed sludge at different temperature. From the study, it was found that the optimum hydrolysis time for pretreating sago palm residue was 72 hours. The pretreated sago palm residue via two-stage chemical pretreatment process gave a 49.63 % decrease in lignin and 81.57 % decrease in crystalline cellulose. It is suggested that the pretreated sago palm residue was easier to digest in reducing sugar by cellulase enzyme (Cellusoft ® CR conc) resulting in a fermented ethanol that was enhanced 3.2 times. Under a fermented temperature of 45°C, the process performance was more efficient in terms of the maximum ethanol yield.

Introduction

Currently, energy is a basic factor in daily life with the main energy source being petroleum. There is continuous population growth, that causes a high demand for energy resulting in diminishing energy resource and unstable oil prices. In order to solve this problem, most researcher try to search for alternative energy that is sustainable and environmentally friendly such as biogas, ethanol, butanol and biodiesel (Kuiprasut, 2008). Ethanol and butanol are the most attractive alternative energy because they have the potentialfor replacing gasoline (Ouephanit et al., 2012). Butanol has overcome ethanol in term of 7.5 times lower of Reid vapor pressure (RVP) and low volatility that is important relating to the function and operation of gasoline-powered, especially carbureted (Ouephanit et al., 2012). Due to high flash point, hence it is safe for use. Moreover, butanol has a high energy

* Corresponding Author
e-mail: tle_turn@hotmail.com

value of 24 % (Ouephanit et al., 2012). However, the production of butanol has not been popular among researchers because of the high investment and the limitation of process production that includes giving a lower yield than ethanol (Sonil et al., 2014). For butanol production, the specific cultures required are *Clostridium* sp. and *Bacillus* sp. (Watchara & Benjamas, 2011). Both *Clostridium* sp. and *Bacillus* sp. are expensive microorganism and the optimum condition should be a completely anaerobic environment and sterile which is difficult to control when the organic waste used is a raw material (Sonil et al., 2014).

Gasohol is an alternative fuel for gasoline engines. It is a mixture between gasoline and ethanol in a different ratio, for example 80:20 (National Science and Technology Development Agency: NSTDA, 2010) and 85:15 (Kuiprasut, 2008). The addition of ethanol not only tends to increase the octane number but also could reduce the use of fossil fuel and be environmentally friendly. Ethanol is the substance produced from lignocellulosic material which is mostly agricultural waste via a fermentation process under oxygen limit condition (Pawongrat, 2015). The purification of produced ethanol is obtained as high as 99.5 % by a distillation process.

Ethanol can be produced from various types of waste material such as agriculture, wood and crop residue (Klaichom, 2011) which consists of cellulose, hemicellulose and lignin. Lignin is the composition enveloping cellulose and hemicellulose. Therefore, the pretreatment is a necessary process for lignin removal and microorganism can digest both cellulose and hemicellulose and further converts to reducing sugar: monosaccharides for example glucose and xylose (Pawongrat, 2015). Reducing sugar is fermented to ethanol simultaneously (Ditkunchaimongkol, 2015).

Large quantities of agricultural waste are abandoned, especially sago palm residue which not only causes problems of odor but also water pollution during the rainy season. For 100 kg of sago starch produced, 40 kg of sago palm residue is generated. Moreover, there is some starch remaining in sago palm residue (54-60%) and it has high cellulose content (Pitchayapon, 2018). Therefore, sago palm residue was considered as one suitable lignocellulosic raw material for ethanol production. Naturally, many species of microbes coexist by interacting with each other, whereas many species of microbes are most effective only when they are present in association with other groups of microbes. In this study, sago palm residue was used as a raw material to produce ethanol by using seed sludge. The optimum condition for pretreatment of sago palm residue and the hydrolysis process of pretreated sago palm residue by Cellusoft ® CR conc enzyme was also studied. The ethanol production by seed sludge was also attempted.

Materials and methods

1. Substrate and seed sludge preparation

The sago palm residue used in this study obtained from Yai Chui Farm, Phatthalung, Thailand. It was dried under sun light for 7 days. The dried sago palm residue was crushed with electrical blender at 14,000 round per minute (rpm) for 3 minutes. The crushed sago palm residue was screened by a 60 mesh sieve. The powder of sago palm residue was kept in desiccator before use.

Cellusoft ® CR conc enzyme was obtained from Brenntag Ingredients (Thailand) Pub Co., Ltd., Bangkok, Thailand. The seed sludge was collected from the biogas plant treating ethanol wastewater of Sapthip Co., Ltd., Lopburi, Thailand, which was first concentrated by sedimentation, and the concentrated sludge was then ground and screened by sieving to remove large solid particles. The sieved seed sludge without heat treatment was directly added to a reactor. The microbial concentration, in terms of the mixed liquid volatile suspended solids (MLVSS) for the start-up in this study was about 71,000 mg/L. The genus of microbes might be found in this study seed sludge was facultative anaerobes such as acidogen and methanogen.

2. Proximate analysis and chemical composition

The proximate analysis and chemical composition of sago palm residue were investigated by using standard method that have been reported in the previous work (Sengar et al., 2012). Proximate analysis is covering with organic and inorganic compound in sago palm residue. Volatile matter (VM) was mostly found in sago palm residue indicating that this studied sago palm residue contained mainly organic compound which was easily digested by microbes. Moreover, the significant chemical composition is cellulose, hemicellulose and lignin. The order of fractions in the sago palm residue sample was hemicellulose > cellulose > lignin > ash.

3. Two-stage chemical pretreatment process

10.00 g of sago palm residue powder was immersed in 0.05% w/v NaOH solution in the ratio of 1:10. The solution of sago palm residue was refluxed at 100°C for 2 hours. After refluxing, 0.05 M H_2SO_4 was added to the solution in the ratio of 1:10 and left for 24 hours. The

solution was allowed to reflux again at 100°C for 30 minutes. The crystalline structure of cellulose in untreated and pretreated sago palm residue were determined by X-ray diffraction method.

4. Hydrolysis

The pretreated sago palm residue was hydrolyzed by Cellusoft ® CR conc in order to break down cellulose to reduce to sugar. Before hydrolysis process, the solution from two-stage chemical pretreatment process was adjusted to pH 5.00 with 0.1 M NaOH. The added enzyme solution was shaken at 150 rpm under the temperature of 50°C for 72 hours. The sample was taken out every 12 hours for analyzing the reduction of sugar by dinitrosalicylic colorimetric (DNS) method (Michael, 1988).

Dinitrosalicylic colorimetric method was analyzed by adding 1.00 ml of 0.03 M dinitrosalicylic solution to 1.00 ml of sample from the hydrolysis step. Then, the reaction was stopped by heat for 10 minutes. Spectrophotometer with the wavelength of 540 nm measured the amount of reduced sugar (Young et al., 2013).

5. Ethanol production

Ethanol was produced by using batch process with a working volume of 220 ml. The reactor was under closed-system for allowing an anaerobic condition. The oxygen was a factor that directly affected the activity of facultative anaerobes in seed sludge (Kanchanatawee, 2012). The solution from hydrolysis step was fed to the reactor containing 20%v/v of the seed sludge. Fermentation process was operated under shaker bath at 100 rpm for 7 days. The system was performed at different fermented temperature of 37, 45 and 55°C without pH control in order to determine the optimum fermented temperature for ethanol production. For each fermented temperature, the sample was quickly taken out of the system for detecting the produced ethanol by HPLC every 4 hours.

Results and discussion

1. Two-stage chemical pretreatment process

Fig. 1 shows the X-ray diffraction patterns of untreated, basic pretreated and two-stage pretreated sago palm residue. The crystalline structure of cellulose in untreated sago palm residue was assigned a sharpen diffractogram at 15.6° and 22.3° (2θ) (Banik et al., 2015). On the other hand, the crystalline structure of cellulose in pretreated sago palm residue gradually disappeared which was consistent with the sharpen diffractogram at 15.6° and 22.3° which became broad. It can be suggested that the two-stage chemical pretreatment process not only has an effect to the decrease in lignin composition but also the crystalline structure of cellulose showed disruption causing intermolecular force: hydrogen bond and β-1,4-glycosidic linkage was weak (Piakong, 2014) therefore, the cellulose and hemicellulose content of pretreated sago palm residue was 81.57 and 28.25% higher than that the untreated sago palm residue, respectively.

Sago palm residue is lignocellulosic material consisting of cellulose, hemicellulose and lignin. Cellulose and hemicellulose are significant components for the production of ethanol. Whereas, the lignin performs as a wall that prevents enzyme to digest both cellulose and hemicellulose in reducing sugar. Therefore, the pretreatment process was not only necessary for removing lignin but also for disrupting the crystalline structure of cellulose simultaneously. When the sago palm residue was pretreated with $NaOH/H_2SO_4$, it had 49.63% lower of lignin and the crystalline structure of cellulose became loosen compared to the untreated sago palm residue. Due to β-1,4-glycosidic linkage in cellulose and hemicellulose structure, they can be broken down to reduce sugar by cellulase enzyme (Charles et al., 2005; Ram & Roshan, 2018; Sonil et al., 2014), which will be discussed in the next section.

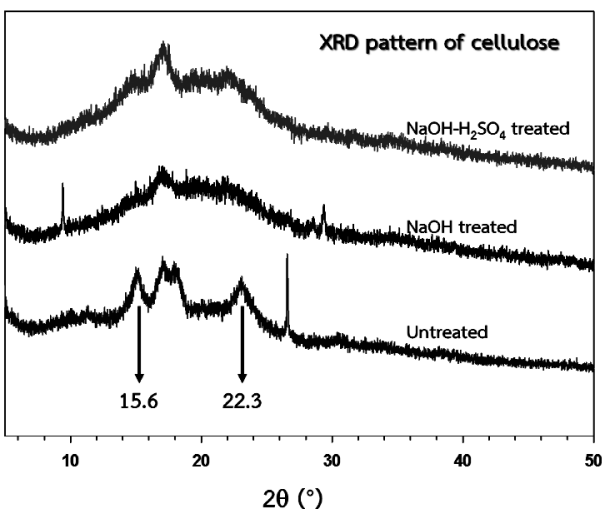
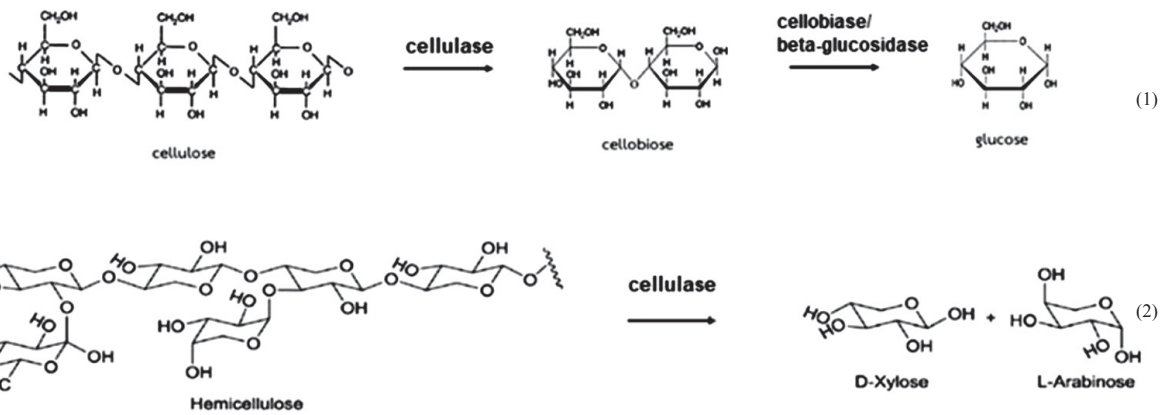


Fig. 1 X-ray diffraction patterns of untreated, basic treated and two-stage pretreated sago palm residue

2. Hydrolysis process

The pretreated sago palm residue was adjusted to pH around 5.0 with 0.1 M NaOH because this pH was an optimum condition for the activity of Cellusoft ® CR

conc enzyme (Kai & Lars, 2014). According to ethanol production process that requires a specific pathway, the Cellusoft ® CR conc enzyme was selected. Cellusoft ® CR conc enzyme can hydrolyzes (1,4)-beta-D-glucosidic linkages in cellulose and hemicellulose structure. The hydrolysis process was operated at a temperature of 50°C with shaking 150 rpm (Tabka et al., 2006). The reduced sugar was analyzed by using spectrophotometer at wavelength of 540 nm with DNS method. From the result, it was found that the concentration of reducing sugar increased with hydrolysis time and attained a maximum value of 19.53 mg/L (or 229.78 mg/g biomass) at a hydrolysis time of 96 hours. The production of reducing sugar from pretreated sago palm residue was about 3.2 time higher than that untreated sago palm residue. This was the same result with other related work (Farzad et al., 2013). Beyond a hydrolysis time of 96 hours, the reduced sugar concentration was constant (Fig. 2). The two-stage chemical pretreatment process result shows sago palm residue had more cellulose and hemicellulose content. Therefore, when pretreated sago palm residue went into the hydrolysis process, it easily converted cellulose and hemicellulose in reducing sugar (Equation 1-2) (Anthonia & Philip, 2015). In this study, it can be concluded that the optimum hydrolysis time for the production of reducing sugar was 96 hours.



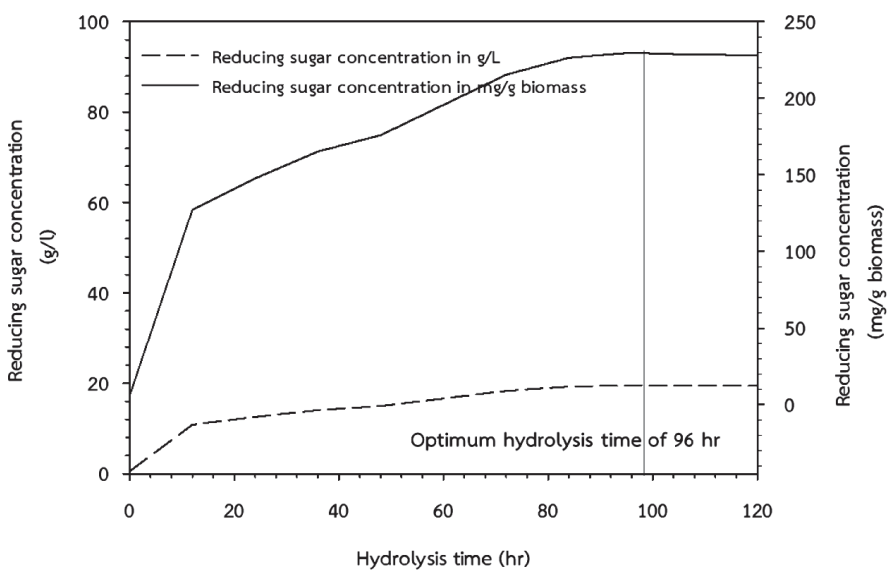


Fig. 2 Reducing sugar concentration at various hydrolysis time at 50°C for 120 hours ethanol production

Fig. 3 shows ethanol concentration at different fermented temperature. Ethanol concentration increased with increasing fermented temperature from 37°C to 45°C. The maximum ethanol concentration was found at a fermented time of 72 hours. However, the ethanol concentration decreased when fermented temperature increased from 45°C to 55°C. Hence, the temperature might be a factor that has an effect on the activity of microbes. The condition for producing ethanol was the same as biogas production system (Laowansiri et al., 2018), which is anaerobic environment, with various factors that have an effect on microbial activity such as temperature, pH, and oxygen content. Among those factors, temperature had the most significant effect on the microbe activity. At a higher temperature (beyond 50°C), the microbial activity particularly non-spore forming microorganisms (facultative anaerobes) was suppressed (Niamsup et al., 2009) because they could not withstand high heat (Lin & Hung, 2008). In this study, a fermented temperature of 45°C is considered an optimum condition for ethanol production using seed sludge.

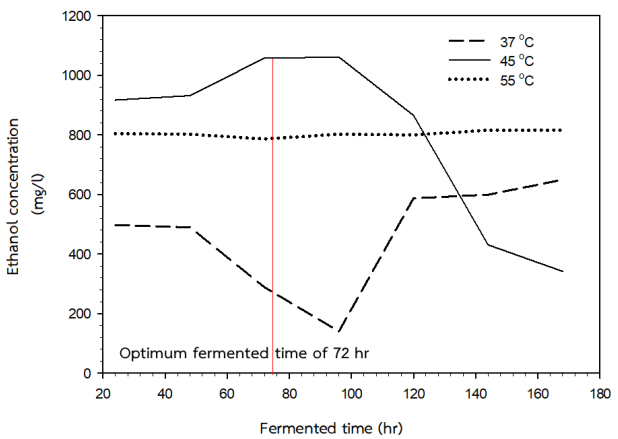


Fig. 3 Ethanol concentration at fermented temperature of 37°C, 45°C and 55°C

Under optimum fermented temperature of 45°C, the ethanol concentration increased with increasing fermented time from 24 to 96 hours which is consistent with the decrease in reduced sugar concentration (Fig. 4). It was suggested that reducing sugar was digested by microbes and further converted to ethanol (Kanchanatawee, 2012). The produced reduction of sugar such as glucose and xylose could be a carbon source for an ethanol producer (Gregory et al., 1998; Sonil et al., 2014). Both glucose and xylose are converted to ethanol via glycolysis which has pyruvate as a main product. The pyruvate changed to acetaldehyde under decarboxylation pathway by decarboxylase enzyme of anaerobe (Shang-Tian et al., 2007; Gregory et al., 1998). The acetaldehyde was transformed to ethanol via the reduction of acetaldehyde pathway by alcohol dehydrogenase enzyme (Shang-Tian et al., 2007; Gregory et al., 1998). Beyond a fermented time of 96 hours, both ethanol and reducing sugar decreased. The result from reducing sugar degraded to small organic acids which were acetic acid (HAc), propionic acid (HPr), butyric acid (HBu) and lactic acid (HLa) (Equation 3-6) under oxidative fermentation of ethanol (Mani et al., 2016; Pongdam, 2017). The produced organic acid might be accumulated in the system and further toxic to the growth of microbe (Kanchanatawee, 2012). Moreover, the pH of the system also decreased from 6.50 to 4.45 whereas, the optimum pH for the growth of microbe was around 6.00-7.00 (Kvesitadze et al., 2012). The toxicity from organic acids accumulation resulted from associated acid and most of produced organic acid found in this study was mainly weak acids that were difficult to break down (Kanchanatawee, 2012; Patcharee et al., 2012).

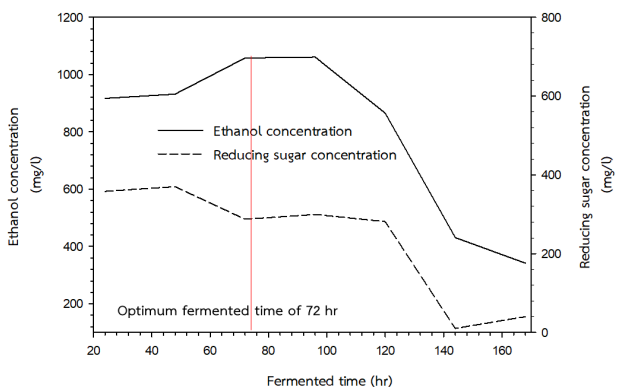


Fig. 4 Ethanol and reducing sugar concentration at an optimum fermented temperature of 45°C

Fig. 5 shows the concentration of small organic acids at different fermented time. The composition of small organic acids found in this study was acetic acid (HAc), propionic acid (HPr), butyric acid (HBu), and lactic acid (HLa). All acid concentrations tended to increase at any given fermented time. Not only was ethanol produced but the gaseous product was also obtained which were methane and carbon dioxide. It was suggested that reducing sugar was a small soluble organic molecule which easily degraded anaerobically by microbes. The

reducing sugar can be degraded to both ethanol and biogas, according to Equation 4-8, respectively (Piakong, 2014; Laowansiri et al., 2018).

$$C_6H_{12}O_6(l) + 2H_2O(l) \rightarrow 2CH_3COOH(l) + 4H_2(g) + 2CO_2(g) \quad (3)$$

$$C_6H_{12}O_6(l) + 2H_2O(l) \rightarrow 2CH_3CH_3COOH(l) + 2H_2O(l) \quad (4)$$

$$C_6H_{12}O_6(l) \rightarrow CH_3(CH_2)_2COOH(l) + 2H_2(g) + 2CO_2(g) \quad (5)$$

$$C_6H_{12}O_6(l) \rightarrow CH_3CHOHCOOH(l) \quad (6)$$

$$CH_3COOH(l) \rightarrow CH_4(g) + CO_2(g) \quad (7)$$

$$CO_{2(g)} + 4H_2(g) \rightarrow CH_4(g) + 2H_2O(l) \quad (8)$$

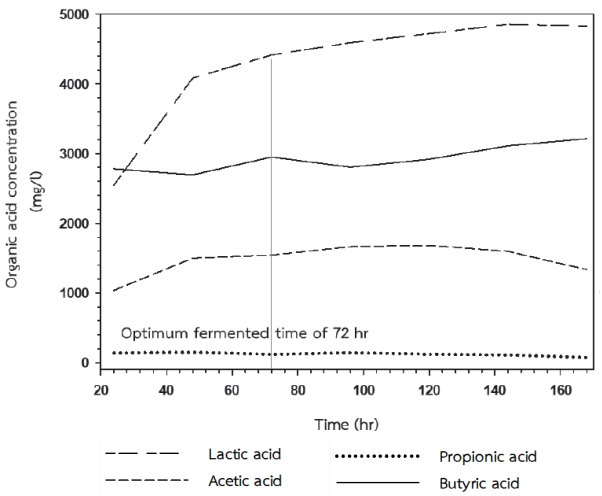


Fig. 5 Organic acid concentration at an optimum fermented temperature of 45°C

The comparison of ethanol production with different types of raw material, fermented temperature and pretreatment process is shown in Table 1. The pretreated raw material gave a higher ethanol concentration than the untreated raw material. The pretreated raw material was easily utilized by microbe since lignin composition as a thick wall enveloped cellulose and hemicellulose was eliminated (Farzad et al., 2013). In addition, the system operated at a higher fermented temperature of 45°C gave a higher ethanol production than that at a low fermented temperature of 37°C, according to Arrhenius' equation. At a higher temperature, the rate constant was higher resulting in a faster reaction rate causing a higher ethanol production performance. Under the same fermented temperature of 37°C, the produced ethanol found in this study was higher than the previous study (Farzad et al., 2013; Kim et al., 2017). Due to the use of sago palm residue as a raw material, the mixed culture from seed sludge was more suitable than that of single culture (Niamsup et al., 2009). The sago palm residue is an agricultural waste in the group of lignicellulosic material. It contains many complex structures such as starch, cellulose and hemicellulose which is difficult to digest by single microorganisms. In this study, seed sludge contained various types of microorganisms: cellulolytic microorganisms, amylolytic microorganisms, microorganisms that use sugar as food and acid-based microorganisms (Songrit & Kositanont, 2014). Therefore, the synergistic property of various kinds of microorganisms helps to degrade and digest sago palm residue affecting to the system by obtaining more ethanol production performance in terms of ethanol yield.

Table 1 Reducing sugar and ethanol concentration produced from different biomass, fermented temperature and pretreatment process

Biomass	Pretreatment process	Fermented temperature (°C)	Time (hr)	Reducing sugar concentration (greducing sugar/ kgbiomass)	Ethanol concentration (gethanol/ kgbiomass)	Ref.
Sago palm residue	NaOH/ H_2SO_4 (0.5 %w/v/0.26 %w/v)	45	96	229.78	62.18	Present study
Sago palm residue	NaOH/ H_2SO_4 (0.5 %w/v/0.26 %w/v)	37	48	229.78	32.82	Present study
Rice straw	-	37	72	102.00	<0.10	Farzad et al., 2013
Rice straw	Alkali	37	72	163.50	1.20	Farzad et al., 2013
empty fruit bunches	Dilute H_2SO_4 (1 %w/v)	30	72	68.00	18.50	Young et al., 2013
Spoilage date palm	-	30	72	915.80	1.15	Hemida & Elsadek, 2012
Coffee residue	H_2SO_4 (4 %w/v)	37	72	229.00	266.00	Kim et al., 2017

Conclusion

In this work, the ethanol production from sago palm residue by using seed sludge was investigated. The sago palm residue was chemically pretreated with 0.5 %w/v sodium hydroxide followed by 0.26 %w/v sulfuric acid via two-stage process. The pretreated sago palm residue gave a 49.63 % decrease in lignin, 44.92 % increase in cellulose and 22.02 % increase in hemicellulose. The maximum reduction in sugar concentration was as

high as 229.78 g glucose/kg biomass at optimum hydrolysis time of 96 hours. The use of dilute acid (<0.50% w/v) was more efficient to break β-1,4-glycosidic linkage of cellulose and hemicellulose structure causing the increase in ethanol production performance in term of ethanol yield. The optimum fermented temperature for maximize ethanol concentration was found at 45°C.

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Seasonal Flight Activity of Adult *Amphipsyche meridiana* Ulmer 1902 (Trichoptera: Hydropsychidae) in an Irrigation Pond Outlet

Thanawan Panich-Pat, Solaya Suksa-Ard & Taeng On Prommi*

Department of Biological Science, Faculty of Liberal Arts and Science, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom Province, 73140 Thailand

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Abstract

Seasonal flight activity of adult *Amphipsyche meridiana* Ulmer 1902 (Trichoptera: Hydropsychidae) in an irrigation pond outlet at Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom Province were investigated, with the influence of air temperature, wind speed, precipitation and relative humidity on its population density being evaluated. Samples were collected monthly from January to December 2016 by light trapping. Monthly sample records for adults collected were 3054, 6396, 576, 6654, 9228, 3222, 3402, 7974, 1950, 3483, 5016 and 5178, respectively. An increase in the number of adults collected was observed from April to May with peaks of 9,228 (16.48%) in May. A remarkable decrease in the number of insects collected was observed in the month after September. There was an increase in the number of adults with an increase in wind speed. Changes in the temperature, relative humidity and precipitation had less influence on seasonal flight activity of adult *A. meridiana.*

Introduction

Trichoptera or caddisflies, one of the largest groups of aquatic insects, are holometabolous insects with aquatic larvae and pupae and terrestrial adults (Wiggins & Currie, 2008). Trichoptera are potentially useful indicators of river and stream health (Chantaramongkol, 1983; Resh, 1992; Stanić-Koštroman et al., 2014). They are relatively easy to identify to species level in the adult stage and show a diverse range of ecological, behavioral and functional feeding modes as larvae. Furthermore, they are good indicators of environmental perturbation. Because they are distributed along the stream continuum, they constitute one of the most interesting groups for studying the ecology of organisms in running water (de Moor, 1999). Their seasonal activity is therefore essential to understanding the ecological impacts (Nowinszky et al, 2014).

Adult Trichoptera that emerge from streams live in the nearby riparian zone where they may select streamside trees as preferred sites to rest while awaiting proper swarming time, to feed in order to increase egg production or to mate (Jackson & Resh, 1991). Provision of suitable habitat for adult aquatic insects, both in terms

* Corresponding Author
e-mail: faastop@ku.ac.th

of its quality and quantity, is an important consideration as the adult stage can be critical in regulating population numbers of aquatic larvae and adults can play an important role in terrestrial food webs (Ormerod & Tyler, 1991).

Documentation for the use of adult caddisflies as bioindicators of water quality in Thailand has been given in the papers of Prommi & Permkam (2010), Prommi & Thamsenanupap (2012), Seetapan & Prommi (2012), Prommi & Thani (2014), Prommi et al. (2014), Prommi (2015) and Prommi et al. (2016). Caddisflies were chosen for this study because they are usually more diverse than other aquatic insect orders (Wiggins, 1996). Adults have been studied widely because they are easily collected by light traps and can be used as a useful tool for bioassessment (Greenwood et al., 2001; de Moor, 1999). Chantaramongkol (1983) recommended light trapping for assessing water quality in large rivers. Knowledge of the taxonomy and ecology of the species has proven valuable in biomonitoring programs because of differences in susceptibilities of the various species to pollutants and other types of environmental disturbances. Genus- or species-level identifications of adult caddisflies are possible and clearly produce more accurate results than family-level identification, thereby giving better ability to assess changes of water quality.

The caddisfly, *Amphipsyche meridiana* Ulmer 1902 (Trichoptera: Hydropsychidae), is an aquatic species that inhabits lake outlets. The main objective of this study was to monitor the occurrence and number of *A. meridiana* in an irrigation pond outlet. This pond received water from the Mae Klong Dam. Water from an irrigation pond is an important water resource for the University, and it serves mainly as a source of drinking water supply and agricultural activities.

Materials and methods

1. Sampling and laboratory analyses

The sampling was carried out in an irrigation pond outlet located at N 14°02.215′, E 099°57.818′ in Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom Province, central part of Thailand (Fig. 1). At each sampling date, adult caddisflies were collected using one unit of 10-watt portable black lights placed over 24 × 30 cm white plastic pans filled with detergent solution. The device was placed ~1 m from the stream edge. Specimens were collected one night in a month for 12 months from January to December 2016. In the laboratory, specimens were sorted and examined under a dissecting stereomicroscope. Specimen identifications were accomplished at the species level using Malicky (2010). Specimen counts from collections at each sampling month were summed. Abiotic factors (air temperature, precipitation, relative humidity and wind speed) in this study were obtained from Nakhon Pathom Meteorological Station year 2016, which is located nearby the sampling site.

Fig. 1 Photographs of the sampling site (A) light traps (B) and specimens contains in the white tray (C).

2. Data analyses

To evaluate the relationship between abiotic factors and adult *Amphipsyche meridian,* a Pearson correlation coefficient was used. Statistical analyses were performed using SPSS software (version 16.0) (http://www.spss.com/).

Results and discussion

The seasonal flight activity of *A. meridiana* was recorded all year round as with many tropical aquatic invertebrates, seems to have a multivoltine life cycle, the year- round favorable environmental conditions resulting in continuous growth and development (Humantinco & Nessimian, 2000). A total of 55,653 adult *A. meridiana* were captured during the sampling period (Table 1 and Fig 2). The number of *A. meridiana* specimens collected was 3054, 6396, 576, 6654, 9228, 3222, 3402, 7974, 1950, 3483, 5016 and 5178, respectively. Peak abundance of flying insects was recorded at the hot-dry season (April to May), which coincided with both high temperature

and wind speed. The abundant flying insect population probably was favoured by increased availability of microhabitats to provide cover and food (plants growing leaves and flowers) (Hill & Hill, 1994). Insect abundance declined to the lowest flying insects (Table 1) in the cold-dry season (September to December) coinciding with low temperature in the same period (Table 1). It has been noted that many tropical insect species become inactive at temperatures below 18°C, as there appears to be a preferred temperature for species, within which the insect thrives (Hill & Hill, 1994). Therefore, in the lowland habitat, seasonality in flying insect abundance could fairly be influenced by weather condition. The temperature, moisture (rainfall) and food supplies vary with season and are important factors for reproduction for insects (Miller & Harley, 1992).

Table 1 Total number of adult *Amphipsyche meridiana* collected over the period from January to December 2016. AT=Air temperature; PT=Precipitation; RH=Relative humidity; WS=Wind speed. All abiotic factors were obtained by Nakhon Pathom Meteorological station.

Month	Total	%abundance	AT (°C)	PT (mm)	RH (%)	WS (m/s)
January	3,054	5.38	26.10	0.00	72.50	4.10
February	6,396	11.39	26.60	0.00	68.00	4.70
March*	576	1.03	30.10	0.20	69.50	5.50
April	6,654	11.85	32.70	0.00	66.00	7.40
May	9,228	16.48	32.00	0.70	68.50	6.70
June	3,222	5.78	30.30	1.70	74.50	5.40
July	3,402	6.01	29.80	4.00	77.00	4.30
August	7,974	14.22	30.10	2.70	75.00	6.20
September	1,950	3.45	29.35	7.00	79.00	4.00
October	3,483	6.25	28.70	7.50	81.50	4.10
November	5,016	9.01	27.80	1.50	79.00	3.60
December	5,178	9.15	25.65	0.00	72.50	5.80
Total	55,653	100				

Remark: * raining during light traps operated.

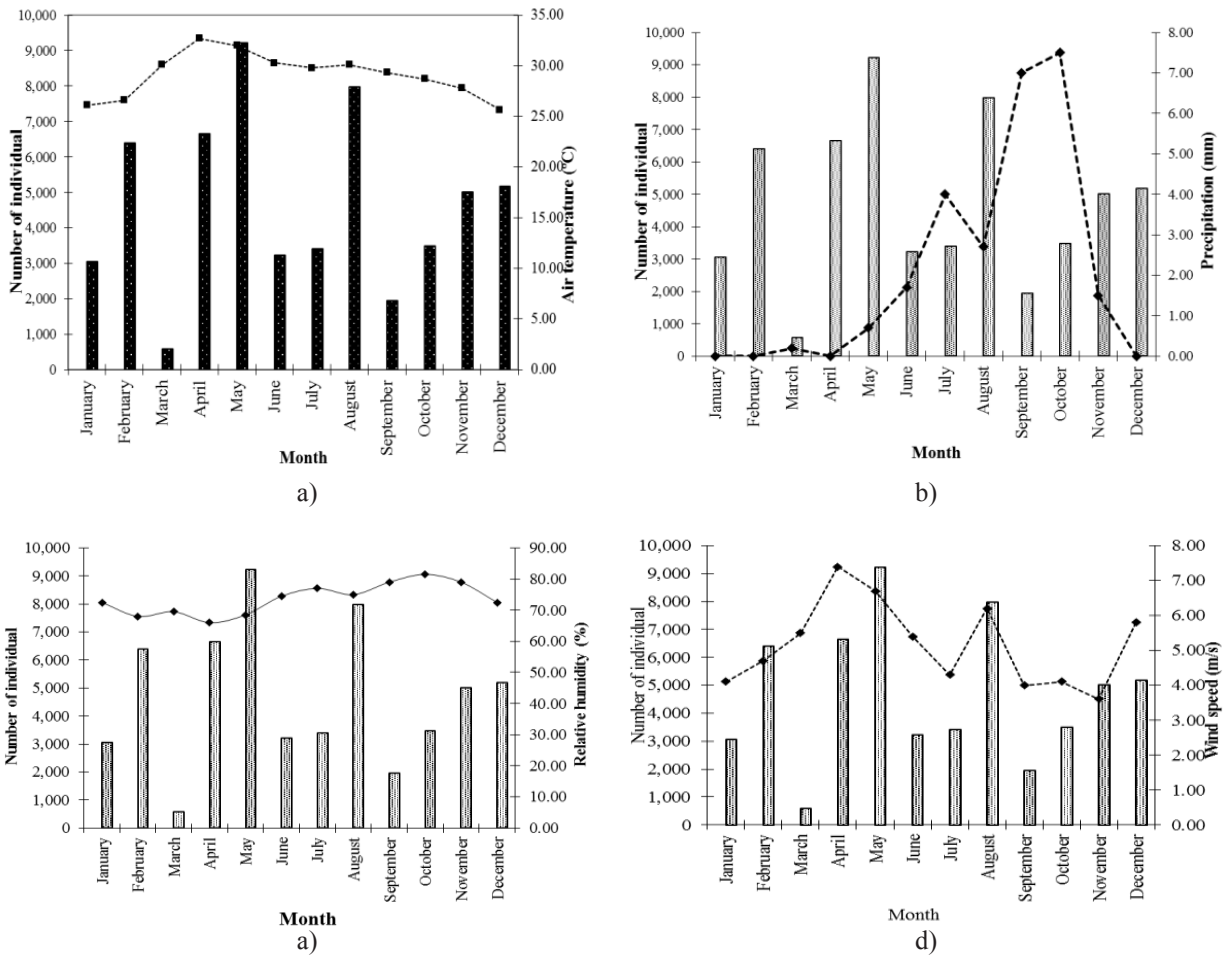

Fig. 2 Seasonal flight patterns of *Amphipsyche meridiana* adults over the period of January to December 2016 in relation to air temperature (a) precipitation (b) relative humidity (c) and wind speed (d).

The number of collected specimens is influenced by the environmental factors. In general, temperature was the climatic factor which influenced population dynamics of adult insects the most. In contrast to the results obtained in this research, air temperature was not significantly correlated with populations of *A. meridiana* ($r = 0.470$) (Fig. 2a and Table 2). In research by Seetapan & Prommi (2012), it was found that adult trichoptera species was highest in richness and abundance during February through to April, which was also the end of the dry season in Northern Thailand. Because of food available and stable habitat in aquatic environment at that time are appropriate for the larval stage, resulting in adults emerging (Lancaster & Downes, 2018).

An increase in precipitation negatively affected the population of adults, being a factor of importance in the occurrence of these insects among the months (Fig. 2b and Table 2). The correlation coefficient (r = -0.221) obtained by the analysis between this factor and the number of *A. meridiana*, confirmed that the increase in precipitation caused a decrease in the number of adults caught. The results showed the importance of seasonality of precipitation for the populations of Trichoptera species similar to those obtained by Prommi & Permkam (2010), who demonstrated a negative effect of precipitation upon the number of adults of Ecnomus vinemar caught in light traps in Ko Hong Hill nature preserve, Southern Thailand.

The relative humidity was a less negative correlation between the number of insects collected (r = -0.358). The driest conditions significantly increased the population density of A. meridiana in the area, enabling the catch of the highest number of specimens (Fig. 2c and Table 2). Likewise, Seetapan & Prommi (2012) verified a negative correlation between relative air humidity and the populations of Trichoptera species in Northern Thailand.

One study pointed out that the wind speed appeared to influence insect flight, with higher wind speeds being associated with lower flight activity (Briers et al, 2003). In this study, wind speed was significantly correlated with populations of A. meridiana (r = 0.592) (Fig. 2d and Table 2). It can be concluded that wind speed had an effect on the population of *A. meridiana*, and this effect is manifested primarily in seasonal fluctuations in this area.

This study was conducted only in the adult stage of *A. meridiana* collected by light traps. The fight period is probably adapted to temperature and the food requirements of the larvae (Byttebier et al., 2012).

Table 2 Pearson's correlation between adult insects and environmental variables in an irrigation pond outlet during January to December 2016.

	AT (°C)	WS (m/s)	PT (mm)	RH (%)	Adult insect
AT (°C)	1				
WS (m/s)	**.586***	1			
PRE (mm)	.549	-.412	1		
RH (%)	-.278	**-.715****	**.836****	1	
Adult insect	.470	**.592***	-.221	-.358	1

Remark: *. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

Conclusion

It can be concluded from this study that the seasonal abundance of flying insects in the irrigation pond outlet changes with weather conditions that eventually trigger the emergence of winged and aquatic insects. Altogether, our results suggest that emergence studies are important for obtaining data on the emergence patterns and faunistics of caddisflies from lowland pond habitat, but also to provide information on the ecology of the investigated area.

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The Development of Khanom-Pum with Sangyod Rice Replacement

Krittin Chumkaew*, Orawan Phuenjcome & Chaiyasit Punfujinda

Department of Food and Nutrition, Faculty of Home Economics Technology, Rajamangala University of Technology Thanyaburi, Pathumthani 12110, Thailand

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Abstract

The objectives of this research were to develop a basic recipe of Khanom-Pum and investigate the proper amount of Sangyod rice suitable for cooking Khanom-Pum. In addition, physical quality, chemical analysis and customers' acceptance of the Khanom-Pum were studied. The results indicated that the proper basic recipe consisted of rice flour, liquid Palmyra palm sugar, and water with the following proportion 24%, 40% and 36%, respectively. The best recipe of Khanom-Pum was 50% replacement with Sangyod rice. Hardness and lightness value (L*, a*, b*) decreaed with increasing the levels of Sangyod rice. The chemical analysis of the Khanom-Pum was 50% covered moisture, protein, fat, fiber, ash and carbohydrate in the following percentage of 1.47, 48.03, 0.12, 1.34, 0.76 and 48.28, respectively and vitamin B1 was 1.06 mg per 100 g. The Khanom-Pum with 50% Sangyod rice flour had more fiber and vitamin B1 than in the basic recipe. In terms of customer acceptance, there were 100 respondents. The results revealed the level of overall satisfaction was at the high level. Regarding future purchase, 94% would buy the product if the product was available in the market. Approximately 75% reasoned that the Khanom-Pum was healthy and 80% accepted the product at the highest level.

Introduction

Khanom-Pum is one type of local Southern Thai desserts, and it is developed from local wisdom and named differently in various local areas such as Khanom-Jok in Pattanee Province, Khanom-Khuen in Nakhorn Si Thammarat Province and Khanom-Pum in Songkhla and Pattalung Province. Khanom-Pum is not widely sold, and it can be seen only in local markets. The main ingredients of Khanom-Pum are rice flour and sugar. Sugar that is frequently used is Palmyra palm sugar found in local areas. It makes the dessert aromatic and provides a natural color as in Palmyra palm sugar. The cooking methods start with soaking rice, grounding it and leaving it for natural fermentation from natural yeast. Then, it is poured into small confection cups and streamed until Khanom-Pum is well cooked. The dessert's shape is like a cup. Its flavors are a combination of sweetness and

* Corresponding Author
e-mail: krittin_c@rmutt.ac.th

sourness. The dessert is aromatic, soft and gummy. It is always served as snacks sprinkled with grated coconut and seasoning salt to add more flavors. Cooking Khanom-Pum requires similar cooking methods and ingredients of Tuay Foofound in the central areas of Thailand since the main ingredients are rice flour and sugar (Maneesri, 2006).

Sangyod rice is a type of local rice variety under Royal Projects in Patthalung Province. Her Majesty Queen Sirikit gave an order to Patthalung Rice Research Center to develop this rice variety. It has been grown since 2000 (Patthalung Rice Research Center, 2010). Sangyod rice is slender with light grains and has been grown for more than a hundred years. When compared with other rice varieties, the color of its seed coat is a mixture between white and red or dark red color. Rice hulls are straw-colored. The color of brown rice is a mixture of white and red. Its grain is 6.53 mm long and 1.75 mm wide. It contains 14.23% of amylose content, which makes the rice soft and gummy when heated (Saeton et al., 2010). Moreover, Patthalung Sangyod rice was certified as a product of geographical wisdom in 2006 as GI rice according to the Act on Protection of Geographical Indication B.E. 2549 (Rice Product Development Division, 2010).

There has been an increase in using Sangyod rice in the form of rice flour in more food products to increase nutritional values. Previous studies have indicated that Sangyod rice contains a large amount of Anthocyanin and fiber (Kaewthanon et al., 2015). The replacement of Sangyod rice flour into Khanom-Pum, the unique dessert of the south, helps to add value and to promote this particular dessert. Moreover, the proper amount of Sangyod rice for cooking Khanom-Pum and the physical quality, chemical analysis and customers' acceptance were determined in this research.

Materials and methods

1. The selection of basic recipes of Khanom-Pum

Three basic recipes of Khanom-Pum were chosen for this experiment. The different ingredients of each recipe are shown in Table 1. The cooking methods start from mixing rice flour with liquid Palmyra palm sugar, adding water, stirring the mixture and leaving the mixture at a temperature ranging from 28 to 30°C. The mixture of Recipe 1 was left for one night. The mixture of Recipe 2 was left for six hours. The mixture of Recipe 3 was left for 10 hours. When bubbles appeared, the mixture from each recipe was steamed in a Chinese steamer with different durations. Recipe 1 was steamed for 20 minutes. Recipe 2 was steamed for 15 minutes. Recipe 3 was steamed for 25 minutes. After Khanom-Pum from each recipe cooled down they were taken out from the small confection cups.

Next, there was a sensory evaluation covering the following aspects: appearance, color, smell, overall flavor, hardness and overall satisfaction with 30 consumers using 9 - Points Hedonic Scale (1 = dislike extremely and 9 = like extremely). The highest average score of the recipe was selected for cooking the Khanom-Pum with Sangyod rice replacement.

Table 1 There basic recipes of the Khanom-Pum

Ingredients	Recipe 1 (%)	Recipe 2 (%)	Recipe 3 (%)
Rice flour	21	31	24
Liquid Palmyra palm sugar	44	31	40
Water	35	38	36
Total	100	100	100

Sources: Recipe 1 (Ritthisak, 2014), Recipe 2 (Kaewboonchu, 2014), Recipe 3 (Poodkhong, 2014)

2. Effect of Sangyod rice levels on Khanom-Pum qualities

2.1 The preparation of Sangyod rice started with rinsing the rice and soaking it in the water with the proportion of 1:1 for 4 hours. Then the rice and the water were blended together until the mixture was soft and smooth. The mixture was measured according to each recipe.

2.2 To study the proper amount of Sangyod rice for replacing the Khanom-Pum in each recipe, the factor used was four different amounts of Sangyod rice in the proportion of Sangyod rice flour as follows 0:100, 25:75, 50:50 and 75:25. The cooking steps shown in Fig. 1 were conducted from Recipe 3 in Table 1 was used.

3. The analysis of food quality and chemical compositions

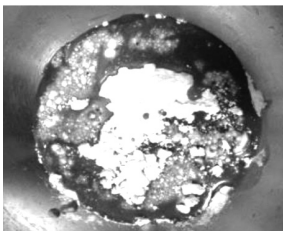
The four recipes of the Khanom-Pum with Sangyod rice replacement were analyzed as follows.

3.1 Sensory evaluation was conducted with consumer's preference in the following aspects: appearance, color, smell, overall flavor, hardness and overall satisfaction using 9- Points Hedonic Scale (1 = dislike extremely and 9 = like extremely), resulting in the highest preferred recipe.

(1) Soak the Sangyod rice

(2) Grind the soaked rice until it is fine

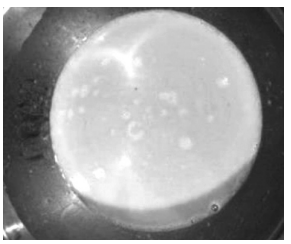
(3) The ingredients of Khanom-Pum



(4) Mix the rice flour with liquid Palmyra palm sugar

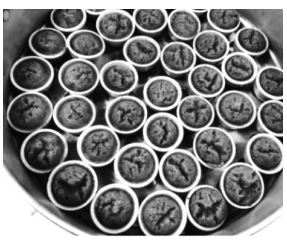
(5) Stir the mixture and add the blended Sangyod rice

(6) Add the water and stir the mixture well



(7) Ferment it for 10 hours until there are some bubbles

(8) Steam the flour in a Chinese steamer for 25 minutes



(9) The Khanom-Pum with Sangyod rice replacement

Fig. 1 Cooking methods of Khanom-Pum with Sangyod rice replacement

3.2 The physical properties of Khanom-Pum were analyzed. Texture analysis was determined by a texture analyzer (TA-XT plus, Stable micro system, UK) by using a 25 mm diameter P36R cylindrical probe to press on the sample with a double-cycle program. The speed of compression was 1 mm/sec for 50 percent of the sample height. The second compression was 15 seconds after the first one. Results were reported as values of hardness and springiness. (Sinchaipanit et al., 2017). The color values (L* a* and b*) were determined by a chromameter (Minolta Co., Ltd, Osaka, Japan).

3.3 The chemical analysis covered moisture, protein, ash, fat, fiber, carbohydrate (AOAC, 2000) and Vitamin B1 (Batifoulier et al., 2005). The Khanom-Pum with Sangyod rice replacement, with the highest acceptance, was analyzed and compared with basic Recipe at the ratio of 0:100 (Sangyod rice 0%:rice flour 100%).

4. Customer acceptance analysis

To understand customer acceptance, there were 100 participants who were university students and university staff of Rajamangala University of Technology Thanyaburi. They were randomly selected and were given a questionnaire covering three sections as follows. The first section covered demographic data such as gender, age, educational levels, occupation and monthly income. The second part was related to sensory evaluation covering appearance, color, smell, overall flavor, hardness and overall satisfaction using 9- Points Hedonic Scale (1 = dislike extremely and 9 = like extremely) with Central location test. The third part was consumers' decision such as purchase intent, reasons for purchase intent and product acceptance.

5. Statistical analysis

The analysis of variance (ANOVA) was conducted to determine whether there were any statistically significant differences using Duncan' New Multiple Rang Test (DNMRT) with confidence interval of 95% (Vanidbhuncha, 2007) and using SPSS for Windows.

Results and discussion

1. The basic recipes

Table 2 shows that in the following aspects: appearance, color and smell, the scores of Recipe 1 and 3 were different from Recipe 2 since Recipe 1 and 3 contained the similar ingredients and cooking steps except the fermentation duration and steaming duration. This led to the similar Khanom-Pum. However, Recipe 2 contained more rice flour than the other two recipes, leading to gummy dough and less duration in fermentation and steaming than Recipe 1 and 3 could lead to a harder Khanom-Pum. The less amount of liquid Palmyra palm sugar than the other recipes made the smell not as aromatic as it should be. In terms of over flavor, hardness and overall satisfaction, the scores of the three recipes were different ($p \leq 0.05$). Recipe 3 had the highest score in all aspects and overall satisfaction. Hence, Recipe 3 was chosen for developing the Khanom-Pum with Sangyod rice replacement.

Table 2 Preference scores of the three recipes of the Khanom-Pum (n=30)

Sensory evaluation	Khanom-Pum		
	Recipe 1	Recipe 2	Recipe 3
Appearance	7.44^{a}±1.14	6.59^{b}±1.06	7.86^{a}±0.96
Color	7.87^{a}±1.16	6.78^{b}±0.96	7.94^{a}±0.72
Smell	7.51^{a}±1.25	7.03^{b}±0.95	7.84^{a}±0.62
Overall flavor	7.23^{b}±1.26	6.59^{c}±1.30	8.13^{a}±0.73
Hardness	7.06^{b}±1.66	6.60^{c}±1.28	8.17^{a}±0.48
Overall satisfaction	7.33^{b}±1.37	6.78^{c}±0.95	8.22^{a}±0.50

Remark: Different superscript in the same column indicate statistical difference among mean values at the 95% confidence level ($p \leq 0.05$)

2. Effect of Sangyod rice levels on the Khanom-Pum qualities

The optimal recipe, Recipe 3, was used to cook Khanom-Pum. Then there was a sensory evaluation for the four levels. Table 3 reveals that in terms of appearance and color, the sample of Sangyod rice: rice flour at the ratio of 0:100 was different ($p \leq 0.05$) from that of 25:75, 50:50 and 75:25 because Sangyod rice replacement added more color to the Khanom-Pum, affecting the appearance of the Khanom-Pum. In terms of smell, overall flavor and overall satisfaction, the samples at the ratio of 0:100 and 50:50 differed from those of 25:75 and 75:25 ($p \leq 0.05$). As a result, The sample at the ratio of 50:50 was selected as the most widely accepted when compared with that of 0: 100 in terms of chemical analysis.

Table 3 Preference scores of the four recipes of the Khanom-Pum (n=30)

Sensory evaluation	Khanom-Pum (Sangyod rice: rice flour)			
	0:100	25:75	50:50	75:25
Appearance	7.30^{a}±1.21	6.47^{c}±0.83	6.89^{b}±0.60	6.80^{bc}±1.39
Color	7.33^{a}±1.06	6.32^{c}±0.90	6.90^{b}±0.76	6.62^{bc}±0.35
Smell	7.41^{a}±1.21	6.75^{b}±0.83	7.35^{a}±0.60	6.75^{b}±1.39
Overall flavor	7.34^{a}±1.25	7.15^{b}±1.09	7.27^{a}±0.71	7.17^{b}±0.22
Hardness	7.35^{a}±1.47	6.95^{ab}±1.12	7.29^{a}±0.50	6.88^{ab}±0.42
Overall satisfaction	7.57^{a}±1.25	7.21^{b}±0.84	7.50^{a}±0.56	7.17^{b}±0.53

Remark: Different superscript in the same column indicate statistical difference among mean values at the 95% confidence level ($p \leq 0.05$)

3. Khanom-Pum quality

In studying food physical quality, the study focused on the texture analysis and color values. The samples at the ratio of 0:100, 25:75, 50:50 and 75:25 were analyzed. Table 4 shows that hardness of 0:100 and 25:75 were different from those of 75:25 ($p \leq 0.05$). The value of hardness decreased when there was a higher amount of Sangyod rice. Springiness of the four samples was not significantly different ($p > 0.05$). Regarding color values, lightness (L*) and redness (a*) of 0:100, 25:75 and 50:50 were significantly different from those of 75:25. While yellowness (b*) of all four samples were different from one another ($p \leq 0.05$). The color values decreased when there was an increase in an amount of Sangyod rice. This result was similar to the result of Tanasombun & Pichaiyongvongdee (2017) in that the color values (L* a* b*) decreased in tofu salad dressing with rice flour gel replacment since the rice flour gel was opaque.

Table 4 Scores of food physical quality of four different amounts of Sangyod rice used in replacing rice flour (texture properties n=4, color n=3)

Sangyod rice: rice flour	Food Physical quality				
	Hardness (kgf)	Springiness^{ns} (mm)	L*	a*	b*
0:100	13.87^{a}±0.14	1.70±0.22	23.83^{a}±1.44	7.86^{a}±0.81	15.33^{a}±0.75
25:75	13.10^{ab}±0.28	1.64±0.33	22.93^{a}±1.25	7.73^{a}±0.25	12.80^{b}±0.26
50:50	12.12^{b}±0.57	1.48±0.12	22.10^{a}±0.55	6.66^{ab}±0.92	10.73^{c}±0.80
75:25	8.15^{c}±1.40	1.37±0.29	17.10^{b}±1.30	5.93^{b}±0.55	8.33^{d}±0.17

Remark: Different superscript in the same column indicate statistical difference among mean values at the 95% confidence level ($p \leq 0.05$)
^{ns} letters mean that there was no statiscally significant difference ($p > 0.05$).

The chemical analysis covered protein, moisture, fiber, ash, carbohydrate and Vitamin B1. The sample of 0:100 (Basic recipe of Khanom-Pum) was compared with that of 50:50 (the Khanom-Pum with replacing 50% of rice flour with Sangyod rice) as in Table 5. It can be seen that chemical qualities such as the amount of fiber and vitamin B1 of the sample of 0:100 had statistically significant difference from that of 50:50 ($p \leq 0.05$). The sample of 50:50 contained a high fiber and vitamin B1, indicating that Sangyod rice contains higher fiber and vitamin than the native rice the 50% Sangyod rice replacement led to an increase in fiber and vitamin B1 at 1.31% and 1.02 mg/100 g, respectively. This result shows that the higher amount of Sangyod rice replacement, the higher amount of fiber and Vitamin B1, which is similar to the result of Tinakorn Na Ayutthaya et al. (2018) in that a higher amount of brown rice flour on sponge cake recipe led to larger number of fiber in the cake.

Table 5 Comparison of chemical compositions of the Khanom-Pum 0:100 and 50:50 (n=3)

Chemical compositions	Khanom-Pum (Sangyod rice: rice flour)	
	0:100	50:50
Protein (%) ^{ns}	1.51±0.03	1.47±0.07
Moisture (%) ^{ns}	47.11±0.22	48.03±0.28
Fat (%) ^{ns}	0.07±0.40	0.12±0.40
Fiber (%)	0.03^{b}±0.02	1.34^{a}±0.34
Ash (%) ^{ns}	1.03±0.03	0.76±0.36
Carbohydrate (%) ^{ns}	50.25±0.25	48.28±0.33
Vitamin B1 (mg/100 g)	0.04^{b}±0.04	1.06^{a}±1.06

Remark: Different superscript in the same column indicate statistical difference among mean values at the 95% confidence level ($p \leq 0.05$)
^{ns} letters mean that there was no statiscally significant difference ($p > 0.05$).

4. Consumer acceptance of the Khanom-Pum with Sangyod rice replacement

The analysis of customer acceptance among 100 questionnaire respondents showed that most of the respondents were female, accounting for 69%. Approximately 36% were 15-24 years old and half of them possessed bachelor's degree. Roughly 52% were university students and 54% earned from 5,001 to 10,000 baht a month.

The analysis of sensory evaluation of the consumers towards the Khanom-Pum with Sangyod rice replacement is shown in Fig. 2. Fig. 2 reveals the average score of all six aspects was at moderate level. It could be explained that the Khanom-Pum with Sangyod rice replacement had an unattractive color. The Khanom-Pum were brown and had brown spots from Sangyod rice. In fact, the Khanom-Pum with Sangyod rice replacement are different from Khanom-Pum with rice flour in central parts of Thailand in that the former is gummy yet soft while the latter is fluffy and soft. This could have an effect on the consumers' satisfaction scores. However, the mean score of overall satisfaction was at the high level. If Khanom-Pum with Sangyod rice replacement was available in the market, 94% of the respondents would buy the product and 75% of them reported that the Khanom-Pum was healthy. Approximately 80% accepted the product at the highest level.

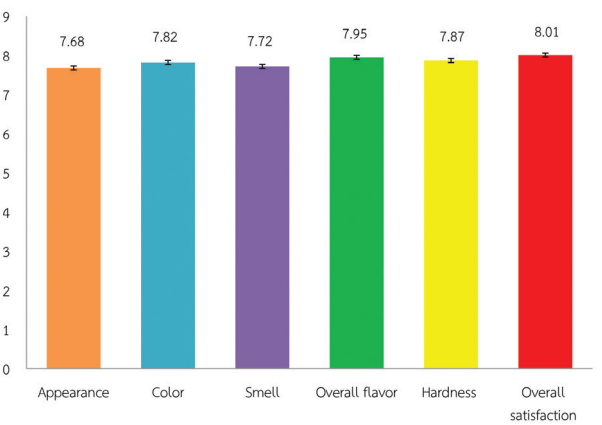


Fig. 2 Average scores of product acceptance of Khanom-Pum with Sangyod rice replacement

In developing the Khanom-Pum with Sangyod rice replacement, there were three levels of replacement 25%, 50% and 75%. The 50% replacement gave the highest score for sensory evaluation because of the proper amount of Sangyod rice, the maintenance of flavor and appearance of Khanom-Pum.

The hardness value decreased when the levels of Sangyod rice increased. This result was similar to the result of Lekjing et al. (2019) which focused on the effects of replacing wheat flour with Sangyod brown rice flour in snack products. Sangyod rice has 14.23% of amylose content (Saeton et al., 2010). This leads to a gummy and soft rice quality after being cooked,and this keeps the consistency of the cooked dough (Patthalung Rice Research Center, 2010). Moreover, using Sangyod rice in the form of frehly milled flour tends to have an effect on lower water absorption (Dechkulchorn, 2006) and that can affect softness and hardness of Khanom-Pum with Sangyod rice replacement.

Lightness (L*), redness (a*) and yellowness (b*) decreased when there was an increase in the amount of Sangyod rice. In fact, Sangyod rice has white with light red color hulls or dark red hulls (Patthalung Rice Research Center, 2010) and Sangyod rice has pigmnets such as anthocyanin, one component of Sangyod rice flour. Anthocyanin can decompose when receiveing heat, oxygen and light (Chanthachot, 2013). When Sangyod rice is added into products, such producst will have darker colors.

The Khanom-Pum with Sangyod rice replacement was more nutritional. It provided 1.31% more fiber and 1.02 mg/100 g of vitamin B1 when compared with the basic recipe. In fact, Sangyod rice contains more fiber and vitamin B1 than rice flour (Bureau of Nutrition, 2001). From the research of Chadakarn & Chanthawong (2011) in developing cookies with replacing wheat flour and grains with 20 % Sangyod rice replacement increased nutritional values of the cookies and the consumers accepted the product. Phadungsilp (2011) studied the effect of Sangyod rice flour fortified in Khanom Thuay-Fu. The research focused on a various amount of Sangyod and native rice flour at 50:50, 75:25 and 100:0. The research found that 50:50 of fortified was the highest acceptance. The sample showed increased energy value, total protein content, fiber, and vitamin B1 and B2 meanwhile low total carbohydrate content. The result yielded the same result as that of Phadungsilp et al. (2012) who conducted a study investigating the replacement of Sangyod rice powder into twist stick. According to Phadungsilp et al. (2012), the twist stick with 50% Sangyod rice replacement had more fiber and vitamin B1 than the stick with 100% rice powder. Rujirapisit et al. (2012) studied nutritional values of 9 species of rice and discovered that Sangyod rice provided lower energy. Moreover, Kaewthanon et al. (2015) reported that Sangyod rice was more nutritional. In fact, Sangyod rice contains more Niacin, vitamin B1 and vitamin B2 as well as other minerals such as calcium and phosphorus. The red color of Sangyod rice is a coloring substance belonging to anthocyanin, a subgroup of flavonoids, which has antioxidant properties (antioxidant) to slow down aging process and reduce risks of diseases such as heart disease and cancer.

In terms of customer acceptance, the results revealed that 94% of the respondents stated that they would buy the product because it was healthy. This result was the same as Chanthachot (2013), who conducted a study about the development of Sangyod brown rice noodels with egg white. The consumers in Chanthachot's study reported that they were interested in the product because of the overall flavor and higher nutritional values. Also, the results of this studied support those of Komchadluek (2017) in that modern consumers pay more attention to their own health and those consumers tend to choose food that is healthy. Results of other polls reveal that 89% of consumers want to purchase healthy food and will make a purchase decision for products with sustainable manufacturing process. This will lead future food and drink producers to use natural ingredients such as protein from plants, natural dyes which give both colors and nutritive values, which is beneficial for consumers' health.

Conclusion

The optimal recipe of the Khanom-Pum with Sangyod rice replacement consists of 12% of rice flour, 12% of Sangyod rice, 40% of liquid Palmyra palm sugar and 36% of water. The Khanom-Pum with Sangyod rice replacement was round due to the circle shape of the small confection cups. Its surface was fluffy and crackly. It was aromatic due to liquid Palmyra palm sugar and Sangyod rice. Also, it was sweet, soft and sticky. This recipe was evaluated in terms of sensory evaluation covering appearance, color, smell, overall flavor, hardness and overall satisfaction, resulting in a moderate level. This recipe provided the following nutritional values: 1.47% protein, 48.03% moisture, 0.12% fat, 1.34% fiber, 0.76% ash, 48.28% carbohydrate and 1.06 mg/100 g vitamin B1. Sangyod rice replacement has an effect on a larger amount of fiber and vitamin B1 in Khanom-Pum. In terms of customer acceptance towards the particular recipe, it was accepted at the highest level.

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Development of Fermented Banana Vinegar: Chemical Characterization and Antioxidant Activity

Wilawan Boonsupa[a]*, Witaya Pimda[b], Kanyarat Sreeninta[a], Chooreeporn Yodon[a], Nawarat Samorthong[a], Benjarut Bou-on[a] & Phanumat Hemwiphat[a]

[a] *Department of Biology, Faculty of Science and Technology, Rajabhat Maha Sarakham University, Mahasarakham 44000 Thailand*

[b] *Faculty of Sciences and Liberal Arts, Rajamangala University of Technology Isan, Nakhon Ratchasima 30000 Thailand*

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Abstract

This study was carried out to examine the chemical properties, antioxidant activities and sensory scores of banana vinegar produced from four banana cultivars, namely 'Khai Pra Tabong', 'Nak', 'Hin', and 'Phama Heak Kuk'. The initial soluble solid contents in the banana juice were adjusted to 25º Brix before taking to fermentation. Alcoholic fermentation was conducted using *Saccharomyces cerevisiae* as the inoculant while *Acetobacter pasteurianus* was used for acetous fermentation. As observed, all samples during the alcoholic fermentation the levels of soluble solids decreased continuously and the level of alcohol were found to increase at the end of fermentation process. Notably, the wine produced from 'Phama Heak Kuk' cultivar exhibited the highest level of alcohol (9.54%) and exhibited the highest levels of antioxidant activity (87.04%). Similar results were observed for all samples during the acetous fermentation, in which the level of alcohol dropped continuously and the levels of acetic acid were noted to elevate at the end of the fermentation process. The highest levels of acetic acid (3.49%) was detected in the vinegars produced from 'Phama Heak Kuk' cultivar while those produced form 'Khai Pra Tabong' cultivar exhibited the highest levels of antioxidant activity (80.59%). Sensory evaluation based on the 9-point hedonic scales showed that the vinegars produced from 'Hin' cultivar showed the highest overall acceptability with an average score of 8.13, equivalent to the hedonic scale of 9, which indicated a high pleasant level of the vinegar preference of the consumers.

Introduction

Due to its availability in several different varieties in every country, vinegar represents one of the most widely used seasonings in the world (Jo et al., 2013). In addition to being primarily used as food seasoning, vinegar plays an important role in the production of food

* Corresponding Author
e-mail: wilawanboonsupa@ymail.com

products since it is applied in a wide variety of products, including sauces, ketchups and mayonnaise (Ho et al., 2017). Moreover, vinegar has long been used in the treatment of many common ailments with claims of anti-infective, antitumor, and hyperglycemic properties (Johnston & Gaas, 2006; Wongsudarak & Nunium, 2013). The production of vinegar is in general low in costs due to the fact that inexpensive raw materials like by-products from food processing, fruit waste, substandard fruit and agricultural surpluses are utilized (Solieri & Giudici, 2009). The beneficial effects of vinegar might be due to bioactive substances such as amino acids, organic acids or phenolic compounds derived from its raw materials (Budak et al., 2014; Ghosh et al., 2016). Moreover, the bioactive compounds in vinegars can be produced and/or increased through the overall vinegar fermentation process (Solieri & Giudici, 2009), where phenolic compounds are transformed into new antioxidative molecules (Shahidi et al., 2008). Additionally, the aroma and flavor of vinegars impacting on consumer acceptance is influenced by the raw materials used, the compounds formed during the fermentation process, and the fermentation type used (Callejon et al., 2008; Liang et al., 2016; Morales et al., 2002; Ubeda et al., 2012). Recently, the demand for fruit vinegars has increased due to their reputation as health food products, which help to promote different kinds of beneficial effects to consumers, such as having antidiabetic effects and lowering cholesterol levels in blood by inhibiting the oxidation of low density lipoproteins (LDLs), among other benefits (Chen et al., 2017).

Musa spp. comprising dessert bananas and plantains, were among the world leading fruit crops as source of energy in the diet of people living in humid tropical regions (Sudhanyaratana et al., 2016). Banana has firm pulp when the fruit is not ripe and soft pulp during maturation. It is known that dessert banana pulp and peel contains some secondary metabolites in their composition, e.g. catecholamines, phenolics, and carotenoid compounds as well as pyridoxine. Many of banana's volatile compounds such as ester and alcohols play an important role in the aromatic properties of banana. (Pereira & Maraschin, 2015). According to Coelho et al. (2017), who studied the chemical composition and antioxidant activity of banana vinegar produced from fruit concentrates, the results revealed the total acidity of banana vinegar (5.4%) and the antioxidant activity by Frap (3.7 mmol Fe_2SO_4/L)

For this purpose, this study was carried out to compare the chemical properties, antioxidant activities and sensory scores of the banana vinegars produced via a two-stage fermentation process from four cultivars, namely 'Khai Pra Tabong', 'Nak', 'Hin' and 'Phama Heak Kuk'. In this context, chemical properties were assessed in terms of alcohol contents, glucose and fructose contents and acetic acid contents. Antioxidant activities were determined by DPPH radical assays and total phenolic contents. Sensory evaluation was performed based on the 9-point hedonic scale in order to determine the consumers preference.

Materials and methods

1. Chemicals and reagents

2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) was purchased from Sigma–Aldrich (Steinheim, Germany). Folin-ciocalteau reagent was from Merck (Darmstadt, Germany) and sodium carbonate (anhydrous) from Univar (Downers Grove, IL, USA). All other chemicals and solvents were purchased from local manufacturers. Deionized water was prepared by a Milli-Q Water Purification system (Millipore, MA, USA).

2. Raw materials and fermentation

Banana fruits of four cultivars, namely 'Khai Pra Tabong' (The short stem is curved and thick peel. When ripe had yellow color, orange flesh and sweet flavor), 'Nak' (The raw banana is bright red, the ripe banana is an orange-red color), 'Hin' (Yellow thick peel, creamy white pulp with a sweet flavor) and 'Phama Heak Kuk' (The raw banana is a dark green color, the ripe fruit is an orange-red color), was used for the production of banana vinegars via a two-stage (alcoholic and acetous) fermentation process. Banana fruits of each cultivar were crushed and mixed with water at a ratio of 1:1 to prepare banana juice. After adjustment of the pH to 4.5 using 5% acetic acid and sugar content up to 25º Brix.

3. Banana vinegar production

Amylase enzyme from Mc-Zyme S.P. Corporation was applied in order to degrade the starch still present in the banana puree for 24 hours at 30ºC. The banana juice was pasteurized for 30 min at 60ºC. Alcoholic fermentation was conducted for 5 days at room temperature under static conditions in plastic vessels containing 2 L of the banana juice inoculated with wine yeast, *Saccharomyces cerevisiae,* (Wine & Scientific Equipment Ltd., Part) at a ratio of 0.75% (v/v). Preparation of yeast inoculum was carried out by mixing 5 g of yeast powder with 60 mL of warm water. At the

end of the fermentation process, the obtained wine was separated from the sediment by allowing it to settle in glass bottles, followed by pasteurization for 30 min at 60ºC and clarification for 45 days at 10ºC. Prior to acetous fermentation, modified from the method of Coelho et al. (2017), the alcohol content of the obtained wine was adjusted to 6%. Acetous fermentation was performed for 15 days under the aforementioned conditions in glass vessels containing 135 mL of the banana wine inoculated with *Acetobacter pasteurianus* TISTR 521 at a ratio of 10% (v/v). Sampling was performed at given timepoints to collect the two-stage fermented banana vinegars by allowing them to settle in microtube and stored at 4ºC before the analyses.

4. Chemical analysis

Analysis of alcohol, acetic acid, glucose and fructose contents modified from the method of Aguiar et al. (2005). The analysis was performed on a Shimadzu HPLC-RID system (Shimadzu, Japan) consisting of Shimadzu LC-20AD pumps and RID-10A refractive index detector. The analytical column was Aminex HPX-87H column (300 mm × 7.8 mm i.d., 9 µm, Bio-Rad Laboratories, Inc., USA) coupled to a cationic exchange precolumn (Bio-Rad Laboratories, Inc., USA). H_2SO_4 (5 mM) was used as the mobile phase. The injection volume was 20 mL with a flow rate of 0.6 mL/min. The column temperature was set at 45ºC.

Total soluble solids values of the wine were measured using an AllA France refractometer (AllA France, France) calibrated with distilled water. The values were expressed as °Brix.

5. Antioxidant activity

Antioxidant activities of the vinegars were evaluated by DPPH radical assay (Brand-Williams et al., 1995) in which 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) radical was used as a stable radical. In brief, 1.5 mL of each sample was added to 1.5 mL of 0.1 mM DPPH radical solution prepared in ethanol, and the mixture was incubated for 20 min at room temperature in the dark. After incubation, absorbance was measured at 517 nm using a Shimadzu UV-1700 spectrophotometer (Shimadzu, Japan) and the DPPH radical scavenging activities were expressed as the percentage of the DPPH radical elimination effect of vitamin C. Control solutions were prepared by dissolving 0.004 g of DPPH in 95% ethanol, followed by adjustment of the solutions to a final volume of 100 mL. DPPH radical scavenging capacity (RSC) was calculated using the equation $\%RSC = (A_C - A_S/A_C) \times 100$, where A_C and A_S denote the absorbance of control and sample, respectively.

6. Total phenolic content analysis

Total phenolic contents of the banana vinegars were determined using Folin-Ciocalteu reagent as described by Singleton et al. (1999). Briefly, 1 mL of each sample was diluted with 9.5 mL of distilled water and was then mixed with 0.5 mL of Folin-Ciocalteu reagent and 2 mL of 10% Na_2CO_3 solution. After 30-min incubation at room temperature, absorbance was measured at 765 nm using a Shimadzu UV-1700 spectrophotometer (Shimadzu, Japan). Results were expressed as mg gallic acid equivalents in 1 mL of sample (mg GAE/mL).

7. Sensory evaluation

Two hundred g of the banana vinegars were mixed with 150 g of honey and 150 g of water to make drinking vinegars and the obtained drinking vinegars were subjected to the sensory evaluation based on the 9-point hedonic scale by using 30 untrained panelists for 5 attributes (sweet, color, odor, taste and overall acceptance) with the scale 9 representing like extremely, 5 representing neither like nor dislike and 1 representing dislike extremely.

8. Statistical analysis

A randomized block design, with three replicates and four samples per replicate, was used to compare the chemical properties, antioxidant activities and sensory evaluation of the banana vinegars produced from four banana cultivars. The results are expressed as the mean ± standard deviation (SD) and data were analyzed using one-way analysis of variance (ANOVA) with Duncan multiple range test (DMRT) to determine the significance between samples. In all cases, $p < 0.05$ was considered significant.

Results and discussion

1. Chemical properties of the banana wines and vinegars

The banana wines produced from four banana cultivars via a 5-day alcoholic fermentation process using *Saccharomyces cerevisiae* as an inoculant were analyzed for their chemical compositions, and the results are presented in Fig. 1A. It was observed that at the end of the fermentation, high alcohol content was detected in all the banana wines, indicating that sugars in the banana juice were rapidly converted to alcohol. The banana wine produced from 'Phama Heak Kuk' cultivar contained the highest alcohol content of 9.54 %, which was higher than that (9.27%) detected in the lychee wines produced

in an earlier study (Chen & Liu, 2016). As given in Fig. 1B, Glucose was rapidly utilized during the production of the banana wine as observed for all samples, with the most rapidly utilized glucose observed after 1 day of the fermentation in 'Nak' cultivar. Notably, glucose was completely depleted in all wine samples after 4 days of the fermentation. Fructose was likely utilized more slowly as compared to glucose (Fig. 1C). The most rapidly utilized fructose were observed in the banana wine produced from 'Phama Heak Kuk', 'Khai Pra Tabong' and 'Hin' cultivar which was decreased more than 'Hin' in 3 days of the fermentation. The rapid utilization of glucose and fructose and the consequent increase in the levels of alcohol confirmed that the yeast dominated the fermentation, which was supported by an earlier study (Taniasuri et al., 2016) which elucidated the rapid utilization of glucose and fructose in the production of durian wine, in which at the end of the fermentation fructose was completely depleted while glucose remained at 0.046 g/100 mL. In Fig.1D, total soluble solid of 4 wine were adjust to 25°Brix after fermenting in 5 days, the results showed that Nak wine (15.47°Brix) had greater TSS than Khai Pra Tabong (12°Brix). From the experiment, it was found that the alcohol content in Khai Pra Tabong wine had greater values than Nak wine because yeast had the ability to consume TSS in the Khai Pra Tabong effectively. During the 15-day acetous fermentation process, Oxidative fermentation is a fermentation process caused by bacteria that requires oxygen to respirate at the cellular level (Pongdam, 2017). The banana vinegars produced from the four banana wines using *A. pasteurianus* were analyzed for their chemical compositions, and the results are given in Fig. 2. All the banana vinegars showed a significant decrease in the alcohol content as it was converted to acetic acid by acetic acid bacteria, which was consistent with the increased acetic acid content. However, the alcohols were not completely depleted, at the end of acetous fermentation the vinegar produced from 'Nak' cultivar contained the highest alcohol content of 0.81 % while that produced from 'Khai Pra Tabong' cultivar had the lowest alcohol content of 0.47 %. In an earlier study (Li et al., 2014) which elucidated that the alcohol content in the *Hericium erinaceus* vinegar was 0% after 9 days of acetic fermentation. Regarding the acetous fermentation, at the end of a 15-day acetous fermentation process,

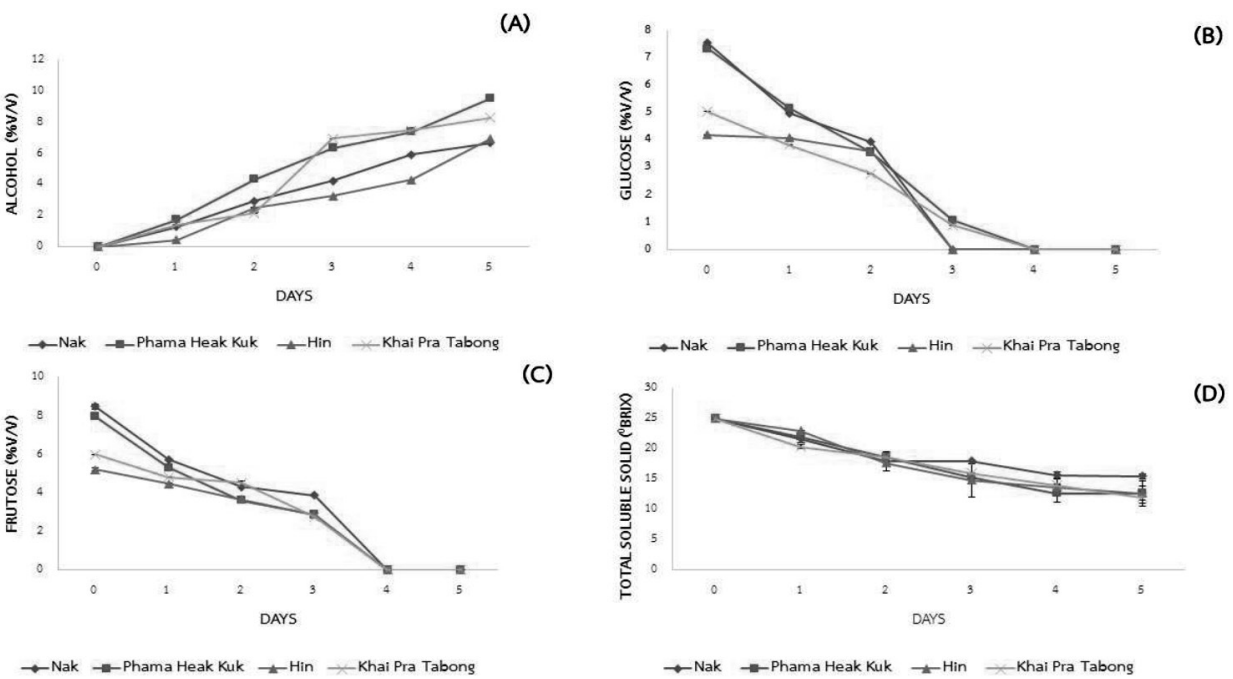

Fig. 1 Physicochemical properties of banana wine during a 5-days fermentation process alcohol (A) glucose (B) fructose (C) and total soluble solid (D)

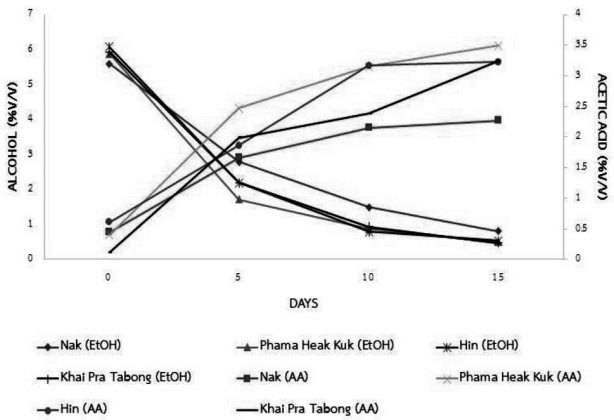

Fig. 2 Physicochemical properties of banana vinegar from banana 4 cultivars during a 15-day fermentation process

acetic acid content was found to range from 2.27% to 3.49%, with the highest value of 3.49% observed in the banana vinegar produced from 'Phama Heak Kuk' cultivar and the lowest of 2.27 % produced from 'Nak' cultivar which was much greater than that obtained in a previous study (Li et al., 2014), in which an acetic acid content of 21.56 mg/mL was detected in the *H. erinaceus* vinegar after 9 days of acetous fermentation. The acetic acid content in vinegar was not up to the standard of fermented vinegar (least 4% acetic acid), this could be caused by the same condition in fermentation had the problem. Ex. The ability of survival of acetic acid bacteria or the deficiency of aeration in fermentation.

2. Total phenolic contents and antioxidant activities

The levels of antioxidant activities of the banana vinegars are presented in Table 1. The results showed that the banana wine derived from 'Phama Heak Kuk' and 'Khai Pra Tabong' cultivar exhibited the highest antioxidant activity of 87.04% and 86.94%, which was greater than that produced from citrus fruit (36.8 ± 0.09%) (Chen et al., 2017). On the other hand, the vinegar produced from 'Khai Pra Tabong' cultivar was observed to exhibit the highest antioxidant activity of 80.59 ± 2.30%, which was much greater than that detected in the purple sweet potato *makgeolli* vinegar (67.63 ± 0.17%) (Chun et al., 2014). The levels of total phenolic contents detected in the banana vinegars produced from different banana cultivars via a two-stage fermentation process are given in Table 2. It was noted that the banana wine derived from 'Nak' cultivar contained the highest levels (229.30 ± 0.59 mg/L) of total phenolics. Similar results were observed for the banana wine produced from the same cultivar, in which the vinegar measured at the end of acetous fermentation exhibited the highest total phenolic content of 243.98 ± 3.35 mg/L, which was much greater than that detected in the purple sweet potato *makgeolli* vinegar (24.73 ± 0.04 mg/L) (Chun et al., 2014). The antioxidant activity and total phenolic content were decreased in vinegar, the results were in agreement with an earlier study of Towantakavanit et al. (2011) which demonstrated that the drop in total phenol level could be due to fermentation process condensation and polymerization reactions as well as the formation of oxidative products and precipitations occur.

Table 1 Antioxidant activities of the four banana vinegars produced via a two-stage fermentation process

Cultivars	DPPH (% inhibition)	
	Wine	Vinegar
Nak	52.48 ± 1.87^{c}	47.44 ± 5.03^{c}
Phama Heak Kuk	87.04 ± 0.00^{a}	68.04 ± 1.49^{b}
Hin	72.08 ± 0.05^{b}	69.30 ± 8.09^{b}
Khai Pra Tabong	86.94 ± 0.05^{a}	80.59 ± 2.30^{a}

Remark: Values with different letters in the same column are significantly different according to Duncan's multiple range test ($p < 0.05$).

Table 2 Total phenolic contents of the four banana vinegars produced via a two-stage fermentation process

Cultivars	Total phenolic content (mg/L)	
	Wine	Vinegar
Nak	229.30 ± 0.59^{a}	243.98 ± 3.35^{a}
Phama Heak Kuk	126.28 ± 0.21^{d}	102.23 ± 0.69^{d}
Hin	214.57 ± 0.23^{b}	198.26 ± 1.61^{b}
Khai Pra Tabong	166.77 ± 0.47^{c}	115.92 ± 9.40^{c}

Remark: Values with different letters in the same column are significantly different according to Duncan's multiple range test ($p < 0.05$).

3. Sensory evaluation

The levels of consumers' preference based on the 9-point hedonic scale of the vinegar drinks; a blend of the vinegars made from different banana cultivars and honey, are depicted in Table 3. The results showed that significant ($p < 0.05$) differences in sweet odor sour and overall acceptability were observed among the drinking vinegars produced from different banana cultivars. The drinking vinegar produced from 'Hin' cultivar displayed the highest level of consumers' preference, with the mean overall acceptability score of 8.13 ± 1.14, which was equivalent to the hedonic scale of 9. In our study, the high levels of consumers' preference of drinking banana vinegars might be attributed to the addition of honey,

Table 3 Sensory scores of the drinking vinegars blended from the four fermented banana vinegars

Curtivars	Sweet	Color	Odor	Sour	Overall acceptability
Nak	7.17 ± 1.90^{ab}	6.57 ± 1.68^{ns}	5.97 ± 1.52^{ab}	6.63 ± 1.59^{b}	7.57 ± 1.55^{ab}
Phama Heak Kuk	6.63 ± 1.47^{b}	6.77 ± 1.3^{8ns}	5.80 ± 1.69^{b}	6.60 ± 1.38^{b}	6.93 ± 1.31^{bc}
Hin	7.87 ± 0.97^{a}	7.30 ± 1.34^{ns}	6.87 ± 1.89^{a}	7.63 ± 1.13^{a}	8.13 ± 1.14^{a}
Khai Pra Tabong	6.37 ± 1.90^{b}	6.50 ± 1.63^{ns}	5.70 ± 1.74^{b}	6.73 ± 1.91^{b}	6.70 ± 1.66^{c}

Remark: Values with different letters in the same column are significantly different according to Duncan's multiple range test ($p < 0.05$).

which was well supported by an earlier study (Marrufo-Curtido et al., 2015) which elucidated that the addition of dietary fiber derived from citrus fruits enhanced the phenolic and volatile profile as well as the judges' preference of the vinegar.

Conclusion

This study was conducted in order to compare the levels of acetic acid, total phenolics, antioxidants and consumers preference of the banana vinegars produced from four banana cultivars via a two-stage fermentation process. The results show that the vinegars produced from 'Phama Heak Kuk' cultivar exhibited the highest level of acetic acid (3.49%) while those produced from 'Khai Pra Tabong' cultivar displayed the highest antioxidant activities (80.59%) measured by means of DPPH radical assay. Meanwhile, the vinegars produced from 'Nak' cultivar were observed to have the highest total phenolics (243.98 mg/L). Sensory evaluation based on the 9-point hedonic scale using untrained panelists showed that the drinking vinegars made from 'Hin' cultivar had the highest overall preference (8.13).

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Development of Local Halal Food Product: Budu Tumih Noni Leaf Rice Crisp

Nutchanet Tayeh*

Food Science and Technology Program, Faculty of Science Agriculture and Technology, Yala Rajabhat University, Yala, 95000 Thailand

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Abstract

This research was conducted to figure out the proper concentration of noni leaf juice to produce noni rice crisp and the appropriate budu-tumih content for topping the crisp in order to be one of local halal food products. The study focuses on the product's chemical composition, physical properties, sensory properties, consumers' acceptance and shelf life. The results reveal that the proportion of noni leaf and water at 120:500 g/ml used in the extraction process and 5 g/piece of budu-tumih content by weight are appropriate in budu-tumih noni leaf rice crisp production. The L*, a* and b* values of the product were 14.27, 4.04 and 2.36, respectively. The a_w was 0.34. For chemical composition, it found that 10.17, 25.97, 61.20, 3.62, 1.90 and 4.14% of protein, fat, carbohydrate, fiber, ash, and moisture, respectively, with a pH of 4.25. The texture property in terms of hardness and crispness was 5.61 and 4.52. The consumer acceptance test shows that the mean of "overall preference" score for the product was at the like to mostly like level (4.99±0.53). It also found that 89% of consumers accept the product and 90% of consumers would purchase the product if it is available in the market. The microbiological quality of the product found the total plate count of $<1x10^6$ cfu/g whereas yeast and mold were <100 cfu/g. The results indicate that the shelf life of the product was at least 10 weeks.

Introduction

The word 'Halal' is an Arabic term referring to what is permissible or lawful in the Islamic law. Halal food is not only concerned about the slaughter of animals, the use of alcohol, the sources of food and beverages, but it is also about standard and process in ensuring the safety and cleanliness of food products (Teng et al., 2013). For many practicing Muslims, keeping a halal diet is an important part of daily life. Halal food has come to represent high quality safety and cleanliness (Ambali & Bakar, 2014).

Rice crisp is a traditional crispy rice snack of Thailand. It is made from rice and has a unique taste and texture. Rice crisp is a snack that has been found since ancient times as one of Thai culture heritage. Up to now, rice crisp is still popular and can be adapted to a variety of taste. (Songpranam, 2013). Many attempts are being

* Corresponding Author
e-mail: nutchanet.t@yru.ac.th

made to improve the snacks' nutritive value and functionality by modifying its nutritive composition with many kinds of vegetables and herbs. The herbs have medicinal and chemical properties for maintaining health or reducing the risk of disease such as high blood cholesterol, cardiovascular disease and high blood pressure disease (Walden & Tomlinson, 2011).

In the south of Thailand, Khao Yum (Rice salad) is a classic traditional Thai dish, It mostly consists of cooked rice, assorted fresh vegetables as well as herbs especially noni leaf. The noni leaf is an example of a plant used as a functional food and has been widely studied due to its apparent beneficial effects on human health. It has been investigated as an alternative in anticancer, antibacterial, and antimicrobial therapies, and in the treatment of esophageal reflux and ulcers in animals (Mahattanadul et al., 2017; Zhang et al., 2016).

Budu-Tumih is a semi-solid fish dish that is popularly eaten as a side dish or as a seasoning in mixed rice, popularly among Muslims in the south of Thailand. Nowadays, the budu-tumih can be found only in the southernmost provinces of Yala, Pattani and Narathiwat. Budu-tumih comprises of many ingredients such as budu, fresh coconut milk, tamarind, sugar, dried chili, garlic, etc. It is commonly used as a dip or flavoring in the Muslim household of Thailand and other countries.

Therefore, the objectives of this research are to bring budu-tumih and noni leaf which is a side dish that is eaten together and to combine them with rice crisps in order to produce a new food product that will increase the value of local halal food as well as to study the product properties.

Materials and methods

1. Study on the appropriate concentration of noni leaf extract to produce noni rice crisps

Noni leaf was collected from 7.5 GY 4/4 color using munsell color book. The juice extract was prepared from 3 proportions of noni leaf and water at 70:500, 90:500 and 120:500 g/ml. Rice crisp was produced by adapting the method from Tachasiriwichai et al. (2015) (Fig. 1). The rice crisp was tested in accordance with the Thai Community Product Standard TCPS: ICS; 119/2011, 2011). (A score must be equal to or greater than 2 and the moisture level of not more that 6%.)

The 3 standard samples of noni rice crisps were tested for sensory quality assessment using 9 point hedonic scale. There were 40 untrained panelists in the study. The sensory quality assessment included appearance, color, odor, taste, crispness and overall preference; the experiment design was Randomized Complete Block Design (RCBD), Analysis of variance (ANOVA) and Duncan's New Multiple Range Test at 95% confidence level.

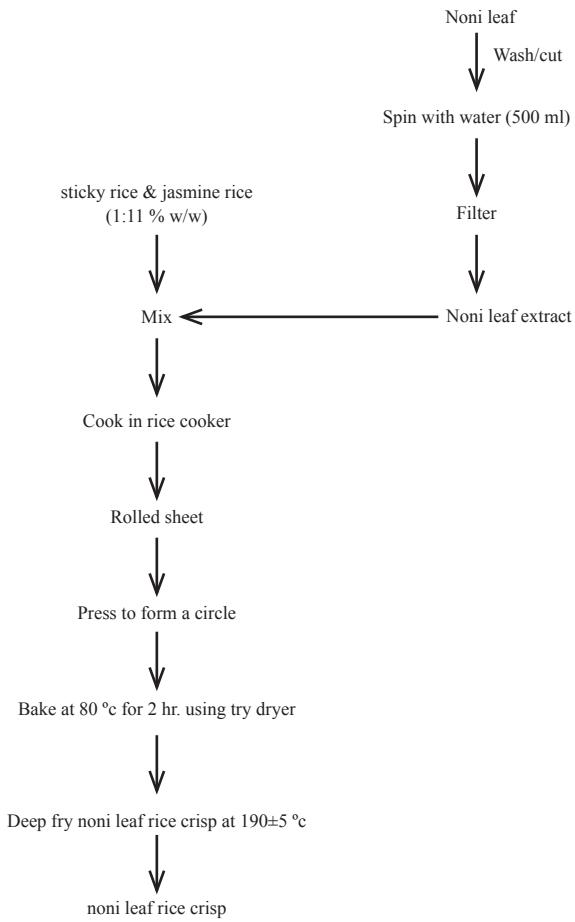

Fig.1 The production process of noni leaf rice crisp (modified from Tachasiriwichai et al., 2015)

2. Study on the appropriate quality of budu-tumih content on the top of the rice crisp

The rice crisp from no. 1 above was topped with budu-tumih. The production process of budu-tumih was modified from the method of Srisuk et al. (2017) as shown in Fig. 2. The amount of budu-tumih per piece of noni leaf rice crisp was varied at 3, 5 and 7 grams and the rice crisp with topping was baked at 80ºC for 1 hour.

The 3 recipes of budu-tumih noni leaf rice crisp were tested for sensory quality assessment using the 9-point hedonic scale (Nicolus et al., 2010) in order to choose

the recipe that has the highest scores. The 40 untrained testers consumed rice crisp regularly. The experimental design used Randomize Complete Block Design (RCBD), analysis of variance (ANOVA) and Duncan's New Multiple Range Test at $p<0.05$.

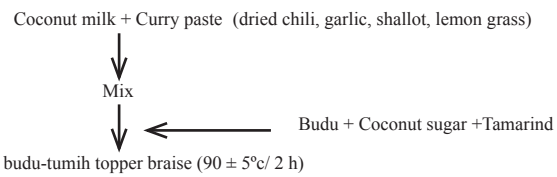

Fig. 2 The production process of budu-tumih (modified from Srisak et al., 2017)

3. Study on the physical properties and chemical compositions of budu-tumih noni leaf rice crisp

The recipe that received the highest scores according to no. 2 above was used in preparing the budu-tumih noni leaf rice crisp in order to test for properties and chemical compositions as follows:

3.1 Physical properties

3.1.1 Water activity (a_w) was measured using a water activity meter (Novasina AG, Neuheimstrasse 12, CH-8853, Lachen, Switzerland).

3.1.2 The rice crisp color was measured at a planar sample surface in which the light (L*), redness/ greenness (a*) and yellowness/blueness (b*) were determined using Hunter lab (Color Aqua Lab s3600090, Hunter Associate Laboratory, VA, USA).

3.1.3 Hardness and crispness were measured by a penetration test using a texture analyzer (TA-XT2 stable Micro System, England). The cracker samples were placed on a fabricated hollow cylindrical base (25 mm inner diameter, 1.5 mm thickness, stainless steel). Force was applied using a 5 mm spherical compression probe (TA7) at the test speed of 0.5 mm/s until the sample cracked. Hardness and crispness were recorded.

3.2 Chemical compositions

3.2.1 The moisture content of the rice crisp was determined by drying a 3 g sample in an oven of 105ºC for 3 hours until a constant weight was obtained (AOAC, 1999).

3.2.2 Crude protein content (g water/100 g sample) was analyzed according to the Kjeldahl method using a factor of 6.25 for the conversion of the nitrogen to crude protein.

3.2.3 Ash content was performed on a 2-3 g sample after combustion in a muffle furnace at 550ºC for 8 h. Calculate the percentage of total ash in the weight of g sample (g ash/100 g sample).

3.2.4 Fat was calculated for weight loss by extraction for 8 h with petroleum ether in a soxhlet apparatus.

3.2.5 Total dietary fiber (TPF) was determined by the method of AOAC (AOAC, 1990).

3.2.6 pH value was determined by dipping the pH electrode into the homogenates of the rice crisp in distilled water (1/1). All measurements were performed at room temperature using pH-meter (WTW Inolab, Weinheim, Germany).

4. Study on the level of consumers' acceptance of budu-tumih noni leaf rice crisp

The rice crisp sample that has been accepted in no.2 was prepared for the consumer test and was kept in polyethylene plastic container at ambient temperature before usage. A survey for acceptance of target consumers that consume rice crisp product at least once per week using questionnaire. Data gained includes demographic data, consumers' liking scores toward the product (using the 5-point hedonic scale), and data of 100 panelist' acceptance (using the binomial (yes/no) scale). The sensory test was used to determine consumer preference of color, odor, taste, crispness and overall acceptance.

5. Study on the shelf life of budu-tumih noni leaf rice crisp

The rice crisp was kept as described in no.4 was tested for the value of a_w, color, and microbial content every 2 weeks for 10 weeks.

6. Statistical analysis

Three replications of the experiment were conducted at separate times and all analyzes were performed in triplicates. Mean and standard errors were calculated. Data gained were analyzed with ANOVA. The results of acceptance test comprised the frequency (percentage) and the average liking score of consumers toward the developed budu-tumih noni leaf rice crisp. Statistically difference was established at $p< 0.05$.

Results and discussion

1. The appropriate concentration of noni leaf extract for the production of noni leaf rice crisp.

The results of the sensory score assessment of 40 untrained panelists' reports in Table 1. From the results obtained, there was no statistically significant difference ($p\geq 0.05$) in the appearance, odor, crispness and overall preference of each recipe. It was found that only the

characteristic of taste in the 3rd recipe was more preferable than the 1st recipe but not different from the 2nd recipe significantly ($p<0.05$). The result indicated that the rice crisp has the taste of noni leaf more pronounced since a higher proportion of noni leaf was used. Thus, the appropriate concentration of noni leaf extract to produce noni rice crisp is the 3rd recipe, which consists of 120 grams of noni leaf and 500 ml of water.

Table 1 Sensory evaluation results for the three noni leaf rice crisp recipes

Sensory characteristic	Sample of noni leaf rice crisp recipes		
	Recipe 1 (70 g noni leaf)	Recipe 2 (90 g noni leaf)	Recipe 3 (120 g noni leaf)
Appearance^{ns}	7.17±0.78	7.47±0.68	7.17±0.83
Color^{ns}	7.13±0.93	7.30±0.65	7.20±0.69
Odor^{ns}	6.97±0.96	7.20±0.84	7.47±0.73
Taste	7.07±0.94^{b}	7.40±0.93^{ab}	7.60±0.77^{a}
Crispness^{ns}	7.23±0.69	7.20±0.08	7.26±0.75
Overall preference^{ns}	7.43±0.72	7.66±0.88	7.70±0.95

Remark: Each value is presented as mean ± standard deviation (n=3)
Different superscripts in the same row indicate significant differences ($p<0.05$)
ns shows that there was no statistically significant difference at ($p\geq0.05$)

2. The appropriate quantity of budu-tumih content of noni leaf rice crisp topper

The sensory quality assessment of 30 untrained panelists' performing with 9-point hedonic scale is shown in Table 2. The results reveal that, the 1st recipe characteristics were significantly different from the 2rd and the 3rd recipe whereas the 2nd and the 3rd characteristics were not significantly different in all characteristics ($p<0.05$) except for the crispness.

Therefore, the selected recipe of budu-tumih at the level of 5 g/piece was used in the product development of the rice crisp due to the use of less budu-tumih. The picture of the 3 rice crisps are displayed in Fig 3.

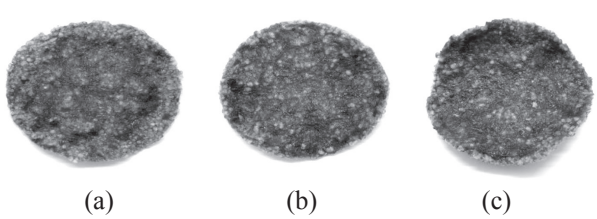

Fig 3. The rice crisp products with difference of budu-tumih topper paste (a) 3 g per piece, (b) 5 g per piece and (c) 7 g per piece

Table 2 sensory evaluation results for the three noni leaf topping with different three budu-tumih contents

Sensory characteristic	Recipe		
	1 (3 g/ piece)	2 (5 g/ piece)	3 (7 g/ piece)
Appearance	7.07±0.98^{b}	7.78±0.73^{a}	7.70±0.98^{a}
Color	7.17±0.69^{b}	7.80±0.66^{a}	7.60±0.89^{a}
Odor	6.90±0.86^{b}	7.73±0.94^{a}	7.43±0.74^{a}
Taste	7.06±0.90^{b}	7.86±0.97^{a}	7.80±0.84^{a}
Crispness^{ns}	7.60±0.81	7.93±0.69	7.66±0.75
Overall preference	7.26±0.73^{b}	8.03±0.71^{a}	7.80±0.88^{a}

Remark: Each value is presented as mean standard deviation (n=3)
Different superscripts in the same row indicate significant differences ($p < 0.05$)
ns shows that there was no statistically significant difference ($p \geq 0.05$)

3. Physical properties and chemical compositions of budu-tumih noni leaf rice crisp.

3.1 Physical properties

The physical properties of the final product are shown in Table 3. The low a_w and pH of the budu-tumih noni leaf rice crisp in which both parameters are highly related to product deterioration. This indicates that the risk of deterioration (by microorganisms, enzymes or none enzymatic reactions) is minimal. The product's a_w values (0.39±0.01) is similar to the study of Sangkabhan (1998) who reported that Khao Tang Na Tang from rice (rice crisp), groundnut and sesame tempeh had the a_w of 0.39. The aw still remains lower than 0.6 in which the level of bacteria are unable to grow (Seman et al., 1980).

The results on the color measurement of the final product in term of Hunter L*, a*, b* color coordinate values were lightness (L*) 14.27±1.27, redness (a*) 4.04 ±0.46 and yellowness (b*) 2.36 ± 0.25. The Hunter Lab a* value represents red color. The red color is derived from the mixture of curry dried chili in which the dried chili has a colorant or pigment called carotene.

The b* value represents a yellow color of paste. In the manufacturing of the budu-tumih, coconut milk is the main ingredient. When coconut milk is heated it changes the color to brown and/or darker. Therefore, budu-tumih shows an attractive color.

Table 3 Physical properties of budu-tumih noni leaf rice crisp

Physical characteristic		Values
Color		
	L*	14.27 ± 1.27
	a*	4.04 ± 0.46
	b*	2.36 ± 0.25
	a_w	0.39 ± 0.01
Texture		
	Hardness	5.61±0.12
	Crispness	4.52±0.09

Remark: Each value is presented as mean ± standard deviation (n=3)

The texture property in terms of hardness and crispness were 5.61 and 4.52. This is similar to the value 4.4 and 4.0 black sesame crispy cracker made from broken Sinlek rice that is reported by Ladnoi & Wongtong (2017). Crispness can be affected by the nature of the material and the structure that the material forms (Zzaman et al., 2017).

3.2 Chemical compositions.

The chemical composition analysis of budu-tumih noni leaf rice crisp has mostly been accepted (2nd recipe), the results reveal that protein, fat, carbohydrate, fiber, ash and moisture content were 10.17%, 18.97%, 61.20%. 3.62%, 1.90% and 4.14%, respectively (Table 4). From Table 4, the moisture content of the rice crisp product was somewhat equal to the 4.08 of Khao-Tang supplemented with calcium from gray feather back fish bone reported by Iamkampeng & Inget (2015). According to the Thai Community Product Standard (TCPS:ICS; 119/2011, 2011), the moisture content in rice cracker products (Khaotang) must contain a maximum of 6 %. The protein content in 10.17% is most probably due to the production process of budu-tumih, which has a fish content in budu of 7.17% - 12.08%. The fat content of the rice crisp was 18.97 because the process of budu-tumih production consist of coconut milk and vegetable oil.

Table 4 Chemical compositions of budu-tumih noni leaf rice crisp

Chemical composition	Noni leaf budu-tumih rice crisp
Protein	10.17±0.20
Fat	18.97±0.28
Carbohydrate	61.20±0.03
Fiber	3.62±0.01
Ash	1.90±0.25
Moisture	4.14±0.38

4. Consumers' acceptance of the budu-tumih noni leaf rice crisp

The results of consumers' acceptance on the finished product which covers the demographic data and the consumers' linking and preference test, using 100 respondents in Yala province are shown in Table 5 and Table 6.

Table 5 Demographic data

Demographic attribute	Frequency (percent)
1. Gender	
- Male	30
- Female	70

Table 5 Demographic data (continued)

Demographic attribute	Frequency (percent)
2. Age	
- Less than 20	-
- 21-25	57
- 26-30	15
- 31-35	15
- 36-40	13
- More than 40 years old	-
3. Occuoation	
- Student	30
- Contractors	13
- Housewife/Steward	10
- Government Official	12
- Personal Businness	15
- Farmer/Gardener	20
4. Education Level	
- High School	3
- Vocational Certificate	17
- High Vocational Certificate	21
- Bachelor's degree	57
- Master's degree	2
- Higher than Master's degree	-
5. Average monthly income	
- Less than 5,000	25
- 5,001-10,000	47
- 10,001-15,000	23
- 15,001-20,000	5
- 20,001-25,000	-
- More than 25,000	-

Table 6 Mean liking scores of the budu-tumih noni leaf rice crisp

Sensory attribute	Mean±SD
Apperance	4.30 ± 1.68
Color	4.62 ± 1.58
Odor	4.08 ± 0.10
Tested	4.53 ± 1.02
Crispness	4.40 ± 0.38
Overall preference	4.99 ± 0.53

Remark: Each value is presented as mean ± standard deviation (n=3)

The results from testing of consumers liking on the 5-point hedonic scale (1-dislike very much and 5-like very much) of the finished product was conducted using 100 respondents. 70% of consumers were females and 30% were males (21-40 age range). The testers mostly rated the highest score of overall preference at 4.99±0.53 point. The characteristic of appearance, color, odor, taste and crispness were 4.30±1.68, 4.62±1.58, 4.08±0.10, 4.53±1.02 and 4.40±0.38, respectively (Table 6). This indicates that the finished rice crisp sensory attributes have a mean of like to like very much.

The results of consumers' acceptance and purchasing decision of the budu-tumih noni leaf rice crisp are shown in Table 7.

Table 7 Consumers' acceptance and purchasing decision of the budu-tumih noni leaf rice crisp

Data	Frequency (percent)
Consumers' acceptance of the budu-tumih noni leaf rice crisp	
- Accept	89
- Decline	11
Purchasing decision of the budu-tumih noni leaf rice crisp	
- Purchase	90
- Not purchase	10

The results from the survey of the consumers' acceptance and decisions on purchasing the budu-tumih noni leaf rice crisp indicates that 89% of the consumers accept the product. 90% of the consumers decided to purchase the product. Therefore, budu-tumih noni leaf rice crisp from this research has a high chance to be produced commercially.

5. Study on the shelf life of the budu-tumih noni leaf rice crisp

The qualities of change in rice crisp were investigated in terms of aw, color and microbial content up to 10 weeks. At first date of storage, a_w was 0.36 and tended to increase slightly until ten weeks as shown in Fig. 4. Water activity range changed to 0.36-0.48 and it was in the scope which was set as 0.60 (Pornchaloempong & Ratanapanon, 2002). The result coincides with the study of Sangkapan (1998) who reported that Khao Tang Na Tang (rice crisp) from rice, groundnut and sesame tempeh has a_w equal to 0.34. The a* values correspond to red-green profile increasing from 1.76 to 2.62 as shown in Fig.5 (b). This change shows that the obvious redness in the color profile. The b* values which represents yellowness increased from 20.32 to 31.02 within 10 weeks as shown in Fig. 5 (c). From the results obtained, the redness of the crisps increased with an increase of a_w. There was higher water absorption and oxygen permeability during storage in accelerated mallard reaction resulting in the red color change (Rattanapanone, 2002). The total plate count of the product was less than $1x10^6$ CFU/g and yeast as well as mold count was less than 100 CFU/g. Thus, it could be implied that the product was safe to consume (Table 8).

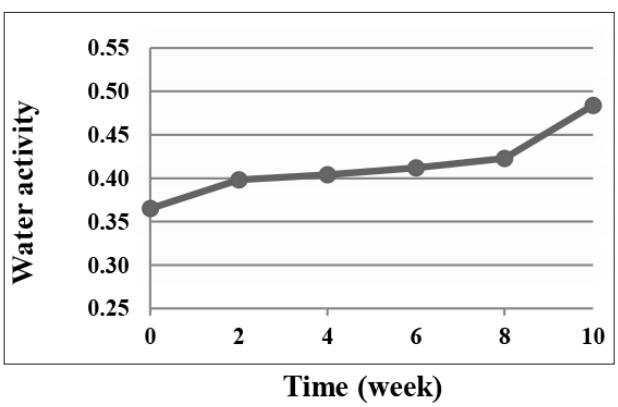

Fig. 4 Water activity change of budu-tumih noni leaf rice crisp during storage period up to 10 weeks

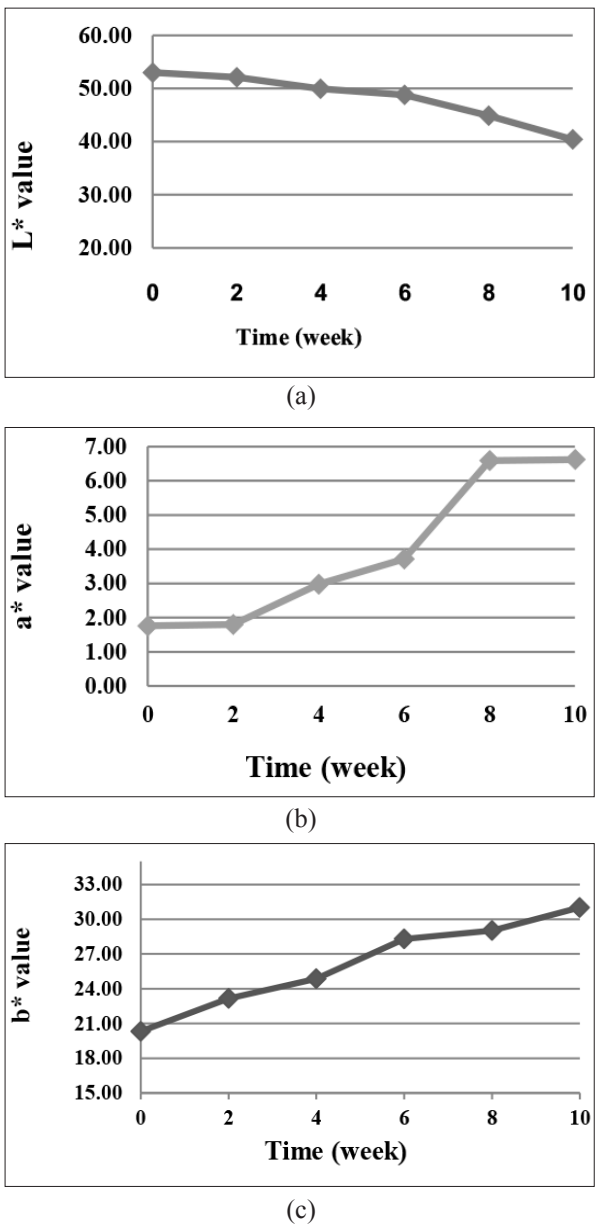

Fig. 5 Color change of budu-tumih noni leaf rice crisp during storage up to 10 weeks: (a) lightness, (b) redness and (c) yellowness

Table 8 Microbial analysis results of budu-tumih noni leaf rice crisp during storage up to 10 weeks in polyethylene plastic container at ambient temperature.

Storage period (week)	Microbial analysis	
	Total plate Count ($x10^6$ CFU/g)	Yeast and Mold (CFU/g)
0	ND	≤100
2	ND	≤100
4	ND	≤100
6	ND	≤100
8	0.05±0.02	≤100
10	0.12±0.01	≤100

Remark: ND = not detect, Each value is presented as mean±standard deviation (n=3)

Conclusion

The purpose of this research was to develop a new rice crisp by adding noni leaf extract and topping with budu-tumih paste as one of local halal food products. The noni leaf rice crisp were dark-green. The results indicate that the proper concentration of noni leaf extract was the ratio of noni and water at 120:500 g/ml and the amount of budu-tumih suitable for the topping of the rice crisp was 5 g/piece (w/w). The product shows an attractive color and odor in which the consumers are satisfied with a high protein content of 10.17%. The product has low moisture content at 4.14%. The product was judged by the average consumers' liking scores in the "like to mostly like" range (4.99±0.53). 89% of consumers accept the product and 90% of the consumers decided to purchase the product if available in the market. From the results of the shelf life study, it was shown that the total plate count was less than $1x10^6$ CFU/g while the yeast and mold count was less than 100 CFU/g at 10 weeks storage period which complies with the Thai Community Product Standard. Hence, the shelf life of the product was at least 10 weeks at room temperature storage. This implies that it is feasible to produce budu-tumih noni leaf rice crisp to commercial scale.

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Lactic Acid Bacteria Isolates from Pla-som, Their Antimicrobial Activities and Fermentation Properties in Pla-som

Sirilak Sanpa[a*], Sirikarn Sanpa[a] & Maitree Suttajit[b]

[a] *Division of Microbiology and Parasitology, School of Medical Sciences, University of Phayao, Phayao, 56000 Thailand*

[b] *Division of Biochemistry, School of Medical Sciences, University of Phayao, Phayao, 56000 Thailand*

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Abstract

Pla-som is a traditional fermented fish product widely consumed in Thailand. However, its hygienic quality and test consistency are often uncontrollable. To minimize the risk of fermentation failure, the use of selected starter culture could result in quality control of the end product. In this study, lactic acid bacteria (LAB) were isolated from eight samples collected from different Pla-som producers in Phayao City using De Man Rogosa Sharpe (MRS) agar. The cell-free supernatant of isolated strains was determined for the antibacterial activity against food borne pathogens (*Escherichia coli, Staphylococcus aureus, Bacillus cereus, Clostridium perfringens, Shigella* sp., and *Vibrio* sp.). In order to exhibit the presence of proteinacious bacteriocin produced by LAB, the crude supernatant of isolated strains were inactivated with proteolytic enzymes, pepsin and trypsin. The results showed that the antibacterial activity of the 14 from 55 bacterial isolates was diminished by the enzymes. Strain ST2 and TT3 were selected based on the highest antimicrobial activity on tested pathogenic bacteria and identified by 16S rRNA sequence analysis as *Lactobacillus paraplantarum* and *Pediococcus pentosaceus,* respectively. Therefore, they were used as starters in Pla-som fermentation. Lower pH value and higher acidity were also observed during the fermentation period. The addition of selected starter cultures significantly decrease fermentation time due to a combination of pH reduction and acid production. The sensory evaluation of the fish product with the mixed starter had the highest overall satisfaction score as tested in 30 volunteers.

Introduction

Pla-som is a traditional fermented fish widely consumed in the northern part of Thailand (Sikhiram, 2018). It is made from fish, sugar, salt, garlic and cooked rice and fermented using natural microbial flora (Hwanhlem et al., 2011). The traditional production of Pla-som was based on spontaneous fermentation due to

* Corresponding Author
e-mail: s.sanpa@gmail.com

the development of the microflora that is naturally present in the raw materials. The fermentation provides a combination of reduced pH and produced organic acid, mainly lactic acid (Saithong et al., 2010; Pringsulaka et al., 2012; Riebroy et al., 2008).

LAB are generally accepted as safe microorganisms that play an important role in food fermentation and preservation either by the presence of natural microflora or the addition of starter cultures under controlled conditions (Paludan-Miiller et al., 1999). The preservative effect exerted by LAB is mainly due to the production of lactic acid. LAB also produces antimicrobial compounds such as hydrogen peroxide, reuterin and bacteriocins (Yang et al., 2012). Bacteriocins are antimicrobial peptides against other bacteria (Yang et al., 2014). In recent years, bacteriocin producing LAB were find to have a potential use as safe additives for food preservation (Diop et al., 2007). Nisin, a bacteriocin produced by *Lactococcus lactis,* is the most thoroughly studied and applied as a commercial additive to certain foods (Woraprayote et al., 2016). Other bacteriocins such as pediocin, may also have potential applications in foods, though they are not currently approved as antimicrobial food additives (Porto et al., 2017).

The traditional fermented fish production has the public health problem of poor hygiene due to microbial contamination during the process, leading to a failure in GMP standardization (Pumipan & Inmaung, 2016). The quality of Pla-som products show the inconsistency of acidity, test, and fermentation time as reported by Chompuming (Chompuming et al., 2010). To minimize the risk of fermentation failure, using a selected starter culture could shorten the fermentation time and resulting in quality control of the end product (Saithong et al., 2010; Visessanguan et al., 2006).

This study aims to search LAB isolated from Pla-som fermented products and to determine their inhibitory effects on food-borne pathogens. Therefore, the LAB was isolated and evaluated for their antimicrobial effects on food borne pathogens including *Escherichia coli, Staphylococcus aureus, Bacillus cereus, Clostridium perfringens, Shigella* sp. and *Vibrio* sp. (Phugan et al., 2018). The effective bacterial strains were selected and used in Pla-som fermentation in which the fish products were finally evaluated for sensory by the testers.

Materials and methods

1. Isolation of lactic acid bacteria

Lactic acid bacteria (LAB) were isolated from eight samples collected from different Pla-som producers in Phayao City. Twenty-five grams of Pla-som samples were added to 225 ml of sterilized 0.1% (w/v) peptone solution. The diluted sample was cultured on de Man Rogosa Sharp (MRS) agar containing 0.4% (w/v) bromocresol green and incubated at 30°C for 48 h (Pringsulaka et al., 2012; Kargozari et al., 2015). The anaerobic cultivation was done by using an anaerobic jar. Colonies that changed the medium color from green to yellow were collected and streaked on MRS agar for purification. Each of the isolated colonies was tested for catalase and Gram strain, then the isolates that were catalase negative and Gram- positive were maintained in MRS broth with 20% glycerol at -20°C.

2. Antimicrobial activity and enzymatic testing of bacteriocin producing LAB

The selected strains were inoculated into fresh MRS broth and incubated at 30°C in anaerobic condition. After 24 h of cultivation, centrifuged at 10,000 rpm for 10 min, the cell free supernatant was collected for antibacterial activity against food-borne pathogens (*E. coli, S. aureus, B. cereus, C. perfringens, Shigella* sp., and *Vibrio* sp.). The agar wells diffusion method was used to determine the antimicrobial activity of LAB strains. The food borne pathogens grown in nutrient broth at 37°C was adjusted to NO 0.5 McFarland standards and spread over the surface of a nutrient agar plate with a sterile cotton swab. The plates were allowed to dry and a sterile cork borer with diameter of 6 mm was used to cut wells in the agar. Each well was filled with 50 µl of the culture supernatant obtained from the LAB strains. After incubation at 37°C for 24 h, the plates were observed for a zone of inhibition around the well. The experiment was carried out in triplicates, lactic acid with pH 2.3 was used as a positive control and MRS broth without bacterial culture was used as a negative control.

To confirm the production of a proteinaceous bacteriocin, cell free supernatant displaying antimicrobial potential after acid neutralization and H_2O_2 elimination was treated with 1 mg/ml of proteolytic enzymes, including pepsin and trypsin (Sigma-Aldrich Corporation, USA) at 37°C for 2 h (Herreros et at., 2005). The cell free supernatant without proteolytic enzymes treatment, was used as a positive control. Antimicrobial activity of the treated cell free supernatant was determined by agar

diffusion bioassay as described above (Diop et al., 2007; Elayaraja et al., 2014).

3. Identification of selected LAB

The selected LAB strains were identified using morphological and biochemical tests and 16S rRNA sequence analysis (Diaz et al., 2013). The selected LAB was characterized by Gram staining, cell morphology, catalase reaction, motility, oxidative-fermentative test, methyl red test, Voges-Proskauer test, gelatin degradation, indole test, citrate test and carbohydrate fermentation. The strains were identified to the species by the scheme devised by Schillinger & Lucke (1989) for lactobacilli with several modifications. The morphology of the isolates was determined under x1,000 magnification using phase-contrast microscopy. The DNA extraction method for 16S rRNA sequencing and sequence data analysis was done as previously described (Anyogu et al., 2014). Genomic DNA was extracted from the isolates using the Microbial DNA Isolation kit. Universal primers F44 (5'RGTTYGATYMTGGCTCAG-3') and R1543 (5'-GNNTACCTTKTTACG ACTT-3') were used for the amplification of the 16S rRNA gene by PCR. A homology search of the sequences was conducted using the BLAST program at the NCBI database.

4. Pla-som preparation and sensory evaluation

The formula of Pla-som consists of 920 g of fish meat, 10 g of garlic, 30 g of salt and 40 g of steamed rice. Pla-som fermentations were carried out with 4 treatments: (1) without inoculation of starter culture (control), (2) inoculation with *Lactobacillus paraplantarum* ST2, (3) inoculation with *Pediococcus pentosaceus* TT3 and (4) inoculation with mixed starter culture (*L. paraplantarum* ST2 and *P. pentosaceus* TT3). In treatment 2-4, the starter cultures were added to the final concentration of 5 log of CFU/g. The mixture of each treatment was transferred into a plastic bag and incubated at room temperature (30±2°C) for 4 days. Their acidity and pH value were recorded during fermentation period. The fermented Pla-som sample was cultured on MRS agar and total plate count agar to determine lactic acid bacteria and total viable bacteria, respectively.

After the 4-day fermentation, all samples were fried in palm oil at a cooking temperature. The sensory evaluation was tested by 30 volunteers using a 5 point Hedonic scaling modified from Seo et al. (2009) and Tinakorn Na Ayutthaya et al. (2018) (1 = dislike extremely, 5 = like extremely). The testers were asked to evaluate the 4-day fermented samples for color, odor, flavor, texture and overall acceptance.

5. Statistical analysis

Statistical analysis was performed using One-way Analysis of Variance (ANOVA) and T-tests for a statistical significance of $P<0.05$ by using SPSS software program version 23. All data are reported as means ± standard deviations (SD).

Results and discussion

1. Isolation of lactic acid bacteria (LAB) and their antimicrobial activity

Lactic acid bacteria (LAB) were isolated from 8 Pla-som samples made in Phayao Province. De Man Rogosa Sharpe (MRS) agar containing 0.4% (w/v) bromocresol green was used as a preliminary screening medium for LAB. There were 55 isolated strains that changed the medium color from green to yellow. Among them, 47 isolates were gram-positive rod or cocci, non-spore forming and negative for catalase test; after that, the isolates were selected for further experiments. These 47 isolates were then analyzed for the antimicrobial activity against food-borne pathogens (*E. coli, S. aureus, B. cereus, C. perfringens, Shigella* sp. and *Vibrio* sp.). The results showed that 33 isolates could inhibit all six food-borne pathogens. After acid neutralization and H_2O_2 elimination, pepsin and trypsin were used for testing of proteinaceous bacteriocin. The cell-free supernatant without proteolytic enzyme treatment was used as a positive control. There were 14 isolates (NL5, TP3, TP4, TP6, TP9, TT3, TT2, MT3, MT4, ST5, ST2, ML3, ML6, and ML1) that lost their antibacterial activity with proteolytic enzymes (Table 1). The results suggested that these 14 strains were able to produce proteinaceous bacteriocin.

During the production of fermented food, LAB played an important role in the formation of flavor and texture and the control of spoilage and pathogenic microorganisms (Gao et al., 2014; Michalak et al., 2018). Among the 14 strains, ST2, which showed strong and broad antibacterial activities against various bacterial strains, was then selected as the starter of Pla-som production. In the fermentation process, various lactic acid bacteria play different roles for improvement of the products; therefore, strain TT3, which is the only one member of Pediococci group, was also selected as starter of Pla-som production.

2. Identification of lactic acid bacteria (LAB)

The selected strains from antimicrobial activity and enzymatic test of bacteriocin-producing LAB were

Table 1 Inhibitory effect of LAB on six food-borne pathogens after treatment with proteolytic enzymes, pepsin and trypsin

Isolate code	Proteolytic enzyme	Inhibition zone (mm)					
		B. cereus	***C. perfringens***	***E. coli***	***S. aureus***	***Shigella* sp.**	***Vibrio* sp.**
NL 5[ab]	Control	11.5	14	12	12	12	10.5
	Pepsin	9.5	12	10.5	9.5	12	10
	Trypsin	10	12.75	10.5	10	11	7.5
TP 3[abc]	Control	7	14	10	11	11.5	13.5
	Pepsin	-	9.5	10	-	10.5	10.75
	Trypsin	7	14	10	9	10.5	10
TP 4[abc]	Control	9.5	10.75	12	13	11.5	13
	Pepsin	-	11.5	12	-	11.25	10.25
	Trypsin	9	-	11.25	-	11	10.5
TP 6[bc]	Control	10	10.5	12	14	11.5	12.5
	Pepsin	9	9	12	-	-	-
	Trypsin	8.5	-	9	-	9	-
TP 9[bc]	Control	11	11.75	13.5	14	12.5	13.5
	Pepsin	-	-	11.75	-	-	-
	Trypsin	10.5	17.5	10	-	-	-
TT 3[bc]	Control	7.5	14.5	12.5	8.75	9.5	11.25
	Pepsin	-	-	12	8	9	-
	Trypsin	6.75	-	12	8.17	9.25	-
TT 2[c]	Control	9.5	11	12	12.5	12.75	14.25
	Pepsin	9.25	-	-	8	-	-
	Trypsin	7.5	10.5	-	-	9	-
MT 3[bc]	Control	9.5	12	12.25	12.5	12.25	14.25
	Pepsin	9	9	9	-	8	-
	Trypsin	8	-	10	-	9	-
MT 4[abc]	Control	9.5	12	12.5	14	11	15.75
	Pepsin	-	9.5	12.5	9	10.5	-
	Trypsin	9	-	12	12	9.5	-
ST 5[abc]	Control	10	12.5	13.5	12.5	12	13.5
	Pepsin	9.5	10	13	10.5	11.75	-
	Trypsin	9	12	12.75	9	11.75	-
ST 2[a]	Control	21	20.5	13	16	12	10.5
	Pepsin	12.5	13.75	12.75	-	12	10.5
	Trypsin	11	13	13	9.5	10.75	10
ML 3[abc]	control	9.5	12.5	12	12	11	11.5
	Pepsin	9.5	12.5	11	11	8.75	11
	Trypsin	9	11.75	11	10	10	11
ML 6[abc]	Control	10	13.5	13	10.5	12	12.75
	Pepsin	8.25	8.75	-	10	10	-
	Trypsin	10	10	12	8.5	-	12
ML 1[abc]	Control	9.5	12	11.5	13	10	10
	Pepsin	9	9	10.75	10	9.5	9.5
	Trypsin	8	12	11	10	10	-

Remark: Mean from triplicate determinations.
Different superscripts in the same column indicate significant differences ($p < 0.05$).

determined for characteristics of Gram staining, cell morphology, catalase reaction, motility, OF test, methyl red test, Voges-Proskauer test, gelatin degradation, indole test, citrate test and carbohydrate fermentation. The strains were confirmed by molecular identification of 16S rRNA (approximately 1,500 bp) sequence, which showed a high similarity more than 99% to *Lactobacillus pentosus, L. paraplantarum, L. plantarum,* and *Pediococcus pentosaceus* (Table 2).

It was reported that *L. paraplantarum* and *P. pentosaceus* could also be isolated from rice bran, pickles, sourdough, fermented sausages and olive fermentation brine (Parente

Table 2 Biochemical and physiological characteristic of LAB and their molecular identification by 16S rRNA sequence

Isolate code	Characteristic														Identification based on 16s rRNA sequencing (%identity)
	Gram stain	Shape	Catalase	Motility	OF test	Methyl red	VP	Gelatin	Indole	Citrate	Carbohydrate fermentation				
											Glucose	Sucrose	Lactose	Manitol	
NL5	Positive	Rod	-	-	+/+	+	-	-	-	-	+	+	+	+	*Lactobacillus pentosus* (99.93)
TP3	Positive	Rod	-	-	+/+	+	-	-	-	-	+	+	+	+	*Lactobacillus pentosus* (99.93)
TP4	Positive	Rod	-	-	+/+	+	-	-	-	-	+	+	+	+	*Lactobacillus plantarum* subsp. *plantarum* (100)
TP6	Positive	Rod	-	-	+/+	+	-	-	-	-	+	-	-	+	*Lactobacillus pentosus* (99.93)
TP9	Positive	Rod	-	-	+/+	+	-	-	-	-	+	-	-	+	*Lactobacillus pentosus* (99.93)
TT2	Positive	Rod	-	-	+/+	+	-	-	-	-	+	+	+	+	*Lactobacillus plantarum* subsp. *plantarum* (99.92)
TT3	Positive	Cocci	-	-	+/+	+	-	-	-	-	+	+	+	+	*Pediococcus pentosaceus* (99.74)
MT3	Positive	Rod	-	-	+/+	+	-	-	-	-	+	+	+	+	*Lactobacillus plantarum* subsp. *plantarum* (99.87)
MT4	Positive	Rod	-	-	+/+	+	-	-	-	-	+	+	+	+	*Lactobacillus plantarum* subsp. *plantarum* (99.87)
ST5	Positive	Rod	-	-	+/+	+	-	-	-	-	+	+	+	+	*Lactobacillus paraplantarum* (99.87)
ST2	Positive	Rod	-	-	+/+	+	-	-	-	-	+	+	+	+	*Lactobacillus paraplantarum* (99.87)
ML3	Positive	Rod	-	-	+/+	+	-	-	-	-	+	+	+	+	*Lactobacillus pentosus* (99.93)
ML1	Positive	Rod	-	-	+/+	+	-	-	-	-	+	+	+	+	*Lactobacillus paraplantarum* (99.87)
ML6	Positive	Rod	-	-	+/+	+	-	-	-	-	+	+	+	+	*Lactobacillus pentosus* (99.93)

Table 3 The changes in total viable count (TVC) and lactic acid bacteria (LAB) in natural fermentation and inoculation with starters

Fermentation Time	0 h		24 h		48 h		72 h		96 h	
	TVC (log cfu/g)	LAB (log cfu/g)	TVC (log cfu/g)	LAB (log cfu/g)	TVC (log cfu/g)	LAB (log cfu/g)	TVC (log cfu/g)	LAB (log cfu/g)	TVC (log cfu/g)	LAB (log cfu/g)
Without starter	7.6 ± 0.1^{a}	3.6 ± 0.1^{b}	7.6 ± 0.1^{a}	4.9 ± 0.1^{b}	7.7 ± 0.2^{a}	5.7 ± 0.2^{b}	7.7 ± 0.1^{a}	6.2 ± 0.2^{b}	7.7 ± 0.3^{a}	6.9 ± 0.1^{b}
Strain ST2	8.1 ± 0.4^{ab}	4.1 ± 0.1^{a}	7.5 ± 0.1^{ab}	5.3 ± 0.3^{a}	7.1 ± 0.4^{ab}	6.5 ± 0.3^{a}	6.9 ± 0.3^{ab}	7.1 ± 0.2^{a}	6.9 ± 0.2^{ab}	6.8 ± 0.3^{a}
Strain TT3	7.5 ± 0.3^{b}	4.5 ± 0.2^{ab}	7.1 ± 0.2^{b}	5.1 ± 0.2^{ab}	6.5 ± 0.3^{b}	6.3 ± 0.2^{ab}	6.9 ± 0.2^{b}	6.8 ± 0.3^{ab}	6.9 ± 0.1^{b}	6.9 ± 0.3^{ab}
Mixed starter culture	7.8 ± 0.2^{ab}	4.6 ± 0.1^{a}	7.5 ± 0.3^{ab}	5.8 ± 0.1^{a}	7.1 ± 0.1^{ab}	6.9 ± 0.3^{a}	6.8 ± 0.3^{ab}	7.1 ± 0.3^{a}	6.8 ± 0.3^{ab}	6.8 ± 0.2^{a}

Remark: Mean ± SD from triplicate determinations
Different superscripts in the same column indicate significant differences ($p < 0.05$).

et al., 2010). *Lactobacillus paraplantarum* L-ZS9 is a probiotic starter isolated from fermented sausage and it is a great producer of class II bacteriocins (Liu & Li, 2016; Zhang et al., 2016). There are many *Pediococcus* strains that produce pediocin, an effective antilisterial bacteriocin (Porto et al., 2017). However, the use of these two bacteria strains, *L. paraplantarum* and *P. Pentosaceus,* as the starter in Pla-som fermentation has been scarcely studied and reported.

3. Pla-som fermentation and sensory evaluation

According to the highest pathogen inhibition, *L. paraplantarum* ST2 and only one member of

Pediococci group, *P. pentosaceus* TT3, were used as starters for Pla-som production. In all Pla-som fermentation with and without inoculation of the starter culture, the numbers of LAB are significantly different (Table 3). Comparison between with and without inoculation of the starter culture, the total viable count in Pla-som without the starter inoculation is significantly higher than Pla-som with the inoculation of the starter culture. On the other hand, the viable lactic acid bacteria in Pla-som without the starter is significantly lower than Pla-som with the starter culture. The result suggest that *L. paraplantarum* ST2 and *P. pentosaceus* TT3 remarkably inhibited the growth of contaminating bacteria as reported by previous studies (Hwanhlem et al., 2011; Visessanguan et al., 2006; Tasaku et al, 2017).

The initial pH and titratable acidity of all the samples were 6.0 and 0.35%, respectively. During the fermentation process, all samples inoculated with the starter culture, both of single culture or mixed culture, exhibited lower pH and higher acidity than natural fermentation without the inoculated starter culture. These samples showed a significant difference from 48 h of fermentation onwards (Fig.1). The manufacturing time of each production could be shortened from 72-96 h to within 48 h and the consistency of acidity and pH levels could be controlled resulting in the qualified Pla-som products, which would be commercially advantageous.

The acceptability of Pla-som inoculated with LAB starter culture was determined by comparing the results of colour, odour, flavour, texture and overall acceptance. The results showed that Pla-som products inoculated with LAB starter were not significantly different from natural fermentation or without the inoculated starter (Table 4). The sensory evaluation was determined with 4-day fermented products, Pla-som inoculated with the mixed starter culture showed the highest overall satisfaction rating of 3.54 from 5.00 satisfaction score.

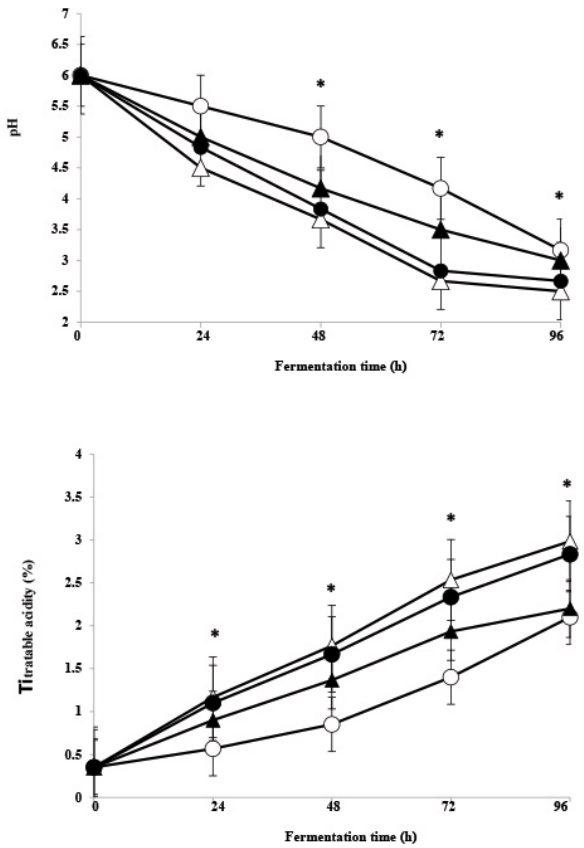

Fig. 1 pH (A) and titratable acidity (B) during Pla-som fermentation. Symbol: open circle, natural fermentation (control); open triangle, inoculated with *L. paraplantarum* ST2; closed triangle, inoculated with P. pentosaceus TT3; and closed circle, inoculated with mixed starter culture.
Error bars represent standard errors, and an asterisk denotes a significant difference between the events within a condition ($p<0.05$).

Finally, both bacterial strains, *L. paraplantarum* ST2 and *P. pentosaceus* TT3, isolated from this study are relatively safe. They were isolated from many food types and were identified as the unharmful species for consumers. The antimicrobial activity of ST2 and TT3 strains against food-borne pathogens contaminated in

Table 4 Sensory characteristics of Pla-som after 4 day fermentation.

	Colour	Odour^{ns}	Flavour	Texture	Overall acceptance
Without starter	3.71± 0.09^{a}	4.10±0.17	3.89±0.19^{a}	2.54±0.11^{b}	3.48±0.06^{a}
Strain ST2	3.37±0.07^{b}	4.20±0.12	3.28±0.25^{bc}	3.03±0.18^{a}	3.47±0.07^{a}
Strain TT3	3.27±0.13^{b}	3.89±0.10	3.23±0.28^{c}	2.94±0.19^{ab}	3.20±0.11^{b}
Mixed starter culture	3.48±0.17^{ab}	3.94±0.10	3.83±0.17^{ab}	3.09±0.13^{a}	3.54±0.14^{a}

Remark: Mean ± SD from triplicate determinations
Different superscripts in the same column indicate significant differences ($p < 0.05$).
ns; not significant

raw materials and food products is demonstrated as a good feature of the strains, which can be further developed as a standard starter used in the fermentation process. Furthermore, the standardization of Pla-som production by ST2 and TT3 strains as a mixed starter culture would definitely upgrade the spontaneous Pla-som fermentation traditionally practiced by the local population.

Conclusion

In this study, lactic acid bacteria (LAB) were isolated from 8 Pla-som samples in Phayao Province. Antimicrobial activity of the bacterial isolates against food-borne pathogens was analyzed. After acid neutralization and H_2O_2 elimination, bacteriocin, which is proteinaceous compound, was positively tested by using pepsin and trypsin. The bacterial strains, ST2 and TT3, were experimentally selected as the starters in Pla-som fermentation. The results show that the pH of the culture inoculated with such selected starters rapidly decreased and the acidity increased within 48 h. The manufacturing time of each production could be shortened from 72-96 h to within 48 h. Besides, the baterioricin in selected bacterial strains was also able to stop pathogenic microorganisms in Pla-som fermentation. Our findings are beneficial to the development and standardization of the Pla-som production from the household level to the reliable SME level.

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Inhibition of *Staphylococcus aureus* by the Cotton Fabrics Treated with the Crude Finish Produced from *Streptomyces* sp. strain AC4

Grissana Pook-In*, Khwanla Seansupa & Sorawit Upakut

Department of Microbiology and Parasitology, Faculty of Medical Sciences, University of Phayao, Phayao, 56000 Thailand

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Abstract

The isolated *Streptomyces* sp. AC4, was able to grow within one week, produced the purple-like pigment in Starch Casein Broth (SCB) and inhibited growth of *Staphylococcus aureus*. The crude samples of AC4 presenting the purple suspension in SCB were separated in two fractions, i.e. supernatants and cell pellets. These fractions were pasteurized for testing the thermal stability of the vegetative cells and spores, and the ability to inhibit *S. aureus*. The investigation showed that cells and spores of *Streptomyces* sp. AC4 in both fractions were inactivated by pasteurization at 73°C. Furthermore, both pasteurized fractions were able to inhibit the growth of *S. aureus*. Then, crude samples were applied to coat cotton fabrics by the simply heat dyeing at 70°C. Using American Association of Textile Chemists and Colorists (AATCC) 147-2004 test for testing antibacterial textile of coating cotton fabrics on *S. aureus*, the investigation indicated that the coating cotton fabrics had inhibition zone of 4.5 mm, indicating that the treated fabrics was able to inhibit *S. aureus*. In addition, AATCC 100-2004 test showed that the treated fabrics present 100% of inhibition of *S. aureus* for 12 and 24 h. Thus, the pasteurized crude samples of *Streptomyces* sp. AC4 will be applied to the natural finish that are environment friendly.

Introduction

The natural compounds of the organism products are investigated for application in various aspects. Especially natural products that could promote skin care and skin health, which is generated by the bioactive compounds that control the growth of the skin flora that live on normal human skin (1×10^5 CFU/cm^2) by the predominant bacterial flora found in the healthy human such as *Staphylococcus epidermidis* and *S. aureus* which are the opportunistic pathogenesis (Teruaki et al., 2017). The human skin flora grows and causes the fiber of textiles to spoil and this may cause unpleasant odor, dermal infection, allergic responses and opportunistic pathogenesis of the skin lesion (Teufel et al., 2010; Singh et al., 2005). These problems were related with consumers of the fabric product. Therefore, the bioactive compounds from the natural products were studied and

* Corresponding Author
e-mail: krissy_seven@hotmail.com

developed for cosmeceuticals and cosmetic ingredients. Recently, Prompamorn and Ratcharin in 2018 studied the *Terminalia chebula* extracts for the gel formulation that showed the crude ethanol extract of *Terminalia chebula* that could protect against dermatitis inducing bacteria including *Staphylococcus aureus* and *Staphylococcus epidermidis* (Prompamorn & Ratcharin, 2018). Parkpoom et al in 2018 reported the leaf extract of *Piper betle* L. as promising for cosmetics with results showing that the test of antimicrobial capacity of the leaf extract of *Piper betle* L., from ethanol inhibited *S. aurues* with the best result having an inhibition zone at the concentration of 12.50 mg/ml. (Parkpoom et al., 2018). Meanwhile, the bioactive compounds for textile products using antimicrobial textiles were also interested in the usage. Currently, the use of fiber and textile are various aspects. In addition, the special fiber or textile containing properties with the ability to inhibit the microflora by itself. This has the capacity to decrease the risk or spread of the opportunistic infection from the flora skin. Moreover, the natural textile is made from natural fibers such as cotton, silk or wool. They have the chemical compounds that are the sources of nutrients such as cellulose, protein or lipid wax for the microbes. Thus, the microorganisms can be the decomposer to reduce the life time of products that the quality of fiber structure destroys. If the textile or natural fiber are coated with the antimicrobial finish, this also extends the life of these products (Singh et al., 2005; Gutarowska et al., 2013). The previous studies focused on the finishing process aimed to improve the quality of the fabric especially finishings that involved treatments with chemical compounds for the potential quality of the fabric including permanent press treatments, water proofing, softening, anti-static protection, soil resistance, stain release and microbial protection that were applied in the fabric treatments process (Moore & Ausley, 2004). On the other hand, the high potential compounds for finishing or dyeing are the synthetic substance which contaminate the natural waters which has become one of the biggest problems in the environment because the major source of dyes and finishes fail to adhere on the fibers during the dyeing step (Guaratini & Zanoni, 2000; Kunz et al., 2002). Therefore, the reasons that the current research about natural resources in the production processes is about natural dyes or finish is to increase the efficiency of the fiber and to reduce environment residual caused from the synthetic compounds. The natural dye and coatings are mostly naturally biodegradable, so it is environmentally friendly (Chequer et al., 2013; Joshi et al., 2009)

The natural dyes or finish are derived from various types of the organism such as plant, fungi, bacteria or actinobacteria. Actinobacteria produce pigments and some species produce bioactive compounds. In addition, some pigments are also a biological substance. The natural resource in finishing and dyeing processes as bioactive compounds are studied in order to improve the quality of the fabric. Various organisms were studied; animal products such as chitosan and sericin, herbal plant products i.e. neem, curcumin, tannin, aloe vera, tea tree and aleppo oak (Gutarowska et al., 2013; Singh et al., 2005; Joshi et al., 2009), and microorganisms such as Monascus pigments and melanin pigments from fungi or actinobacteria. In a previous study, Ali et al studied the melanin pigments that were produced from *Streptomyces virginiae* strain and considered the activity of the bioactive compounds to apply in printing and dyeing of the wool fabric. The result showed that the culture broth of this strain could affect against *S. aureus, Pseudomonas aeruginosa* and fungi (Ali et al., 2011).

In addition to the compounds for finishing, the steps to operate the coating at the optimum temperatures have been a concern because the fiber of textile material such as, cotton, rayon, nylon, wool and some other fibers, dye well at temperatures of 100ºC or below. While polyester and some other synthetic fibers dye more conveniently at temperatures above 100ºC (Perkins, 1991). Thus, the thermo stability of the bioactive compounds for the antibacterial finishes were attempted at the optimum conditions to immobilize the fibers and remain the ability of bioactive compounds too.

The aim of this research is to study using the purple crude samples from *Streptomyces* sp. strain, AC4, cultivated in SCB media. The crude samples (the supernatants and the cell pellets) were tested to inhibit the *S. aureus*. Then, the study of the pasteurization of the crude samples by heat treatment. Furthermore, this results were applied to determine the pasteurization of the crude samples before the coating step. The cottons were coated with the crude finish then the treated fabrics was determined by AATCC Test Method 147-2004 and AATCC Test Method 100-2004 for assessing the antimicrobial properties of textiles. This coating cotton could help to promote the skin's hygiene. In addition, the process of the coating is environment-friendly and going apply to the manufacture of textile coating.

Materials and methods

1. Preparation of the starter of *Streptomyces* sp. AC4 and cultivation

The soil actinobacteria, *Streptomyces* sp. AC4, from the collection of the isolated strains of the Plant Genetic Conservation Project under the Royal Initiation of Her Royal Highness Princess Maha Chakri Sirindhorn (RSPG) were cultivated by Starch Casein Agar (SCA: soluble starch 10 g, casein 0.3 g, KNO_3 2 g, NaCl 2 g, $MgSO_4.7H_2O$ 0.05 g, $CaCO_3$ 0.02 g, $FeSO_4.7H_2O$ 0.01 g, K_2HPO_4 2 g, agar 15 g for distilled water 1000 ml) at 30°C for 4 to 7 days. The purple color colony from creating purple-like pigments were observed and subsequently selected. This pure colony for 3 to 5 colony were inoculated to the Starch Casein Broth (SCB) approximately 150 ml and incubated in incubator shaker at 30°C for 4 days (starter). The starter was then transferred to the new SCB (750 ml in the 1000 ml flask) approximately 5 to 10 percentages of final volume and incubated in incubator shaker at 30°C for 7 days. The cultured samples were collected with the incubation times at 0, 24, 48, 72, 96, 120, 144 and 168 h to plot the growth curve performed by the cell dry weights and to calculate the basic characteristics of the growth kinetics as the doubling time (t_d) and specific growth rate (μ) by the formula of the growth kinetics according to the mathematical equation described by Fattah et al. The exponential curve fitting function generated the equation (Fattah et al., 2018).

Exponential phase equation: $y = Ae^{Bx}$, where A and B are numbers

Doubling time equation (t_d): $y = Ae^{Bx} \rightarrow x = \ln (y/A)/B$

When $y = 1$, $x1 = \ln (1/A)/B$

When $y = 2$ (i.e. when y is doubled), $x2 = \ln (2/A)/B$

$t_d = x2 - x1 = [(\ln2 - \ln A) - (\ln1 - \ln A)]/B$ by $\ln1 = 0$

$t_d = \ln2/B = 0.693/B$

Growth rate (μ):

$\mu = \ln2/t_d$

The exponential curve fitting function was computed by the Excel 2016 program with the graph of the growth curve.

2. Preparation of the crude samples

The crude samples were prepared by the cultured media that were divided into two parts including the supernatant and the cell pellets. The cell pellets were then separated from the supernatant by centrifugation at 5,000 rpm for 10 minutes. Next, the supernatant was removed to a new tube and cell pellets were stored in -80°C refrigerator and grinded by the mortar.

The parts of the cracked-cell pellets and the supernatants were used for the experiment of inhibiting the test organism and stabilizing of active compounds after the heat treatments. The cracked-cell pellets were resuspended by the distilled water for 1:10 w/v with the radio of the maximum concentration for the crude samples to permeate the 0.45 µm filter paper. The aseptic processes were sterilized to the aseptic conditions by 0.45 µm filter papers and pasteurized by the heat treatments (the details are shown in the next experiment). Furthermore, the parts of the undiluted supernatants were sterilized by the same methods with the cracked-cell pellets and then the samples were tested for disc diffusion.

Finally, the supernatants and the cracked-cell pellets were mixed as the crude finishing. The ratio of grams of the cracked-cell pellets to volume of the supernatants 1:10 (w/v) for the experiment of coating cotton fabrics. The mixture of the crude finishing were pasteurized by the optimal temperature that was selected by the result of the stabilizing of active compounds after the heat treatments. The optimal temperature for pasteurizing the crude finishing were determined by the inactivate growth of the vegetative cells and spores of the tested actinobacteria and stabilizing of the antibacterial activity.

3. Heat treatment of the crude samples for the pasteurization

The crude samples were treated by the autoclave at the pasteurization temperature of 62°C for 30 minutes and 73°C for 15 minutes. After the pasteurization, the ability of the crude samples to inhibit bacteria were tested by the disc diffusion method. The growth of the vegetative and spore of the actinobacteria, *Streptomyces* sp. AC4 were examined by the dropped plate method on SCA of 10 µl crude samples then cultivated at 30°C for 7 days.

4. Experiment of the crude samples inhibit to the growth of the test microorganisms

The ability of the crude samples to inhibit bacteria were tested by the disc diffusion method. The selected microorganisms which were used in the assays were *Staphylococcus aureus* and *Klebsiella pneumoniae,* according to the standards methods of American Association of Textile Chemists and Colorists (AATCC) for assessing the antimicrobial properties of textiles. The bacteria, *S. aureus* ATCC 25923 and *K. pneumoniae* ATCC 8216, were observed as the tested microorganism.

The pure colony of the test microorganisms were cultured in Muller Hinton Broth (MHB) (HiMedia) at 37°C for 16 to 18 h. The cell suspension was then adjusted to the standard concentration of the McFarland turbidity standards No. 0.5 (1.5 x 10^8 CFU/ml). The bacteria were spread by swabbing on Muller Hinton Agar (MHA) then onto the circular filter papers (6 mm diameter) and were placed on the surface of the MHA. The 20 µl of crude samples was dropped on each filter paper and incubated at 37°C for 18 to 24 h. A drop of distilled water, MHB and SCB was used for the negative control. Moreover, the chloramphenicol disc (30 mg/disc) was applied for the qualitative and positive control of the disc diffusion method. The diameter of an inhibited area (inhibition zone) was measured by the unit in millimeters.

5. Coating cotton Fabrics by crude samples of *Streptomyces* sp. AC4

The circular cottons (45 mm diameter) and the square cotton (25x40 mm) were used. The cotton fabrics were washed by the solution of laundry soap, sodium carbonate (Na_2CO_3) and water (5g:8g:500ml) that were mixed by boiling. This volume was used for 50 g of the cotton. The cotton fabrics were boiled with the solution for 1 h. The fabrics were rinsed by water for 5 times and the cotton was dried before applying the coating. The crude samples as the crude finishing were prepared by mixed suspension of the cracked-cell pellet and the supernatant at ratio 1:10 w/v, as mentioned previously. The coating process used heat at 70°C for 1 h by the ratio of grams of dry cotton material to volume of crude finishing 1:10 (w/v). The coating products were rinsed by water 3-5 times and dried after the coating. The coated fabrics were defined as the treated fabrics for the estimation of the antibacterial activity of the treated cotton (AATCC Test Method 147-2004 and AATCC Test Method 100-2004) while the non-coated fabrics were the untreated fabrics for the control.

6. Estimation of the antibacterial activity of the treated cotton by qualitative methods, AATCC Test Method 147-2004

The treated and the untreated fabrics were tested by modified AATCC Test Method 147-2004 (Antibacterial Activity Assessment of Textile Materials: Parallel Streak Method) for assessment of the qualitative analysis (AATCC 147:2004; Pinho, et al., 2015). The square cottons (25x40 mm) were used and prepared the inoculum of the pure *S. aureus* into 10 ml of Brain Heart Infusion Broth (BHI) (HiMedia) and incubated at 37°C for 24 h. Then, the 1.0±0.1 ml from the inoculum in the enrichment medium, BHI (approximately 1.0x10^7 cells/ml), was transferred into the new BHI 9.0 ml (AATCC 147:2004; Pinho, et al., 2011; Pinho, et al., 2015). The diluted inoculum was streaked on BHI agar (approximately 15±0.5 ml in the petri dish) by making five streaks approximately 60 mm in length, spaced 10 mm on the surface of the central area of the media without refilling the loop and performed the same condition on Baird-Parker Agar (BP) (HiMedia) for the parallel control of the characteristic of *S. aureus*. The pasteurized step of the test swatches (treated cottons) was used by the autoclave at 73°C for 10 min. The test swatches were pressed by contact across the five inoculum streaks on the agar surface with the same condition as the untreated cottons for the control of the experiment. All of the plates were incubated at 37°C for 24 h. The incubated plates were matured by the unit in millimeters of the inhibition zone. The average width of zone was calculated using the equation, W = (T-D)/2, where: W = width of clear zone of inhibition in mm; T = total diameter of the test specimen and clear zone in mm; D = diameter of the test specimen in mm.

7. Antibacterial assessment of the treated cotton by quantitative methods, AATCC Test Method 100-2004

The circular swatches (45 mm) were used. The treated and the untreated fabrics, as mentioned previously, were tested by modifying AATCC Test Method 100-2004 (Antibacterial Finishes on Textile Materials: Assessment of) for assessment of the quantitative evaluation with *S. aureus*, the test bacteria (AATCC 100:2004; Pinho, et al., 2011; Pinho, et al., 2015). The pasteurized step of the test swatches was used by the autoclave at 73°C for 10 min. The number of swatches was considered by the property of the fiber type and fabric construction by the amount of fabric which could absorb the 1.0±0.1 ml of inoculum without free liquid. The number of swatches used per jar are reported. The number of cotton swatches in the experiment used by two cotton swatches (45 mm diameter) that amount of fabric will absorb the 1.0 ml of inoculum.

Inoculation of fabrics when using *S. aureus*, 24 h culture in BHI and the dilution of the organism to McFarland No.0.5 made in peptone water. The size of inoculum per two sample applied 1 ml showed counts of final concentration as 1-2x10^5 cells/ml. The two swatches were placed in sterile petri dishes and drop 1 ml of the inoculum carefully onto the swatches and used 1 ml peptone water to drop for the negative control. The inoculated swatches stacked in the 250 ml wide-mount

glass jar with screw cap. Each of incubation time (contact time) placed the swatches separately in the jar as 0, 6, 12 and 24 h. After incubated at the contact time, the sample added 100 ml of the normal saline (neutralizing solution) to each of the jars. The 10-fold serial dilution made by the normal saline and plated on the BHI agar and BP (parallel control of the characteristic of *S. aureus*) by dropping plate methods for 10 µl per drop (in triplicate). Finally, all of the plates were incubated 37°C for 24 h.

The bacterial counts were reported by percentage of reduction (% reduction; R) and the number of bacterial per sample. Consequently, the report was "absent (0) colony" counted at 100 dilution (in the jar mixed between swatches and neutralizing solution as the undiluted) as "less than 100". The percent reduction of bacteria by the formulas: R = 100(C-A)/C, where: R = % reduction, A = the number of bacteria recovered from the inoculated treated test specimen swatches in the jar incubated over the desired contact period, C = the number of bacteria recovered from the inoculated untreated control specimen swatches in the jar immediately after inoculation (at "0" contact time).

8. Data analysis

The different inhibition zones were measured in triplicate and compared by performing One-way ANOVA ranked with Duncan's multiple range tests. Statistical Package for the Social Sciences (SPSS) 24 Version was used for analysis of recorded data. The statistical results with $p<0.05$ were determined to be statistically significant. The formula of the growth kinetics as the doubling time (t_d) and specific growth rate (µ) and the graphs were performed and computed by the basic math calculations in Excel 2016.

Results and discussion

1. Culturation and characterization of the crude samples of *Streptomyces* sp. AC4

The pure actinobacteria isolated from soil samples and identified belong to *Streptomyces* by 16SrDNA sequencing. The actinobacteria were known that *Streptomyces* species could produce plenty of the secondary metabolites as bioactive compounds such as antimicrobial compounds (Lam, 2006). The test strain, AC4, consistent with the research that was performed by screening of inhibited the gram-positive bacterial growth (the result not shown). *Streptomyces* sp. isolated strain, AC4, was used by property of inhibition of *S. aureus* growth (next result). Their ability produced the purple-like pigment on SCA. When cultured in SCB, AC4 growth presented the purple suspension and difficultly separated between cell and supernatant by centrifugation. Although, most of actinobacteria grow slowly in the habitat (Goodfellow, 1985) but the researchers screened some of them that could produce the bioactive compounds and grow significantly to apply to produce the benefit products. The basic characteristics of the growth kinetics of the strain AC4 showed the result that AC4 strain belonged to the significant growth of the actinobacteria group which produced the bioactive compounds by the doubling time (t_d) of the strains AC4 incubating in SCB as 72.19 h and specific growth rate (µ) as 0.0069 h^{-1} (Fig. 1). In addition, the research of Sejiny (1991) noted that the *Streptomyces* MY18, which produced antibiotic agents, showed the progressive to increase the biomass and had the highest antibiotic activity as recorded in the stationary phase of the growth during the first 4-7 days of incubation. The characteristic of significant growth might be applied to produce the benefit products.

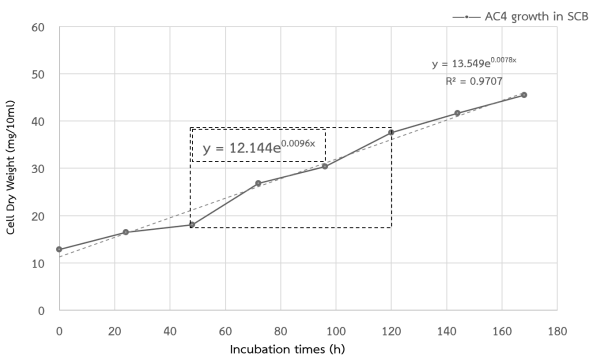


Fig. 1 The growth curve of the actinobacteria, *Streptomyces* sp. AC4

This *Streptomyces* strain, AC4, was performed by screening of inhibition the growth of the gram-positive bacteria, *S. aureus*, that relates to the skin flora and opportunistic pathogens. So, *S. aureus* was related with the fabric products that are consumed in the human. Then, the antibacterial activity to *S. aureus* of the crude samples from AC4 were interested to apply to the research of the antimicrobial textiles.

2. Antibacterial activity of the crude samples by disc diffusion method

The cultivated suspension of AC4 was then separated into two parts including the undiluted supernatants and the cracked-cell pellets (re-suspending with the distilled water 1:10 w/v that was sterilized with the 0.45 µm filter paper). The test bacteria of inhibition that concerned the skin flora and the standard organisms by the standard methods of American Association of Textile Chemists and Colorists (AATCC) for assessing the antimicrobial properties of textiles as *S. aureus* and *K. pneumonia*. The qualitative and positive control of the tested bacteria could be reliable with the standard range for the inhibition zone of the chloramphenicol disc (30 mg/disc) for *S. aureus* and *K. pneumonia* as 19.22±0.55 mm and 24±0 mm, respectively (CLSI, 2016). The ability of the crude samples to inhibit bacteria was tested by the disc diffusion method at the standard concentration of 1.5 x 10^8 CFU/ml on Mueller-Hinton agar (MHA). The diameter of the *S. aureus* inhibited zone of the supernatants and the cracked-cell pellets was measured as 17±0.5 mm and 18.00±0 mm, respectively. Both of the tested samples could not inhibit *K. pneumonia* (Table 1). Ali et al. (2011) studied the melanin pigments that were produced from *Streptomyces virginiae* strain which could have the proper activity of the bioactive compounds to apply in printing and dyeing of the wool fabric. The result showed that the culture broth of this strain could inhibit against *S. aureus*, *Pseudomonas aeruginosa* and fungi (Ali et al., 2011). There were bacteria on normal human skin (1×10^5 CFU/cm^2) by the predominant bacterial flora found in the healthy human such as *S. aureus* (Teruaki et al., 2017). The human skin flora grows and causes the fiber of textiles to spoil and this may cause unpleasant odor, dermal infection, allergic responses and opportunistic pathogenesis of the skin lesion (Teufel et al., 2010; Singh et al., 2005). The problems were related with consume of the fabric product. Therefore, the study of the crude samples from AC4 strains for coating the cotton were interesting. The crude samples of AC4 had narrow spectrum for gram-positive bacteria and did not effect to gram-negative bacteria the result shows the standard gram-negative bacteria of the standards methods as *K. pneumonia*. However, the other characteristic could pasteurize, significantly grow for 3-7 days and be environmental friendly from the natural finish by itself (Ali et al., 2011). The result of the characteristic of the crude samples of AC4 could apply to the study of the antibacterial coating.

3. Antibacterial activity of the crude samples after treated by pasteurized temperature

After the pasteurization, the ability of the crude samples to inhibit *S. aureus* was tested by the disc diffusion method (Table 1 and Fig. 2B). The diameter of the *S. aureus* inhibited zone was measured for the cracked-cell pellets at 62°C and 73°C as 20.41±0.40 mm and 20.16±0.95 mm, respectively, which are not significantly different. For the undiluted supernatants, the diameter of inhibited zone was measured at 62°C and 73°C as 19.30±0.72 mm, 18.19±0.44 mm and the statistical results were not different. The statistical results showed that both the cracked-cell pellets and the

Table 1 Antibacterial activity of the crude extraction after treated by pasteurized temperature and the thermal stability of the vegetative cells and spores of *Streptomyces* sp. AC4 in the crude extract

Temperature (°C) for treatment of crude samples	Inhibition zone (diameter in mm)				The number of the vegetative cells and spores of *Streptomyces* sp. AC4 (CFU/ml)	
	S. aureus		*K. pneumoniae*			
	Supernatant	Cell Pellets	Supernatant	Cell Pellets	Supernatant	Cell Pellets
No treatment	17.00±0.50^b	18.00±0^b	0^b	0^b	>300	>300
62	19.30±0.72^a	20.41±0.40^a	0^b	0^b	2.95x10^4	>300
73	18.19±0.44^{ab}	20.16±0.95	0^b	0^b	0	0
Chloramphenicol* (30 mg)	19.22±0.55		24.00±0			

Remark: Values were means ± SD
a, b with different letter in the column show significant statistical difference ($p < 0.05$)
* = The chloramphenicol disc (30 mg/disc) are applied for the qualitative and positive control of the disc diffusion method.

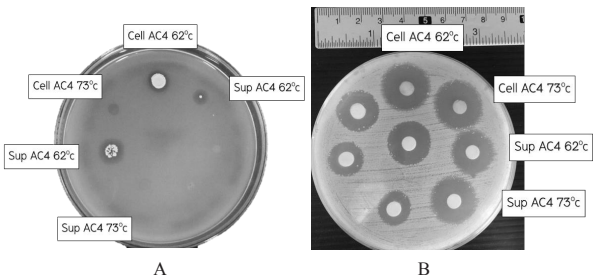


Fig. 2 The thermal stability of *Streptomyces* sp. AC4 growth and antibacterial activity after heat treatments (A) The growth of the vegetative and spore of the actinobacteria, *Streptomyces* sp. AC4 (B) the ability of the crude extract to inhibit *S. aureus* after heat treatments by the disc diffusion method on MHA (Sup: Supernatant, Cell: the cracked-cell pellets)

supernatants at 62°C had a higher inhibition zone that is significantly different when compared to the non-heat treatment of the crude samples (the cracked-cell pellets was measured as 17±0.5 mm and 18.00±0 mm, respectively) and were not different to the cracked-cell pellets and the supernatants treated at 73°C. The stability of the antibacterial activity for the high temperatures of finishing were important because the high temperatures were suitable for immobilization of the dye or finishes. The steps to operate the coating at the optimum temperatures have been a concern because the fiber of textile materials such as cotton and some other well dyed fibers at the high temperatures at 100ºC or below (Perkins, 1991). The result of the stability of the antibacterial activity supported the coating step and produced the antibacterial fabric from the conventional methods of the dyeing. The diameter of inhibited zone with the undiluted supernatants and the cracked-cell pellets after being treated at 62°C and 73°C, were not different from the statistical results but the choice of the temperature for the coating process were considered to be consistent with the pasteurization due to the crude samples from microorganisms used as finishers.

4. The effect of the thermal stability of the vegetative cells and spores of *Streptomyces* sp. AC4 in the crude samples

After the pasteurization, the growth of the vegetative and spore of *Streptomyces* sp. AC4 was examined by the dropped plate method on SCA of 10 µl crude extract then cultivated at 30°C for 7 days. The result showed that the cracked-cell pellet and the supernatant at 62°C could not inhibit the growth of the vegetative and spore with the colony count more than 300 colony and $2.95x10^4$ CFU/ml, respectively, but the higher the temperature could inhibit the growth of AC4 at 73°C (Fig. 2A).

The pasteurization at 73°C killed all the vegetative and spore of *Streptomyces* sp. AC4 and surprisingly the ability of the crude extract to inhibit bacteria could still exit hence this might be the reasons for selecting this temperature for pasteurization of the crude samples. In addition, the crude finish of AC4 could apply to coat the cotton with the traditional process by heat at 70-100°C because their anti-*Staphylococcus aureus* activity could tolerate the temperature for pasteurization at 73°C. Thus, the ability of *S. aureus* inhibition could be available for the coating process at 70°C. The optimal temperature for dyeing the cotton with cellulose component was at 70-100°C (Perkins, 1991).

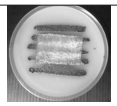
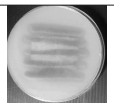
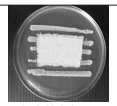
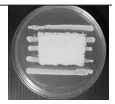
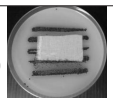
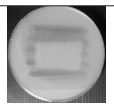
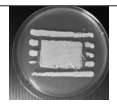
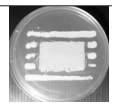
5. Antibacterial activity of the treated fabrics by qualitative methods

The supernatant and the cracked-cell pellet were mixed and pasteurized as the crude samples (crude finish) for the coating. The untreated (control) and treated fabrics were tested by AATCC Test Method 147-2004 for assessment of the qualitative analysis. The result shows that the treated fabrics had the average width of inhibited zone as 4.5 mm (Table 2). For the parallel control of the characteristic of *S. aureus* on BP, the five inoculum streaks on the agar surface were black line that showed the characteristic of the pure *S. aureus* on BP. However, the inhibited zone on BP could not be measured because BP is a hydrophobic media, nevertheless the process of coating and the crude finish was hydrophilic compound. The treated fabrics of AC4 showed clear ability to antimicrobial activity against *S. aureus* by Antibacterial Activity Assessment of Textile Materials: Parallel Streak Method. This method was quick and easily to screen and determine the antibacterial activity of diffusible antimicrobial agents on the treated swatches. The size of the inhibition zone was different. This does not mean that the treated sample had more or less biocidal. The zone size of inhibition depends on the permeated property of the antibacterial finish to diffuse into the agar, therefore, it does not rely on the efficacy of the bioactive compounds (Varesano et al., 2011).

6. Antibacterial assessment of the treated cotton by quantitative methods

The fabrics were tested by AATCC Test Method 100-2004 for assessment of the quantitative evaluation with *S. aureus*. The calculation of percent reduction of the inoculated untreated fabrics at contact time for 24 h was a significant increase in the number of bacteria over the number of bacteria at contact time for 0 h. The result at 0 h on BHI and BP show $4x10^5$ CFU/sample and $1.3x10^5$

Table 2 Inhibition of *S. aureus* of the treated cottons by AATCC Test Method 147-2004

Samples	Inhibition zone (mm)			
	BP		BHI	
	Front side	Back side	Front side	Back side
Control (untreated fabrics)				
	No inhibition zone			
AC4 (treated fabrics 1:10 w/v)				
	4.5 mm			

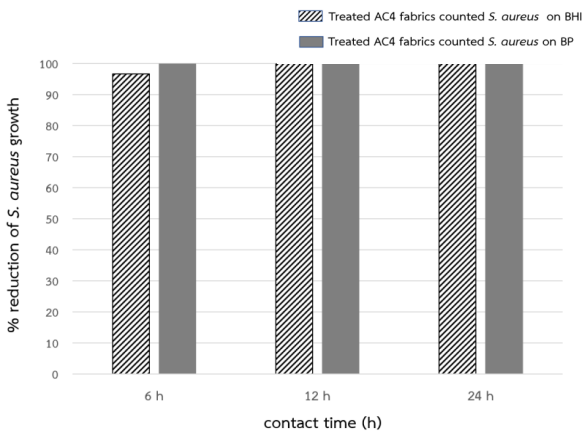


Fig. 3 The percentage of reduction (% reduction) of *S. aureus* growth by the treated fabrics

CFU/sample, respectively. The results of percentage of reduction (% reduction; R) showed that the treated fabrics could reduce *S. aureus* growth 96 to 100% for contact time at 6, 12 and 24 h (Fig. 3) on BHI. The reduction activity was determined that prevented microbial growth and killed bacteria bio-cidal activity. Additionally, the parallel control of the characteristic of *S. aureus* could count on BP for contact time at 12 and 24 h, that shows the treated fabrics could reduce *S. aureus* growth as 100% as same as the result of percent reduction on BHI agar (Fig. 3). For the parallel control of the characteristic of *S. aureus* on BP, the colony on the agar surface were black color that shows the characteristic of the pure *S. aureus* on BP for the easy counts the colony forming of *S. aureus*. The research showed the information that the cotton contained of largely cellulose, natural waxes and proteins. The mainly hydroxyl groups on the cellulose created a high potential of the water absorption that could be useful for immobilization the water-soluble compounds (Chequer et al., 2013). In this study, the hydroxyl groups that presented on the cellulose could immobilize the antibacterial agents from the *Streptomyces* sp. AC4 crude extract by the capability of *S. aureus* inhibition on the cotton fabrics which could remain and reduce *S. aureus* growth to 100%.

This study shows that the crude finish from *Streptomyces* sp. AC4 culture could correspond the cotton fabrics by the traditional batch process as the simply heat dyeing and the capability of *S. aureus* inhibition on the cotton fabrics could be exit.

The finishing was concerned with chemical agents purposed to improve the quality of the fabric such as microbial protection of fabric applied in the finishing process. Nevertheless, the contamination of natural waters from chemical synthesis from finishing and dyeing process is a problem for human health and environment because the major source of dye and finish loss immobilized during the textile fiber coating step and releases harmful effluents into the environment. So, the economical use of this natural resource in production processes has gained special attention (Chequer et al., 2013). Furthermore, the crude finish from *Streptomyces* sp. AC4 is a natural product during the finishing step and will release friendly effluents. Nonetheless, during the produced metabolite process, the microorganisms showed a significant growth of the organisms as evidenced by *Streptomyces* sp. AC4 which could culture for 4-7 days (t_d in SCB as 72.19 h and μ as 0.0069 h^{-1}) and create the bioactive compounds.

Conclusion

Streptomyces sp. AC4 crude samples could inactive its cells and spore by the temperature for pasteurization at 73°C with the ability to inhibit remaining that showed the diameter of the *S. aureus* inhibited zone of supernatant and the cracked-cell pellet was measured as 18.19±0.44 mm and 20.16±0.95 mm, respectively. The immobilization of the antibacterial agents with the cotton fabrics were performed by the traditional batch process as the simply heat dyeing at 70°C. The agents were treated by two steps of high temperature but the capability of *S. aureus* inhibition on the cotton fabrics could be remain that showed clear zone of inhibition against *S. aureus* as 4.5 mm on BHI by using AATCC

147-2004 test on *S. aureus*, the investigation indicated that the coating cotton fabrics had inhibition zone of 4.5 mm, indicating that the treated fabrics was able to inhibit *S. aureus*. In addition, the results of % reduction showed that the treated fabrics could reduce *S. aureus* growth as 100% for contact time at 12 and 24 h when the inoculated treated fabrics were incubated and calculated in both media (BHI and BP). Although the *Streptomyces* sp. AC4 crude extract was the narrow spectrum for gram-positive bacteria, the other characteristic could pasteurize, significantly grow for 3-7 days [the doubling time (t_d) of the strains AC4 incubating in SCB as 72.19 h and specific growth rate (μ) as 0.0069 h^{-1}] and be environmental friendly from the natural finish by itself which were useful to improve and optimize the proper condition of finishing that could apply to produce the antibacterial fabrics in the future.

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Anti-aging and Health Benefits from Thai Food: Protective Effects of Bioactive Compounds on the Free Radical Theory of Aging

Patipanee Khanthapok[a] & Suchada Sukrong[b]*

[a] *Community Public Health Sub-Department, Faculty of Science and Technology, Phranakhon Si Ayutthaya Rajabhat University, Phranakhon Si Ayutthaya, 13000 Thailand*

[b] *Research Unit of Herbal Medicine Biomaterial and Material for Dental Treatment, Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, 10330 Thailand*

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Abstract

Traditional food is an important representative for each country. Thai dishes are appreciated not only by people in Thailand but also worldwide. The unique flavor, meticulous preparation, and dish decorations of Thai food certainly reflects the background, culture, tradition, and characteristics of Thailand. In addition to its deliciousness, Thai food is also recognized as a healthy and functional diet. Various healthy ingredients, such as local vegetables, home-grown culinary herbs, and spices are usually contained in Thai dishes. The biological activities of ingredients in Thai foods, including antioxidant, anti-inflammatory, and antimicrobial effects suggest that the appropriate consumption of such a diet can influence the anti-aging process. Hence, the recipes of selected famous Thai dishes are described regarding their ingredients, phytochemicals, phytonutrients, and biological activities associated with anti-aging effects. Furthermore, the free radical theory related to aging and the possible role of Thai food in health benefits are also discussed for a more comprehensive explanation of the anti-aging effects of Thai diets.

Introduction

Food is an intangible part of a nation's cultural heritage which embodies its country's background, characteristics, and tradition. The legacy of national food and cooking develops from the beginning of a nation's history. Thailand is one of the oldest countries in the world and accordingly has a long history, a unique culture, and delicious food. Today, foreigners associate Thailand with its remarkable Thai cuisine. With Thai food's strong reputation for flavor and meticulous preparation, Thai cuisine has been known internationally for over a decade. The unique flavor of Thai food is a balance of different tastes, including sour, sweet, salty, and spicy (Nitiworakarn, 2014). The variety and flavor of Thai food differs among the four different regions of Thailand: North, Northeast, Central, and South. Thai food recipes have been modified slightly to satisfy the palate of people coming from

* Corresponding Author
e-mail: Suchada.Su@chula.ac.th

different parts of Thailand and foreign countries. However, all the main ingredients, preparation procedures, and signatures of each dish remain the same (Nitiworakarn, 2014). Thai ancestors emphasized not only the taste of food but also its health benefits, using knowledge from Thai folk tradition to create many healthy and delicious dishes for prolonging lifespan and maintaining good health and bodily functions (Sirisunthorn, 2013). Various vegetables, home-grown culinary herbs, and spices are always present in Thai dishes. These ingredients are rich in nutrients with medicinal properties. The biological activities of Thai culinary herbs and spices have been demonstrated in many studies including antioxidant (Tangkanakul & Trakoontivakorn, 2014), anti-inflammatory (Sirikanokvilai et al., 2014), antibacterial (Nugboon & Intarapichet, 2015), antihyperglycemic (Wongsa et al., 2012), and hypocholesterolemic effects (Duangjai et al., 2011). Because of the information mentioned above, Thai food has been accepted as a healthy and functional diet (Singsomboon, 2015).

Today, the progression of medical care and technology increases human life expectancy. However, this longevity is normally accompanied by aging, chronic and age-related diseases, undesirable physical traits, memory loss, and a decline in physical and physiological functions (Ho et al., 2010; Scapagnini et al., 2016). Accordingly, most people attempt to have a healthy lifestyle and prevent the onset of age-associated diseases including physical deterioration. Cosmetic surgery and the intake of synthetic vitamins and/or food supplements seem to be top strategies that have been chosen and used for those purposes. Those strategies and other similar approaches may provide a youthful appearance or the desired aesthetic results in a short time, but such changes are not sustainable. The keystone of successful aging is having good health. Good nutrition combined with regular physical activity is one of the most common practices for obtaining and maintaining good health.

The consumption behavior of most people is quite different from that in the past. Increasingly rushed lifestyles cause most people choose to consume fast food, takeaways, or ready-to-eat meals instead of home-prepared food for the sake of convenience (Sirichakwal et al., 2015; Townshend & Lake, 2017). These foods provide poor nutrition and are high in meat, carbohydrates, and fat. A poor diet affects a person's health and can contribute to untimely aging and age-related diseases (Willcox et al., 2009). In recent years, health-consciousness has risen worldwide. To achieve a longer and healthier life, many people have changed their consumption behavior by consuming more wholesome diets (Chang, 2014; Waratornpaibul, 2014). In addition to Mediterranean (Renna et al., 2015) and Okinawan diets (Willcox et al., 2009), Thai food is among the healthiest and most functional diets. Consuming Thai dishes helps maintain good health and postpone the aging process and is suitable for people of any age or gender. Therefore, this review is focused on the anti-aging and health benefits of Thai diets. The recipes of selected popular Thai foods and some essential ingredients used in those recipes are described. In this context, the biological activities and bioactive components of these selected dishes and their condiments, in addition to the free radical or oxidative stress theory of aging and the possible role of Thai food in anti-aging and health benefits are also discussed.

Famous Thai foods

Thai food is characterized by full-flavored dishes. Both Thais and foreigners are fascinated by its exotic and intense flavors. Thai dishes are high in vegetables and low in glycemic index, red meat, and fat. Many Thai foods consumed by foreigners are favored for their taste and health benefits. In this review, several famous Thai foods and dessert (Fig. 1) are selected from recipes that are commonly consumed in Thailand and their popularity among foreigners (Cable News Network, 2017) and described. Some essential ingredients used in the recipes for these dishes are summarized in Table 1. The active components and the biological activities exhibited by the essential ingredients are also presented.

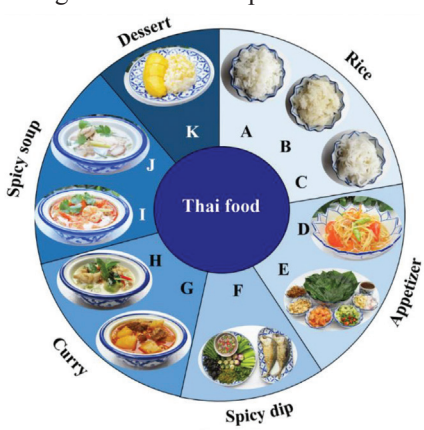

Fig. 1 Selected famous Thai foods: (A) steamed non-glutinous rice, (B) steamed glutinous rice, (C) rice noodle, (D) spicy green papaya salad, (E) wild betel leaf wraps, (F) spicy shrimp paste dip, (G) Massaman curry, (H) green curry, (I) hot and sour soup, (J) coconut milk soup with galangal, and (K) sweet sticky rice and ripe mango.

1. Rice

Most Thai dishes are served with cooked rice (*Oryza sativa* L.). For example, non-glutinous (Fig. 1A) and glutinous (Fig. 1B) rice and other rice products, and rice noodle (Fig. 1C) are an important part of Thai meal. Specifically, Thai dishes are usually accompanied by steamed non-glutinous rice, while steamed glutinous rice is often served with regional dishes from Northern and Northeastern Thailand. Rice noodle or "Kanom Jeen" is served along with Thai coconut milk-based curries. Rice, a staple food as well as an economic crop of Thailand, acts as a major source of carbohydrates in Thai meals. It also provides protein, lipids, dietary fiber, vitamins, essential amino acids, minerals, and bioactive compounds (Lim, 2013; Nakornriab, 2018). Many rice cultivars have been cultivated for domestic consumption and exports, which Thai jasmine rice or "Hom Mali rice" is the most well-known rice cultivar (Rice Department, 2016). Many studies have reported the diverse biological activities of rice. Grains of white, red, and black rice exhibit antioxidant effects at different levels. The bioactive compounds in black rice grains, including catechin, cyanidin-3-glucoside, and peonidin-3-glucoside play an important role in the antioxidant activity (Jiamyangyuen et al., 2017). Protocatechuic acid contributes to the antiproliferative activity of cooked Thai purple rice (Chatthongpisut et al., 2015) and anti-inflammatory potential of cooked black rice (Bhawamai et al., 2016). Ethanolic extracts of white and colored rice grains exhibit an antiglycation effect (Daiponmak et al., 2014) that is beneficial to the management and treatment of diabetes (Ramkissoon et al., 2013). Therefore, the low glycemic index (GI) rice cultivars are being bred concerning health and diabetes (Vanavichit, 2015). Phenolic compounds are responsible for the anti-advanced glycation end product (AGE) activity, resulting in the reduction of cellular oxidative damage (Daiponmak et al., 2014). Hence, the nutritional, anti-aging, and health benefits of Thai food arise not only from the cooking ingredients but also from rice.

2. Appetizers

For example, Thai style appetizers consist of various fresh vegetables such as cucumbers, tomatoes, yard-long beans, culinary herbs, tamarind and lime juices, garlic, shallot, and chili pepper. The sour and sweet flavors combined with a touch of spiciness of Thai appetizers can increase appetite.

2.1 Spicy green papaya salad or "Som Tam"

Spicy green papaya salad has spread from the Northeast region to other parts of Thailand. A basic recipe of spicy green papaya salad contains shredded green papaya, garlic cloves, yard-long beans, wild tomatoes, dried shrimps, and roasted unsalted peanuts (Fig. 1D). Other ingredients such as salted eggs, fresh blue swimmer crabs, and crispy fishes are added to the basic recipe to create new varieties of this Thai-style salad. This dish is seasoned with fish sauce, palm sugar, tamarind and lime juices, and chili peppers. The large amount of dietary fiber from fresh vegetables accompanied by the organic acids from the tamarind and lime juices can help improve gastrointestinal function (Padayachee et al., 2017) and defecation (Bhardwaj et al., 2014). Garlic possesses various phytochemicals that play an important role in its biological effects including organosulfur compounds or thiosulfinates and flavonoids (Corzo-Martínez, et al., 2007; Bayan et al., 2014). Some of the notable antioxidant activities of garlic result from quercetin (Nuutila et al., 2003) and diallyl disulfide (Chekki et al., 2014), while catechin, vanillic acid, and ferulic acid are responsible for its anti-inflammatory effects (Moutia et al., 2016). The immunomodulatory activities of fructooligosaccharides isolated from aged garlic extracts has been demonstrated *in vitro* (Chandrashekara & Venkatesh, 2016). Allicin and other *Allium* thiosulfinates exhibit antihypertension activity in hypertensive patients (Bhardwaj et al., 2015) and show α-glucosidase inhibitory effects in diabetic rats (Al-Malki, 2016). Moreover, carpaine in the flesh of green papaya also has strong inhibitory effects against both α-amylase and α-glucosidase. There is also scientific evidence supporting the use of papaya for type 2 diabetes treatment, which originated from Nigerian folk medicine (Oboh et al., 2013).

2.2 Wild betel leaf wraps or "Miang Kham"

Wild betel leaf wraps are healthy appetizers. Many Thai culinary herbs are used as the main ingredients in the recipe. This one-bite appetizer usually consists of wild betel leaves, roasted coconut flakes, small lime wedges, roasted unsalted peanuts, dried shrimp, small pieces of ginger, chili peppers, shallots, and a sauce made from shrimp paste and palm sugar (Fig. 1E). To prepare this dish, a betel leaf is rolled into a cone shape, and all the ingredients are put into it. Then, one teaspoon of sauce is added on top. Many scientists have studied the phytochemicals and biological activity of wild betel leaves. The flavonoids rutin and vitexin in wild betel leaves exhibit antioxidant efficacy by protecting human umbilical vein endothelial cells from oxidative stress (Ugusman et al., 2012). The anti-inflammatory and antipyretic effects of wild betel leaf have also been

demonstrated in rats (Ridtitid et al., 2007). Shallots are rich in well-known antioxidants including, allicin, quercetin, anthocyanins, and kaempferol (Swamy & Gowda, 2006). Dipropyl disulfide and dipropyl trisulfide play an important role in the antioxidant and antimicrobial activity of the essential oils (Mnayer et al., 2014) and extracts (Raeisi et al., 2016) of shallots. The anti-inflammatory (Mohammadi-Motlagh et al., 2011), immunomodulating (Mlcek et al., 2016), anti-obesity (Woon & Toh, 2014), anti-hypertensive (García-Trejo et al., 2016), and hypoglycaemic (Luangpirom et al., 2013) effects of shallots have also been reported. Furthermore, the antispasmodic effect of ascalonicoside B on the isolated guinea pig ileum supports the use in traditional Thai medicine of shallot for alleviating gastrointestinal disturbance (Corea et al., 2005). Ginger is another potent source of antioxidants. The major phenolic compounds found in fresh ginger rhizomes are gingerols and its analogues, the shogaols (Koch et al., 2017), which contribute to the antioxidant and anti-inflammatory effects (Dugasani et al., 2010). Ginger has been found to exhibit antidiabetic and antihyperlipidaemic activities (Jafarnejad et al., 2017), analgesic potential (Darvishzadeh-Mahani et al., 2012), and gastroprotective effects (Chantharangsikul et al., 2016).

3. Spicy dip

Thai spicy dip is an indispensable dish in all four regions of Thailand, as it provides spicy, sour, sweet, and salty flavors. The common ingredients of Thai dipping sauces are chili pepper, garlic cloves, and shallot. The varieties of spicy dip and the other ingredients vary depending on regions and types of diet. For instance, Central cuisine uses shrimp paste to enhance the saltiness and intenseness of the dipping sauces, while fermented fish are used in Northeastern cuisine (Sirisunthorn, 2013) and fermented soybean paste are used for replacing shrimp paste in the vegetarian dipping sauces (Wittanalai et al., 2011). Various types of fresh and cooked vegetables are typically served along with this menu as a side dish. Interestingly, more than one hundred different recipes for spicy dip have been listed in Thai cookbooks since the Ayutthaya era (Sirisunthorn, 2013).

Spicy shrimp paste dip or "Nam Phrik Ka Pi" is a signature dish of the Central region of Thailand. It is made from shrimp paste, garlic cloves, shallots, chili peppers, and turkey berry and is flavored with lime juice, fish sauce, and palm sugar. This dipping sauce is often served along with fried Thai mackerels and vegetables (Fig. 1F). Shrimp paste, which is made from fermented krill or mysid shrimps, contains essential and non-essential amino acids especially, glutamate, aspartate, lysine, and leucine (Chotechuang, 2012; Kleekayai et al., 2015). Thai shrimp paste has shown strong antioxidant and angiotensin I-converting enzyme (ACE) inhibitory activities (Kleekayai et al., 2015). Capsaicinoids cause the hot, spicy, and pungent tastes of chili peppers (Barbero et al., 2016) and have remarkable antioxidant and anti-inflammatory activities (Chen et al., 2015). The extracts of chili peppers exhibit immunostimulant (Yamaguchi et al., 2010), antidiabetic (Nantakornsuttanan et al., 2016), and neuroprotective (Watcharachaisoponsiri et al., 2016) effects. Turkey berry, a small *Solanum* fruit, has been discovered to exhibit strong antioxidant and antihyperlipidaemic effects (Gupta & Gohain, 2015) and anti-inflammatory (Rammohan & Reddy, 2010), antihypertension (Mohan et al., 2009), and hepatoprotective (Ramamurthy et al., 2016) activity. Thai spicy dip is always served with local vegetables such as Asiatic pennywort, bitter cucumber, and water morning glory (Tharasen & Lawan, 2012), the edible parts of which contain strong antioxidants including β-carotene, xanthophyll, and zeaxanthin. Interestingly, another potent antioxidant, lutein, is found in only the young stems of water morning glory (Tharasen & Lawan, 2012).

4. Coconut milk-based curry

Curry pastes and coconut milk are the relevant ingredients of Thai coconut milk-based curry. Thai curry pastes are a mixture of various culinary herbs and spices including garlic, shallot, coriander root and seed, galangal rhizome, lemon grass, kaffir lime peel, chili pepper, peppercorn, and cumin seed. Curry pastes are named according to the color of these main condiments and additional essential ingredients used in each recipe. Coconut milk is important ingredient of Thai curry. It is an oil-in-water emulsion pressed from the solid endosperm of coconut (Raghavendra & Raghavarao, 2010). Thai folk wisdom uses it as a liquid medium in many Thai food recipes, particularly curries and soups. Coconut milk serves as a natural solvent during food preparation by extracting both polar and non-polar compounds from various herbs and spices in Thai curry pastes (Sapabguy & Yasurin, 2015). Thai curry paste extracted with coconut milk has higher antimicrobial activity than that extracted with water (Lazuardi et al., 2012). Coconut milk itself is rich in nutrients and phytochemicals such as protein, fatty acids, and phenolic compounds (Nadeeshani et al., 2015) and has exhibited antioxidant effects (Alyaqoubi et al., 2015). The phenolic

compounds such as ellagic, *p*-coumaric, and ferulic acids contribute to the antioxidant activity of coconut milk (Nadeeshani et al., 2015). The low-molecular-weight proteins isolated from coconut milk have the antimicrobial effects against microbes, *Debaryomyces hansenii,* and *Candida albicans* (Algar & Mabesa, 2015).

4.1 Massaman curry or "Kaeng Massaman"

Massaman curry is one of the most famous Thai curries (Fig. 1G). It is a mild coconut milk-based soup containing peanut, potato, and pineapple that provides delicate sweet, salty, and sour flavors. Massaman curry is made with yellow onions, potatoes or sweet potatoes, roasted peanuts, pineapple, Siam cardamoms, bay leaves, cinnamon sticks, and Massaman curry paste. Generally, either beef or chicken is used in the preparation of Massaman curry. Tamarind juice, palm sugar, and fish sauce are added for seasoning. Traditional Thai medicine uses pineapple to aid in the process of digesting meat. Cardamoms and bay leaves help deodorize the smell of meat in Massaman curry. The antioxidant efficacy of Siam cardamom arises from the compound kravanhin B (Yin et al., 2013). In Thailand, the bay leaves in Thai Massaman curry are from *Cinnamomum porrectum.* This bay leaf-like condiment contains abundant phenolic acids and exhibits antioxidant activity (Saetan et al., 2016). Massaman curry paste, which contains plentiful antioxidant-rich spices (Table 1), has demonstrated strong antioxidant activity in vitro (Siwarungson & Lertpringkop, 2016). Various phytochemicals are derived from Massaman curry paste, including gallic acid, cinnamaldehyde, and eugenol from clove (Embuscado, 2015; De La Torre Torres et al., 2017); myristophenone from mace and nutmeg; rhamnetin from peppercorn (Embuscado, 2015); linalool from coriander seed; cinnamon stick and bay leaf (De La Torre Torres et al., 2017); cuminaldehyde from cumin seed; oleoresin from chili pepper; and quercetin and kaempferol from shallot and garlic (Peter, 2006). Numerous studies have reported the health benefits of the culinary herbs and spices contained in Massaman curry, including antioxidant (Masuda et al., 2015), anti-inflammatory (De La Torre Torres et al., 2017; Opara & Chohan, 2014), and hypoglycaemic, antidiabetic, and antipyretic activities (Peter, 2006).

Table 1 Culinary herbs and spices composed in selected Thai foods.

Ingredients	Scientific name	Appetizer		Spicy dip	Curry		Soup		Dessert
		Spicy green papaya salad	Wild betel leaf wraps	Spicy shrimp paste dip	Massaman curry	Green curry	Hot and sour soup	Coconut milk soup with galangal	Sweet sticky rice and ripe mango
Herbs									
Chili pepper	*Capsicum annuum*	✓	✓	✓		✓	✓	✓	
Chili spur pepper	*Capsicum annuum*					✓			
Coriander leaf	*Coriandrum sativum*						✓	✓	
Coriander root	*Coriandrum sativum*				✓	✓	✓		
Galangal rhizome	*Alpinia galanga*				✓	✓	✓	✓	
Garlic	*Allium sativum*	✓		✓	✓	✓			
Ginger	*Zingiber officinale*		✓						
Kaffir lime leaf	*Citrus hystrix*					✓	✓	✓	
Kaffir lime peel	*Citrus hystrix*					✓			
Lemon grass	*Cymbopogon citratus*				✓	✓	✓	✓	
Lime	*Citrus aurantifolia*	✓	✓	✓			✓	✓	
Sweet basil leaf	*Ocimum basilicum*					✓			
Tamarind	*Tamarindus indica*	✓							
Shallot	*Allium ascalonicum*		✓	✓	✓				
Yellow onion	*Allium cepa*				✓				
Spices									
Bay leaf	*Cinnamomum porrectum*				✓				
Cinnamon	*Cinnamomum spp.*				✓				
Clove	*Syzygium aromaticum*				✓				
Coriander seed	*Coriandrum sativum*				✓	✓			
Cumin seed	*Cuminum cyminum*				✓	✓			
Dried chili spur pepper	*Capsicum annuum*				✓				
Mace	*Myristica fragrans*				✓				
Nutmeg	*Myristica fragrans*				✓				
Peppercorn	*Piper nigrum*				✓	✓			
Siam cardamom	*Amomum krervanh*				✓				

Remark: The symbol (✓) indicates ingredients used in recipe.

Table 1 Culinary herbs and spices composed in selected Thai foods. (Continued)

Ingredients	Scientific name	Appetizer		Spicy dip	Curry		Soup		Dessert
		Spicy green papaya salad	Wild betel leaf wraps	Spicy shrimp paste dip	Massaman curry	Green curry	Hot and sour soup	Coconut milk soup with galangal	Sweet sticky rice and ripe mango
Vegetables									
Coconut flake	*Cocos nucifera*		✓						
Brinjal	*Solanum aculeatissimum*					✓			
Aubergine	*Solanum melongena*					✓			
Green papaya	*Carica papaya*	✓							
Turkey berry	*Solanum torvum*			✓		✓			
Yard-long bean	*Vigna unguiculata*	✓							
Wild betel leaf	*Piper sarmentosum*		✓						
Wild tomato	*Solanum lycopersicum*	✓							
Fruits									
Pine apple	Ananas comosus				✓				
Ripe mango	*Mangifera indica*								✓
Others									
Coconut milk	*Cocos nucifera*				✓	✓		✓	✓
Shrimp paste	*Acetes spp.*		✓	✓	✓	✓			

Remark: The symbol (✓) indicates ingredients used in recipe.

4.2 Green curry or "Kaeng Khiao Wan"

Green curry is a greenish coconut milk-based soup that has a medium spicy taste. It is usually served with either steamed rice or rice noodle. Thai eggplants, turkey berry, sweet basil leaves, kaffir lime leaves, fresh chili spur peppers, and green curry paste are the basic ingredients of this curry, which can be made with pork, beef, chicken, fish, or seafood. Thai green curry made with chicken is the most popular variety among both Thai people and foreigners (Fig. 1H). The condiments of Thai green curry paste are shown in Table 1. *In vitro* studies have demonstrated the antioxidant effect (Siwarungson & Lertpringkop, 2016) and anti-inflammatory activity (Sirikanokvilai et al., 2014) of digested Thai green curry. The flavonoids and phenolic compounds in the culinary herbs composing green curry paste are responsible for its antioxidant and anti-inflammatory activities (Settharaksa et al., 2012). Thai eggplant, an edible fruit of the genus *Solanum,* has three varieties that can be used in green curry preparation: brinjal, aubergine, and turkey berry. Various studies on the biological activities of these *Solanum* fruits, including the antioxidant and antihaemolytic potential of a protease inhibitor in brinjal (Meenu Krishnan et al., 2015), anti-inflammatory activity of the flavonoids, sterols, and saponins in turkey berry (Rammohan & Reddy, 2010), antidiabetic and antihypertension effects of the phenolic compounds in green aubergine (Kwon et al., 2008), and cardioprotective activity of nasunin in aubergine (Das et al., 2011). Sweet basil leaf has an anise-like aroma. Active compounds including rosmarinic acid and essential oils contribute to its potent antioxidant activity (Avetisyan et al., 2017), anti-inflammatory and antigenotoxic capacities (Güez et al., 2017), and hypolipidaemic effect (Touiss et al., 2017).

5. Spicy soup

Spicy soup normally contains lemon grass, fresh galangal rhizome, kaffir lime leaves, and coriander roots. It is garnished with coriander leaves and flavored with fish sauce, lime juice, and chili peppers. Thai soup is typically made with some basic culinary herbs and ingredients and is used in curry. The difference between Thai soup and Thai curry is that the shrimp paste is not used as one of the main ingredients in any recipes of Thai soup. In traditional Thai medicine, the consumption of spicy soup can relieve the fever and gastrointestinal disturbance. Lemon grass, kaffir lime leaves, and galangal rhizome are responsible for the antipyretic property and ability to act as a gastrointestinal remedy of spicy soup (Sirisunthorn, 2013). Thai spicy soups are divided into two types based on the appearance of the liquid medium, which can divide Thai spicy soup into two types: clear soups and creamy soups. The latter type is made with coconut milk or evaporated milk.

5.1 Hot and sour soup or "Tom Yam"

Hot and sour soup is one of the most famous Thai dishes (Fig. 1I). The variety of this soup depends on the choice of meat, which can include shrimp, beef, pork, chicken, fish, and seafood. Interestingly, all of the ingredients and mixed pastes of hot and sour soup have potent antioxidant and antimicrobial effects (Siripongvutikorn et al., 2005). The fresh culinary herbs in hot and sour soup are a rich source of antioxidants, including β-carotene from chili peppers and kaffir lime leaves (Siripongvutikorn et al., 2005); chlorogenic acid, isoorientin, and swertiajaponin from lemon grass (Campos et al., 2014); and naringin and vitamin C from

lime juice (Boshtam et al., 2011). Lemon grass (Campos et al., 2014) and kaffir lime leaves (Laohavechvanich et al., 2010) exhibit protective effects against oxidative damage in human umbilical vein endothelial cells (HUVECs) and HepG2 cells, respectively. The anti-inflammatory properties of lemongrass result from its luteolin glycosides (Francisco et al., 2014) and essential oil (Boukhatem et al., 2014). The hepatoprotective effects of lemongrass extracts have been demonstrated in rats as well (Arhoghro et al., 2012; Saenthaweesuk et al., 2017).

5.2 Coconut milk soup with galangal or "Tom Kha"

Coconut milk soup with galangal is a coconut milk and galangal-based soup (Fig. 1J). It is similar to hot and sour soup in both main ingredients and flavors. However, compared to hot and sour soup, this soup has a milder taste and contains chili pepper. The taste of the dish is a combination of salty, sour, and sweet flavors arising from fish sauce, lime juice, and coconut milk, respectively. The antioxidants mainly originated from galangal rhizome, lemon grass, kaffir lime leaves, chili peppers, and coconut milk (Ayusuk et al., 2009). Galangal, a key ingredient of this soup, exhibits different biological activities that have important health benefits. The antioxidant activity of galangal has been determined (Ayusuk et al., 2009), and the presence of gallic acid and ellagic acid in galangal extracts contributes to their antioxidant effects (Nampoothiri et al., 2015). Galangin, an abundant phytochemical in galangal rhizomes (Kaur et al., 2010), plays an important role in anti-inflammatory (Baldo & Serrano, 2016), antidiabetic (Sivakumar et al., 2010), anti-obesity, and antihyperlipidemic activities (Kumar & Alagawadi, 2013). Another potent anti-inflammatory agent, 1′-acetoxychavicol acetate is also presented in galangal rhizomes (Ichikawa et al., 2006).

6. Dessert

Most Thai desserts are made from sugar, rice, rice products, and coconut. Coconut milk, in addition to the mature flesh and the water of young coconut, has been used in the preparation of Thai sweets for a long time. Tropical fruits in Thailand, such as mango, banana, and durian, are also used as the main ingredients in many Thai dessert recipes.

Sweet sticky rice and ripe mango or "Khao Niao Mamuang" is one of the best summer desserts in Thailand. Sliced ripe mangoes are served with steamed sticky rice that is mixed in thick coconut milk and sugar, covered with extra thick coconut milk, and sprinkled with roasted mung bean (Fig. 1K). Thai mangoes have a unique aroma and a mild sweet flavor. They are usually served in both Thai restaurants and street food markets. The flesh of ripe mango possesses many powerful antioxidants including, mangiferin, ellagic acid, β-carotene, and vitamin C (Oliveira et al., 2016). Mango flesh extracts have demonstrated antioxidant (Kim et al., 2010) and anti-inflammatory (Kuganesan et al., 2017) effects, which result in part from the presence of mangiferin (Pardo-Andreu et al., 2008; Gong et al., 2013) and ellagic acid (Favarin et al., 2013; Kilic et al., 2014). The antigenotoxic and cytoprotective activities of mangiferin have also been confirmed in vivo (Viswanadh et al., 2010).

Aging processes and Thai food

Thai nutritionists have suggested that the intake of Thai diets could affect the aging process and help maintain good health. The bioactive compounds and phytochemicals in Thai dishes have been claimed to play an important role in their anti-aging effects and health benefits (Nitiworakarn, 2014; Sirisunthorn, 2013). However, one question that is raised from this information, "How does Thai food affect the aging process?" needs to be clarified. The understanding of aging and how it occurs would help answer this question.

1. What is aging?

All living organisms, including humans, cannot avoid aging. Aging is a progressive biological process that causes considerable functional deterioration of the human body, such as declining physical and physiological functions, memory loss, and increased disease vulnerability (Ho et al., 2010). Normally undesirable physical traits, such as grey hair, skin wrinkles, patchy and sagging skin, and dark spots, are signs of aging (Cannon, 2015; Calasanti et al., 2016). Humans try to understand the aging process and use the obtained knowledge to delay the onset of aging. Different theories have been proposed for the explanation of this natural process. The free radical or oxidative stress theory is one of the main hypotheses and could be used to simplify the explanation of how aging occurs.

Oxygen-centered free radicals or reactive oxygen species (ROS) are unstable molecules containing unpaired electrons. They rapidly react with other substances to neutralize themselves (Lobo et al., 2010). These unstable molecules attack all types of biomolecules, including proteins, lipids, and nucleic acids, via redox

reactions, thereby leading to aging, mutations, cellular damage, and cell death (Vaiserman et al., 2016). Free radicals are involved in chronic illnesses and age-associated diseases (Daiponmak et al., 2014). ROS such as hydroxyl, peroxyl, and superoxide radicals originate from both internal and external sources (Durak, 2014; Bhattacharya, 2015). Cellular metabolism, especially the electron transport chain in mitochondria, inflammatory response, and phagocytosis, are the main endogenous ROS generators (Scapagnini et al., 2016). The exogenous sources include environmental pollutants, cigarette smoking, chemicals, radiation, and high energy diets full of high sugar, protein, and fat-containing foods (Lobo et al., 2010). Normally, the human body has both enzymatic and non-enzymatic antioxidant defense systems to maintain the balance between free radicals and antioxidants by eliminating excess ROS (Urquiza-Martínez & Navarro, 2016). A large amount of ROS and insufficient antioxidative defense systems cause the accumulation of free radicals in cells, which causes oxidative stress and oxidative damage (Momtaz & Abdollahi, 2012). Many studies have reported the substantial function of oxidative stress in the aging process and cellular senescence. Cellular oxidative damage including the damage of mitochondrial DNA, DNA, and the cell membrane; loss of structural integrity; and protein dysfunction also contributes significantly to age-related and chronic diseases, especially type 2 diabetes, hypertension, atherosclerosis, cardiovascular diseases, and cancer (Fig. 2) (Scapagnini et al., 2016; Daiponmak et al., 2014). These diseases are more harmful than undesirable physical traits.

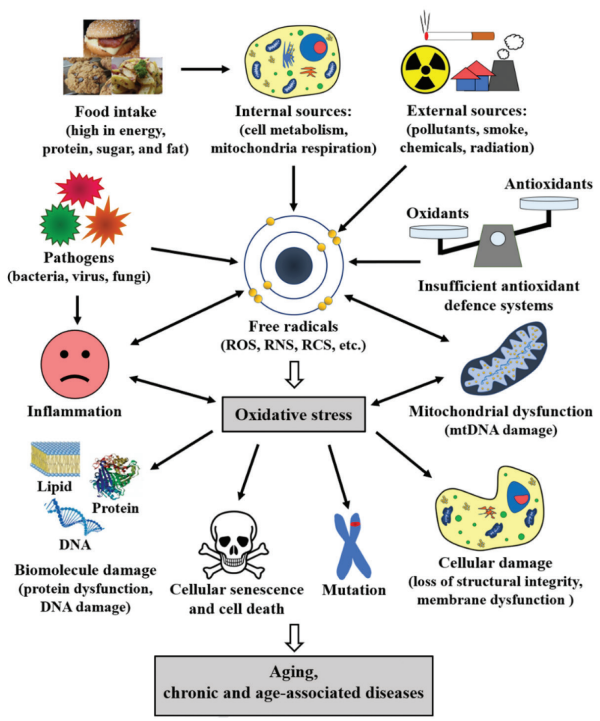

Fig. 2 Proposed mechanism of the free radical or oxidative stress theory of aging

Fig. 2 shows that free radicals are produced by both internal and external sources. The imbalance between free radicals and antioxidants in favor of free radicals results in oxidative stress. Excessive free radicals interact with biomolecules and cells, thereby causing aging, mutations, cellular damage, cell death, chronic illness, and age-associated diseases. ROS: reactive oxygen species, RNS: reactive nitrogen species, RCS: reactive chlorine species.

2. How does Thai food affect the aging process?

Reducing the excess free radicals in human body can delay the aging process and reduce the risk of chronic and age-related diseases. Antioxidants can decrease the level of excess free radicals and protect cells from oxidative damage by scavenging, neutralizing, deactivating, or stabilizing free radicals before they can react with biomolecules and cells (Bhattacharya, 2015; Urquiza-Martínez & Navarro, 2016). Natural antioxidants present in the human body include glutathione (GSH), pyruvate, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Momtaz & Abdollahi, 2012). However, oxidative stress frequently occurs because the antioxidant defense systems are not capable of eliminating the excess ROS, thereby resulting in the imbalance between ROS and antioxidants. Therefore, exogenous antioxidants from different sources are supplemented to either diminish ROS or elevate the efficiency of the endogenous antioxidant systems.

Exogenous antioxidants originate from a person's daily diet. Food ingredients such as vegetables, spices, and fruits are a rich source of plant-derived antioxidants. Plant-derived antioxidants in diets are mainly phenolic compounds, flavonoids, and vitamins. These compounds can be divided into water-soluble and lipid-soluble antioxidants (Lobo et al., 2010). Well-known hydrophilic antioxidants include vitamin C or ascorbic acid, anthocyanins, catechins, and gallic acid, while vitamin E or α-tocopherol, β-carotene, and quercetin are the most prominent representatives of lipophilic antioxidants (Scapagnini et al., 2016; Asif, 2015). By acting as hydrogen donors, electron donors, radical scavengers, or

singlet oxygen quenchers, these antioxidants can maintain the amount of cellular ROS at a non-toxic level thereby reducing oxidative stress and cellular oxidative damage (Lobo et al., 2010; Bhattacharya, 2015). Trace elements from diets, including iron (Fe), selenium (Se), copper (Cu), manganese (Mn), and zinc (Zn) from diets, are essential cofactors for antioxidant enzymes. For instance, Cu/Zn, Fe, and Se are required for the optimum catalytic activity of SOD, CAT, and GPx respectively (Vural et al., 2010). Natural, semi-synthetic, or synthetic antioxidants are delivered to consumers in the form of dietary supplements. However, these supplements supply only antioxidants while excluding other nutrients. The excessive intake of instant antioxidants could have adverse effects on human health because of their pro-oxidant activities (Yordi et al., 2012). Food offers appropriate amounts of both natural antioxidants and nutrients that are necessary for maintaining bodily functions and good health.

Thai food is a very healthy and functional diet. As indicated by the descriptions the explanations of selected Thai dishes provided above, Thai food is a rich source of nutrients and antioxidants. Based on the oxidative stress theory of aging, the antioxidants derived from Thai diets can help increase the level of cellular antioxidants, eliminate excess oxidants, prevent free radical formation, and break the chain reaction of oxidative stress (Lobo et al., 2010; Vaiserman et al., 2016; Bhattacharya, 2015).

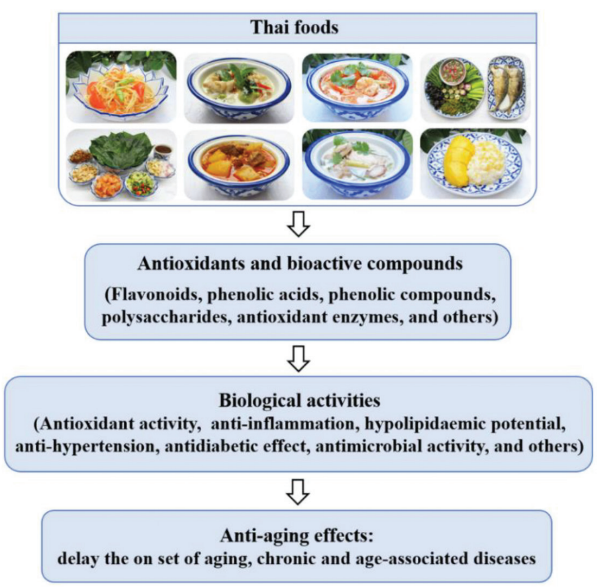

Fig. 3 The role of antioxidants and bioactive compounds derived from antioxidant-rich and healthy diets in anti-aging effects.

Fig. 3 shows that exogenous antioxidants eliminate excess oxidants, enhance endogenous antioxidative defense systems, and maintain the balance between oxidants and antioxidants. The biological activities of antioxidants and bioactive compounds influence the aging process and reduce the risk of chronic and age-associated diseases. Micronutrients contained in some ingredients, such as Se in garlic (Corzo-Martínez, et al., 2007), Fe in shallot (Swamy & Gowda, 2006), and Zn in rice (Lim, 2013), also optimize the catalytic activity of endogenous antioxidant enzymes (Vural et al., 2010). This information indicates that the consumption of Thai food can influence the aging process by balancing the level of oxidants and antioxidants in cells and increasing the efficiency of the endogenous antioxidant systems. Moreover, it suggests that Thai food can delay and/or prevent the onset of age-associated diseases and age-related chronic illness.

Conclusion

Ultimately, the classic quote "You are what you eat" is true. A person's consumption behavior always reflects the health conditions of that person. In addition to good taste, the health benefits of diets have recently been taken into consideration as well as good tastes. Thai food is a healthy diet providing both intense flavors and health benefits, including anti-aging effects. In this context, many studies have indicated the role of Thai food and its healthful ingredients in anti-aging and maintaining good health. The antioxidants and bioactive compounds of condiments comprised in Thai dishes diminish free radicals in human body resulting in the reduction of oxidative stress the postponement of the aging process. The explanations and scientific evidence of antioxidants, bioactive compounds, and biological activities associated with the anti-aging efficacy of Thai food described in this review suggest that consuming Thai foods may delay the onset of aging and prevent the development of age-related and chronic diseases.

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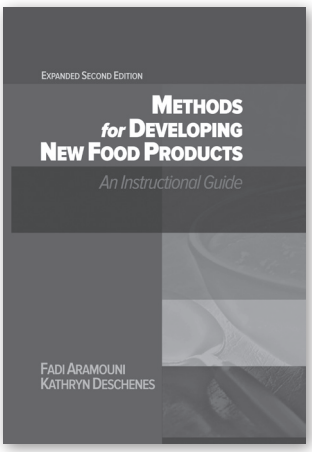
Book Review

Varaporn Vittayaporn



Book name:	Methods for Developing New Food Products, Expanded (Second Edition)
Authors:	Aramouni, F. & Deschenes, K.
Publisher:	Destech Publications
Hardcover:	430 pages
Language:	English
ISBN-10:	1605954322
ISBN-13:	978-1605954325

Methods for Developing New Food Products Second Edition is a comprehensive overview of the foundation components in new food products' lifecycle from conceptualisation to commercialisation. The text is an outstanding tool for teachers and educators, a helpful reference for industry professionals and an exceptional resource for students to go through the basics of the food industry and new product development. The text provides a solid foundation upon which to build specialized interests and on-the-job experience. The book frequently responds to a typical question by students, "But when will this information be useful in real life?"

The topics progress in a logical, simply comprehensible arrangement - starting with an overview of product development and discussing consumer preference and market trends, chemical and physical properties of food components, sensory analysis of a product and methods of evaluation, experimental design in product development, basic units of operation (how a product is manufactured), shelf life testing, on-pack labelling, quality control, and more. The text is filled with practical information and examples.

There are several key features contained in the book, including but not limited to:

- Consistency in formatting
- Essential vocabulary and analytical questions, making the text an intuitive learning tool
- Practical case studies, enabling the applicability to the readers, and giving readers a hint of expected difficulties that could potentially be encountered during the initializing of a new food product.

In studying Methods for Developing New Food Products, Second Edition, students will go through the fundamentals of new product design and development. More importantly, learners will acquire an understanding for the many interrelationships with different facets of

the commercialization cycle (e.g. ideation, economic and production feasibility, marketing and interfacing with the consumer, regulatory compliance and quality control). Learners are prepared for a successful career in the food industry by the efficacy of addressing these vital topics.

Reviewer

Asst. Prof. Dr. Varaporn Vittayaporn
School of Culinary Arts, Suan Dusit University, Thailand
e-mail: varaporn.vit@dusit.ac.th

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