ผลของแกนสับปะรดในผลิตภัณฑ์ปลาร้าถั่วเหลืองต่อการเจริญเติบโตของจุลินทรีย์และคุณลักษณะทางประสาทสัมผัส

EFFECT OF PINEAPPLE CORES IN AN IMITATED FERMENTED FISH (PLA-RA) PRODUCTS MADE BY SOYBEAN ON THE GROWTH OF MICROORGANISMS AND SENSORY ATTRIBUTES

นิจฉรา ทูลธรรม* *ชัยวุฒิ บัวเนี่ยว เฉลิมพร นามโยธา จันทิมา ตาพั้ว บุบผา มะลิกรรณ์ พรปวีณ์ แสงระพี*

Nitchara Toontom*, *Chaiwut Bourneow*, Chalermporn Namyotha, *Jantima Tapua, Bubpha Malikan, Pornpavee Sangrapee*

คณะสาธารณสุขศาสตร์ มหาวิทยาลัยมหาสารคาม

Faculty of Public Health, Mahasarakham University.

***Corresponding author, e-mail:** *nitchara@yahoo.com*

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บทคัดย่อ

วัตถุประสงค์ของงานวิจัยนี้คือการพัฒนาผลิตภัณฑ์ปลาร้าถั่วเหลือง ซึ่งเป็นผลิตภัณฑ์เลียนแบบปลาร้าโดยการใช้ถั่วเหลืองและแกนสับปะรด ผลของการเติมปริมาณแกนสับปะรดที่แตกต่างกัน (ร้อยละ 2.91 5.66 และ 8.26 โดยน้ำหนัก) ต่อการเปลี่ยนแปลงของจุลินทรีย์และคุณลักษณะทางประสาทสัมผัสระหว่างกระบวนการหมักถูกศึกษา ผลิตภัณฑ์ปลาร้าถั่วเหลืองที่หมักเสร็จสิ้นจะถูกวิเคราะห์ความสามารถในการต้านอนุมูลอิสระ 2,2-diphenyl-1-picrylhydrazyl (DPPH) และการยอมรับโดยผู้บริโภค จำนวน 50 คน เทคนิค Principal Component Analysis (PCA) จะถูกใช้เพื่อสังเกตความสัมพันธ์ระหว่างจุลินทรีย์ คุณลักษณะทางประสาทสัมผัส ความสามารถในการต้านอนุมูลอิสระ 2,2-diphenyl-1-picrylhydrazyl (DPPH) และคะแนนการยอมรับของผู้บริโภคที่มีต่อผลิตภัณฑ์ปลาร้าถั่วเหลือง ผลการวิจัยพบว่า ผลิตภัณฑ์สูตรที่มีปริมาณแกนสับปะรด ร้อยละ 8.26 มีค่า pH ที่เหมาะสมสำหรับกระบวนการหมักในวันที่ 2 โดยมีค่า pH เท่ากับ 5.56 และสิ้นสุดกระบวนการหมักในวันที่ 7 ด้วยค่า pH 5.04 และพบว่าผลิตภัณฑ์ดังกล่าวมีเจริญเติบโตของเชื้อราและยีสต์ในปริมาณต่ำที่สุด (3.00×10^6 CFU/Kg) และพบการเจริญเติบโตของจุลินทรีย์ผลิตกรดแลคติกในปริมาณสูงที่สุด (4.75×10^3 CFU/Kg) เมื่อเปรียบเทียบกับสูตรอื่น ๆ ($P \leq 0.05$) นอกจากนี้ยังพบว่า สูตรที่มีปริมาณแกนสับปะรด ร้อยละ 8.26 มีความสามารถในการต้านอนุมูลอิสระ 2,2-diphenyl-1-picrylhydrazyl (DPPH) สูงที่สุด ($P \leq 0.05$) ด้วยค่า IC_{50} 1.28×10^5 mM ของกรดแอสคอร์บิก คุณลักษณะทางประสาทสัมผัสที่สำคัญของสูตรนี้ คือ กลิ่นหมัก กลิ่นเปรี้ยว กลิ่นข้าวคั่ว และรสอูมามิ โดยทุก ๆ คุณลักษณะทางประสาทสัมผัสของสูตรนี้ได้รับคะแนนการยอมรับอยู่ในระดับค่อนข้างชอบ (6.23-6.89) สรุปได้ว่า การเติมแกนสับปะรดในปริมาณ ร้อยละ 8.26 ช่วยเร่งการหมักผลิตภัณฑ์เลียนแบบปลาร้าที่ผลิตจากถั่วเหลือง และยังช่วยเพิ่มประโยชน์ต่อสุขภาพของผลิตภัณฑ์

คำสำคัญ: ปลาร้า ถั่วเหลือง แกนสับปะรด คุณลักษณะทางประสาทสัมผัส สารต้านอนุมูลอิสระ

Abstracts

The purpose of this study was to formulate the imitated fermented fish (Pla-ra) made by soybean and pineapple cores. Effects of addition of pineapple cores with different contents (2.91, 5.66 and 8.26% w/w) on changes of microorganisms and sensory attributes during the fermentation process were studied. 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant capacity and consumer acceptances (n=50) of the finished products were studied. Principal Component Analysis (PCA) was applied to observe relationships among the microorganisms, sensory attributes, 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant capacity and consumer acceptances of the finished imitated fermented fish products. The results were found that the product with 8.26% of pineapple cores had the optimum pH for the fermentation process on day 2 with a pH of 5.56 and ended on day 7 with a pH level of 5.04. The product had the lowest number of molds and yeasts (3.00 x10^6 CFU/Kg) and the highest number of lactic acid bacteria (4.75 x10^3 CFU/Kg) when compared to other products ($P \leq 0.05$). Also, the product with 8.26% of pineapple cores had the highest 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant capacity ($P \leq 0.05$) with IC_{50} of 1.28 x10^5 mM ascorbic acid equivalent. Its feature sensory attribute was a fermented odor, sour odor, roasted rice and umami taste. Moreover, this product obtained acceptance scores with the rather like level of all attributes (6.23-6.89). In conclusion, the addition of pineapple cores 8.26% accelerate the fermentation of imitated fermented fish product made by soybean and also enhance the health benefits of the product.

Keywords: Fermented fish, Soybean, Pineapple cores, Sensory attribute, Antioxidant

Introduction

Pla-ra is a fermented fish product that can be made cheaply from various fish raw materials. It is a famous ingredient of the north-eastern food of Thailand, such as Namprik Pla-ra, Som Tam Pla-ra, Kang Lao, Namya, and Pla-ra Sub [1-2]. The many foods added Pla-ra are popular among consumers. However, there are many other consumers, such as vegetarian who consume these foods but do not wish to eat fish or other meat. Soybean is a good alternative raw material that can be used to produce vegetarian Pla-ra. Soybean has long been prized among vegetarians for both their high protein content and versatility [3]. The quality of soy protein is higher than that of other legumes (protein digestibility corrected amino acid scores for soy protein from different soy foods ranges from about 0.9 to 1.0) [4] and soybean is the only legume to provide ample amounts of the essential omega-3 fatty acid, α-linolenic acid (ALA) [5]. In addition, soybean and the foods made from it are essentially unique dietary sources of isoflavones [6]. The soy isoflavones extract had strong antioxidant activity [7-8]. Also, fermentation enriched isoflavones and improved the biological functionality of soybean product (e.g. antioxidant activity, alleviation of hormonal disorders in postmenopausal women, etc.) [10]. Generally, fermentation of soybean products is conducted by *Bacillus* and *Aspergillus* species which take a long period of time to complete due to the fermentation process [9].

Waste utilization in fruits and vegetable processing industries is one of the important and challengeable jobs around the world. It is anticipated that the discarded pineapple fruits as well as its waste materials could be utilized for further industrial purposes, e.g. fermentation, extraction of bioactive components, extraction of functional ingredients etc [11]. Core pineapple wastes are recommended as good sources of organic raw materials and are potentially available for fermentation process. It has one of the most important chemicals, namely bromelain which is a protease enzyme. The presence of bromelain is reported in core of pineapple fruit and is used to ferment meat for improving meat texture. Moreover, it could digest fish tissues in a short period [12]. Also, core pineapple contains high amounts of crude fiber promoting on the digestive system to function effectively. Researchers have focused on the utilization of core pineapple primarily for fermentation and expected that it could enhance in nutrients of the fermented food products. The addition of pineapple in order to use of its enzyme for fermenting soybean products has been not observed, especially aspects of accelerated fermentation and sensory profile of soybean products.

Objectives

This research aims to produce an imitated product of fermented fish (Pla-ra) made by soybean and pineapple cores which is an agricultural waste. The effects of addition of pineapple cores with different contents on changes of microorganisms and sensory attributes during the fermentation process were observed. In addition, the finished products were determined in antioxidant activity and consumer acceptance.

Methods

Chemicals and Microbiological Media

2,2-diphenyl-1-picrylhydrazyl (DPPH) and gallic acids were purchased from Sigma Chemical Co. Potato dextrose agar (PDA) was purchased from Hi-media Co. Ltd.. Man rogosa sharpe agar (MRS) was purchased from Hi-media Co. Ltd.

Soybean sample

Fresh soybean (*Glycine max* L. Merill) samples of Chiang Mai 60 variety were purchased from Loei Field Crop Research Center (Loei Province, Thailand), dried at 60°C until <13% of moisture content reached and kept in vacuum package at 4° C until used.

Process of the imitated fermented fish product made by soybean

Soybeans were washed and soaked in tap water (1:3.5 soybean to water ratio) for 16 h at ambient temperature (~25°C). After decanting the water, soaked soybeans were boiled at 100°C until they were cooked (approximately 4-6 hours). The cooked soybeans were placed in a glass bottle with lid and take it exposure to the sun for 3 days. The soybeans were mixed with salt, soybean paste (Tao-jeow) and roasted rice. After that, the mixture was mixed with pineapple core producing 0, 2.91, 5.66 and 8.26% (Table 1). The fermentation was carried out in airtight glass containers at 30°C for 7 days or until the pH value was a range of 5.0-5.5.

Table 1 Formulas of the imitated fermented fish product made by soybean.

Treatments	Formulas adding pineapple cores	Soybean (%)	Salt (%)	Roasted rice (%)	Soybean paste (%)
Control	0%	50	34	10	6
3gPC	2.91%	48.54	33.01	9.71	5.83
6gPC	5.66%	47.17	32.08	9.43	5.66
9gPC	8.26%	45.87	31.19	9.17	5.50

Determination of pH

The pH of the products was measured daily interval for 7 days. The sample was measured for the pH value at ambient temperature with a pH meter (Satorious, USA) which was calibrated with pH 4.0 and 7.0 [13].

Microbial determination

The microbial determinations of the products were conducted on every day for 7 days. Twenty five grams of samples were mixed with 225 ml of sterile peptone water (0.1%). The mixtures were then homogenized by stomacher for 2 min, and diluted using the ten-fold serial dilution for microbial evaluation. Yeasts, molds and lactic acid bacteria (LAB) counts were determined during fermentation. For yeasts and molds counts, 0.1 mL of each decimal diluted sample was spread on the potato dextrose agar (PDA) with 10% of tartaric acid. The growth of yeasts and molds was also observed after the plates were incubated at 37°C for 3-5 day [14]. Determination of LAB counts was conducted according to the modified method of Kim and others [15]. A thousand microliter was transferred to a plate, and MRS agar was poured onto the plate and incubated at 37°C for 48 h. The standard plate count method was used to enumerate the bacteria, wherein the number of colonies was multiplied by the dilution factor and reported as the number of colonies forming units (CFU) per kilogram of sample.

Determination of DPPH antiradical activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the samples was measured according to the modified method of Brand-Williams and others [16]. The reaction mixture contained 3 ml DPPH working solution (0.1mM DPPH in ethanol) to which was added 1 ml samples. The mixture was shaken and held for 30 min in the dark at room temperature (30±1°C). The absorbance was then read at 515 nm using a UV-visible spectrophotometer (Cecil, Aquarius 7400). The inhibition percentage of the absorbance of the DPPH solution was calculated using the following equation (1)

$$\text{Inhibition \%} = [\text{Absorbance of control - Absorbance of sample/absorbance of control}] \times 100 \quad (1)$$

Then, the DPPH radical scavenging activity was then calculated in the term of IC_{50} (concentration providing 50% inhibition/scavenging). Ascorbic acid was used as a standard.

Sensory profiling

The sensory profile of each formula was conducted on every day for 7 days. A generic descriptive analysis method [17] was applied. Ten panelists who were screened and recruited from MSU panelist database, based on their sensory sensitivity and ability to describe sensations perceived from food products. They should participate in discussion sessions for orientation and development of terms. Panelists undertook a 24-hour training program. They were trained to use a 15-cm unstructured line in the training sessions to evaluate the sensory intensity (10 training sessions). The panels were asked to describe the perceived sensations of appearance, aroma, taste, and texture of the samples. The initial list of attributes was revised to clarify and remove subjective, duplicate, or ambiguous terms. The final attributes were developed by all panelists. Also, definitions of the attributes were conducted. Then, the final attributes and definitions were used for further training of the panelists. After the training, the panelist's performance was tested to determine reliability and validity on all attributes. If the panelists performance was consistent and valid on the test samples (known intensities), then they would be qualified to start working on the sample evaluation. Each sample (2.5 g) was served in plastic cups coded with three-digit numbers. All samples were presented in the booths with a red light to mask any color interference. Panelists were asked to rinse their mouth with water between each sample. Each member received the same sample in triplicate without knowledge of its nature to ensure the quality of the results.

Consumer acceptance test

The finished product of each formula was evaluated. Acceptance on product sensory quality was evaluated by using a 9-point category hedonic scale (9 = "like extremely" and 1 = "dislike extremely"). Fifty consumers, whose age ranged from 18 to 35 years were recruited. All consumers were presented with random 3-digit coded samples. Balance first-order and carry-over-effect design [18] was applied for serving plan on samples. The samples were presented and assessed in red masking light in a sensory booth to reduce color interference effects.

Statistical analysis

A completely randomized design was used to study the antioxidant activity and microbial determination. The experimental design of sensory profiling and consumer acceptance test was a randomized complete block. Data were subjected to analysis of variance (ANOVA). Duncan's new multiple range test (DMRT), with a level of significance of 0.05. Principal Component Analysis (PCA) was applied to present relationships among all parameters. Statistical analyses were performed by using the using IBM SPSS Statistics 20.

Results

pH value during fermentation

The pH values of the products were shown in Figure 1. The pH values were significantly different among the samples ($P \leq 0.05$). At the end of fermentation, pH values of all products varied between 5.04 and 5.44. It was found that the pH values of the products decreased with fermentation time and amount of pineapple cores increased. After observing throughout the fermentation period, formula adding 8.26% of pineapple cores had the lowest pH when compared with other formulas ($P \leq 0.05$).

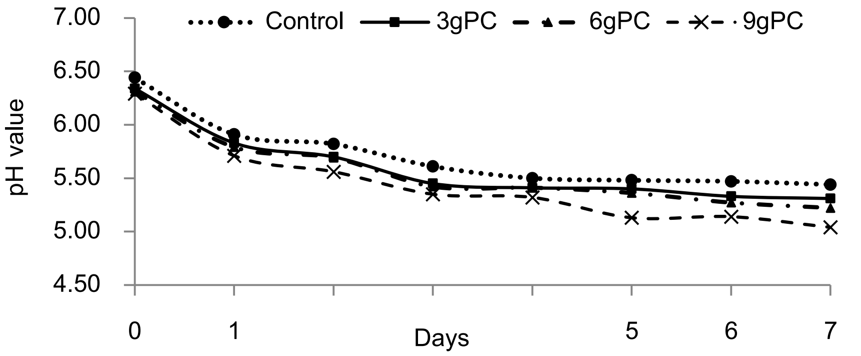


Figure 1. pH value during fermentation of the imitated fermented fish product made by soybean adding different amounts of pineapple cores.

Microbial changes during fermentation

Numbers of colonies on culture media of the imitated fermented fish product made by soybean adding different amounts of pineapple cores were shown in Figure 2. Increasing amounts of pineapple cores significantly decreased yeasts and molds counts. However, LAB growth increased with amounts of pineapple cores increased ($P \leq 0.05$). The yeasts and molds counts of all products varied between 3.00 $\times 10^6$ and 46.2 $\times 10^6$ CFU/Kg. The LAB counts of all products varied between 0 and 4.75$\times 10^3$ CFU/Kg. Formula adding 8.26% pineapple cores presented the lowest of yeasts and molds counts, while it had the lowest of LAB counts. A significant difference in microbial changes was observed at day 2. During fermentation, it was found that yeasts and molds growth decreased when the fermentation time increased. Whereas, LAB growth increased with the fermentation time increased.

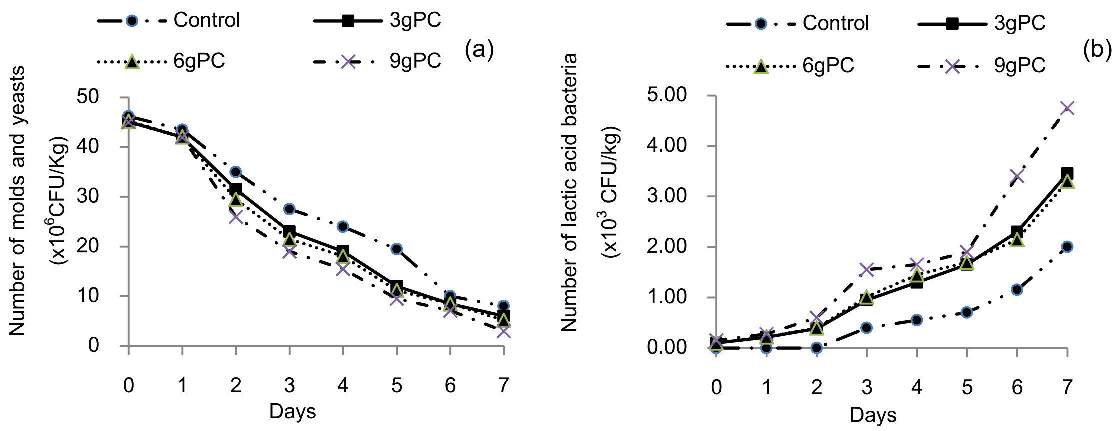


Figure 2. Numbers of molds and yeasts (a) and lactic acid bacteria (b) during fermentation of the imitated fermented fish product made by soybean adding different amounts of pineapple cores.

Antiradical activity of the imitated fermented fish product made by soybean adding different amounts of pineapple cores

The IC_{50} of imitated fermented fish made by soybean adding different amounts of pineapple cores are shown in Figure 3. DPPH antiradical activity of all formulas reveals antioxidant potency based on IC_{50} values equivalent to ascorbic acid. IC_{50} value, which is the inhibition concentration of solvent extract required to decrease initial DPPH concentration by 50%. A lower value of IC_{50} indicates higher antioxidant activity. The DPPH antiradical activity increased as the dosage of pineapple cores increased. The DPPH antiradical activity of formula adding 8.26% pineapple cores showed higher antioxidant activity than other formulas with IC_{50} values 1.28 x10^5 mM ($P \leq 0.05$).

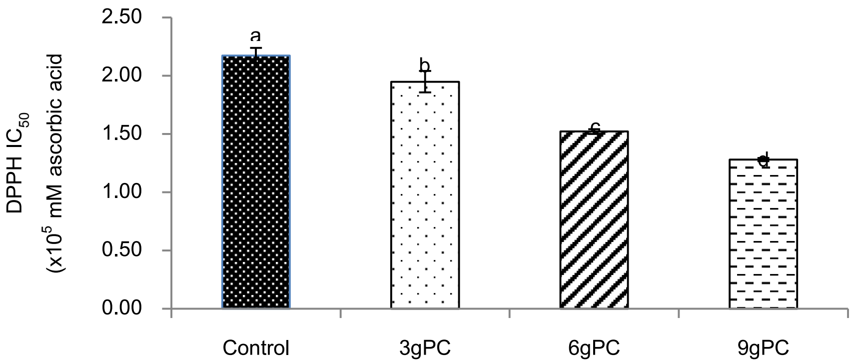


Figure 3. DPPH IC_{50} of the imitated fermented fish product made by soybean adding different amounts of pineapple cores

Note: Different letters refer to a significant difference ($P \leq 0.05$).

Sensory profile during fermentation

The sensory lexicon was developed and agreed by 10 panelists including attributive terms and agreed definitions shown in Table 2. The sensory rating scores of individual products for brown color, tao-jeow odor, roasted rice odor, fermented odor, sour odor, salty, umami and peanut butter were defined and elucidated in this case.

Table 2 Sensory attributes and references developed for the imitated fermented fish product made by soybean

Sensory attributes	Definitions	References
1. Brown color	Brown color characteristic	Dark chocolate
2. Tao-jeow odor	Odor/aroma characteristic like Tao-jeow	Tao-jeow
3. Roasted rice odor	Odor/aroma characteristic like roasted rice	Roasted rice
4. Fermented odor	Odor characteristic like fermented fish (Pla-ra)	Fermented fish (Pla-ra)
5. Sour odor	Odor characteristic like sour of pineapple	Pineapple
6. Salty	Basic taste produced by aqueous solutions of substances like sodium chloride	2 g of salt is dissolved in 1 liter of water. 30 ml of dissolution in 50 ml plastic cup [19]
7. Umami	Taste characteristic like broths (miso soup)	Miso soup
8. Peanut butter taste	Taste characteristic like peanut butter	Peanut butter

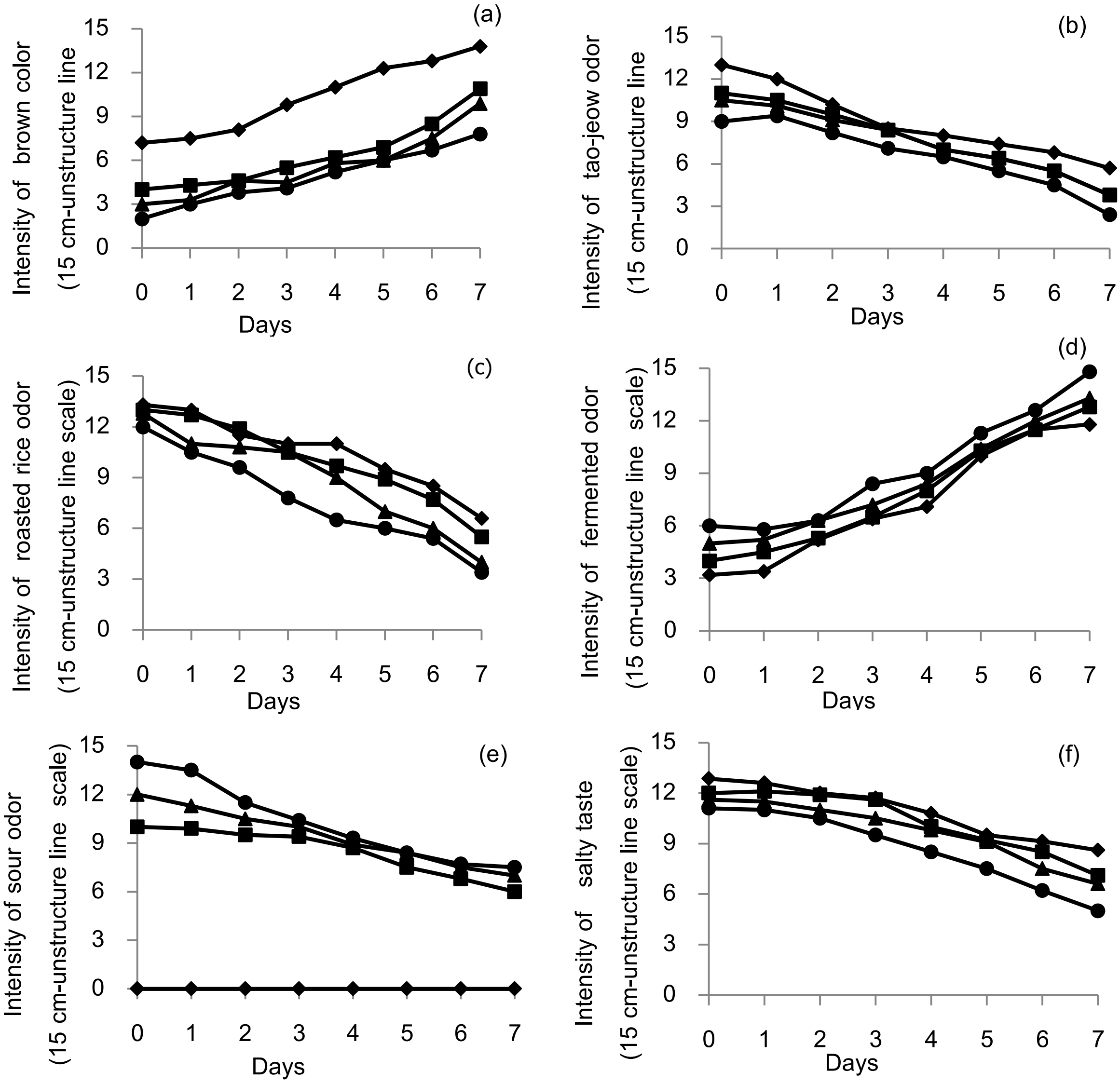
Figure 4. shows changes of sensory attributes of the imitated fermented fish product made by soybean during fermentation. The results were found that attributes of tao-jeow odor, roasted rice odor, sour odor, salty and peanut butter tended to decreases. Formula adding 8.26% of pineapple cores had the lowest intensities of tao-jeow odor, roasted rice odor, peanut butter and salty. Whereas, the product had the highest intensity of sour odor ($P \leq 0.05$).

Consumer acceptances

Mean scores of 50 consumers on a brown color, tao-jeow odor, roasted rice odor, fermented odor, sour odor, salty, umami and peanut butter likings are shown in Figure 5. Mean difference reveals that formula adding 8.26% of pineapple cores got the highest liking scores of almost of attributes with a range of rather liked (6.23-6.89). While, the commercial product was rated the lowest scores of all attributes when compared with the experimental formulas (P≤0.05).

Principle component analysis

Principal Component Analysis (PCA) illustrates an overview of the characteristics of all samples. The bi-plots of quality values-products PCA shown in Figure 6a-b here are composed of 2 Principal Components (PC) which explain 96.86% of the data variability. The first components (PC1) explain 81.27% of the variance in the data set, which means the sensory profile of the imitated fermented fish products made by soybean, can be mainly interpreted by the PC1. PCA illustrations which show that formula adding 8.26% of pineapple cores is highly positive relationship to consumer acceptances, fermented odor, sour odor, roasted rice odor, umami and LAB counts. Also, IC_{50} of DPPH presented negative relationship to the formula.



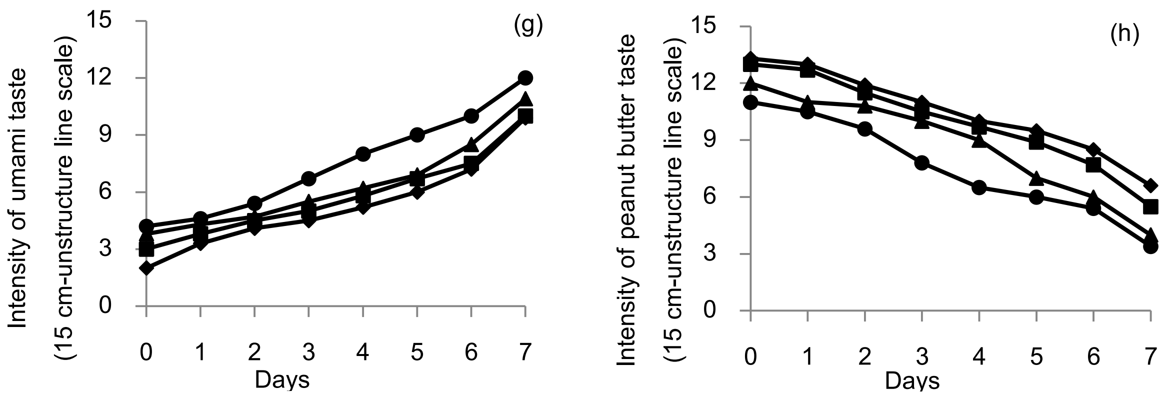


Figure 4. (a-h) Changes of sensory attributes the imitated fermented fish made by soybean during fermentation (Cotrol, 3gPC, 6gPC, 9gPC), (a) brown color, (b) tao-jeow odor, (c) roasted rice odor, (d) fermented odor, (e) sour odor, (f) salty, (g) umami and (h) peanut butter.

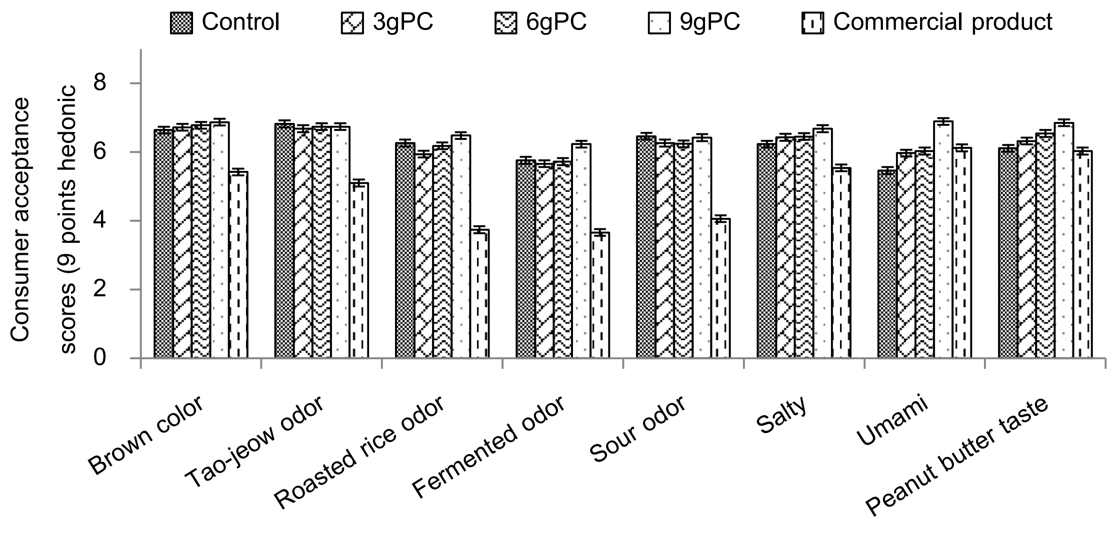
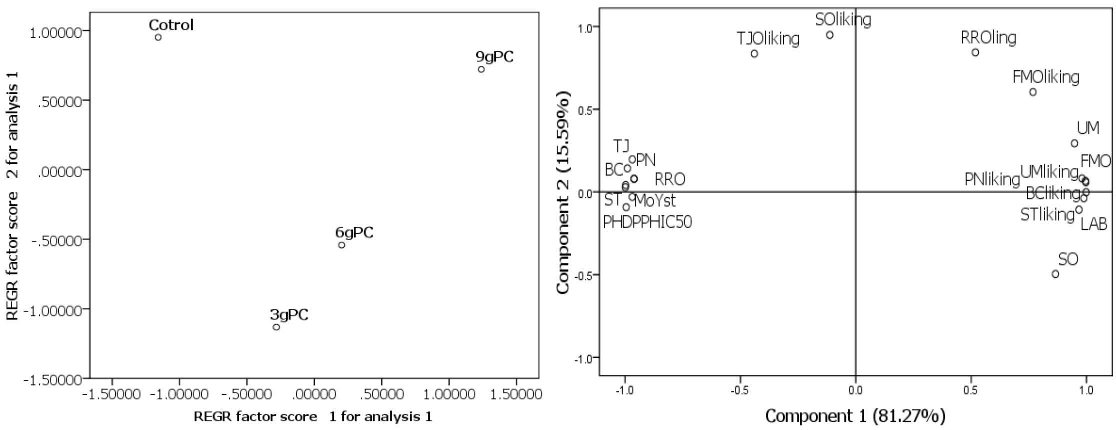


Figure 5. Means scores of consumer acceptances on sensory attributes of the imitated fermented fish product made by soybean.



(a) (b)

Figure 6. (a-b) PCA bi-plots on quality values of the imitated fermented fish product made by soybean adding different amounts of pineapple cores

Note. molds and yeasts (MoYst), lactic acid bacteria (LAB). brown color (BC), tao-jeow odor (TJO), roasted rice odor (RRO), fermented odor (FMO), sour odor (SO), salty (ST), umami (UM) and peanut butter (PB).

Conclusion and Discussions

The pH values of all products varied between 5.04 and 5.44. These were in a range of the optimum pH range of Pla-ra products (pH 5.0 and 6.0) [20]. After observing throughout the time course of fermentation, pH of the products was decreased with amount of pineapple core and fermentation time increased. This may be pH effect of the pineapple core which contained a pH value of 4.0 [21].

Nadpranil and Leenanon [22] mentioned that fermentation was caused by the production of lactic acid in fermented fish. Thus, the fermentation time increased is resulting in decreased pH. As Table 1, the formula adding 8.26% of pineapple cores showed lower pH than other samples at the end of fermentation ($P \leq 0.05$). It may be due to the acid existing in the pineapple cores enhancing the acidity of the products and then causes the pH of the product to decrease. Roda and others [23] reported the pH of pineapple cores at 3.93±0.10. This agreed with Abdullah and Hanafi [24] and Sasaki and others [25] who reported the pH of pineapple juice in the range value of 3.6-4.6. ($P \leq 0.05$). The low pH results in the inhibition of microorganisms that cause spoilage and pathogenic bacteria [26], while this range of pH value promote the growth of main microorganism of fermented soybean, namely molds, *Aspergillus oryzae*, *Aspergilus soyae*, *Rhizopus oligosporrus* and yeasts *Saccharomyces rouxii*, and lactic acid bacteria such as *Pediococcus halophilus*, *Bacillus subtilis* or *Steptococcus* [27].

At the beginning of fermentation, formula adding 8.26% of pineapple cores presented the lowest of yeasts and molds counts, while it had the lowest of LAB counts. This may be because the pH of the formula is not suitable for the growth of yeast and mold, but it promote on the growth of LAB. Likewise, 8.26% pineapple core added formula showed the lowest of yeasts and molds counts, and had the highest of LAB at the end of fermentation. In addition, it can be fermented faster than other products ($P \leq 0.05$), considering pH of the product. Another possible reason, this may be due to more content of bromelain enzyme in the 8.26% pineapple core can digest soy protein more effective than other samples, resulting in more producing substrate for microorganism. Abdullah and Hanafi [24] mentioned that pineapple cores is a substrate suitable for cultivation of bacteria, i.e. potentially used as a carbon source for organic acid fermentation.

DPPH antiradical activity of formula adding 8.26% of pineapple cores showed the highest antioxidant activity at IC_{50} values 1.28 x10^5 mM ascorbic acid equivalent. On the contrary, control (0%

pineapple core) showed the lowest antioxidant activity ($P \leq 0.05$). This indicates that adding pineapple cores enhances antioxidant activity. Kongsuwan and others [28], who studied bioactive compounds and antioxidant capacities of phulae and nanglae pineapple, mentioned that pineapple fruit contained a high level of vitamin C, phenolic compounds and β-carotene which constitute natural sources of antioxidants. Loh and others [29] reported that pineapple core contained 6.14 $x10^5$ mM gallic acid equivalent of phenolic compounds, 2.63 $x10^5$ mM quercetin equivalent of flavonoid and had 5.47 $x10^2$ mM gallic acid equivalent of DPPH EC_{50}.

In addition, fermentation promotes antioxidant activity in the soybean products. Samruan and others [30] reported that fermentation enhanced the DPPH antiradical activity of soybeans. Soybean and different soybean products were known to contain phenolic compounds. The concentration of these compounds in soybean was reported to increase after fermentation. Isoflavones was the main source of the biologic activities of fermented soybean foods which acts as antioxidant also. Glycoside isoflavones in soybeans were hydrolyzed by β-glucosidase produced by microorganisms, thereby increasing aglycone isoflavones during fermentation [31]. Moreover, soybean proteins during fermentation could be hydrolyzed into peptides and amino acids [32-34]. This improved the antioxidant functionality of fermented soybean product.

The sensory attribute changes of the products were found during fermentation. The intensity of roasted rice odor, tao-jeow odor, sour odor, salty taste and peanut butter taste decreased with increasing time of fermentation. This may be explained that increased proportion of pineapple core increase moisture content, resulting in dilution of the ingredient concentrations. In addition, an important reason may be because of the product ingredients (i.e. soybean, tao-jeow and roasted rice) which were substrates of microorganisms were used. Generally, fermented soybean products (e.g. soy sauce) were made by a two-step fermentation process from wheat flour and soy beans with a mixture of molds, yeasts and bacteria. The first step is the fermentation with mold to produce proteases and amylase enzymes for digesting protein and polysaccharide in food source (i.e. koji) which is a starter culture [35-36]. This step provides characteristic aroma and flavor to the soybean products. The second step is the fermentation made by yeast and bacteria in the presence of high salt concentration (18-20%) [37]. However, the fermentation of this study was monitored after adding 30% salt concentration in the product. The principal microorganisms which cause the phenomenon in this study were likely to be halophilic bacteria, such as *Pediococcus halophilus* (presently *Tetragenococcus halophilus* [38]). It can grow in 18% NaCl or greater [39]. The bacteria can produce lactic acid to decrease the pH. In addition, the bacteria play an important role in the quality control of the fermentation process and have an influence on fermented soybean products such as flavor and taste [40]. As Figure 4, attributes of brown color, fermented odor and umami were recognized to increase by the fermentation time. Formula adding 8.26% of pineapple core was rated with lower intensity of brown color than other formulas ($P \leq 0.05$). This may be because acidity obtained from pineapple cores inhibited Maillard reaction. The brown color of the product may be caused by Maillard reaction. Hashimoto and Nakata [41] reported that coloration of fermented soybean product is caused by melanoidin. It was formed by non-enzymatic reaction with sugar and amino acid. The compounds were increasingly produced more by the time of fermentation. The sugar and amino acid are aminocarbonyl reaction. Sugar contributes to this aminocarbonyl reaction. Pentoses, such as xylose and arabinose, have higher reactivity than hexoses such as glucose and galactose. For increasing fermented odor, it may be because of biosynthesis of the microorganisms. Hauck and others [42] reported that the precursors of 4-hydroxy-3[2H]-furanones are formed during soybean fermentation. Also, microorganisms may produce ethanol and aroma components, specifically phenolic compounds (e.g. 4- ethylphenol) which add characteristic aromas to fermented soybean products. In an aspect of increasing umami taste, formula adding 8.26% of pineapple cores was recognized as having high intensity of umami taste when compared with other formulas ($P \leq 0.05$). This may be explained that it involved to the production of extracellular enzymes from the pineapple cores and microorganisms during soybean fermentation. The enzymes, such as protease and carbohydrase complexes to digest the proteins and carbohydrates contained in the raw ingredients into peptides, amino acids, and sugars [32-34]. The action of the protease complex is releasing glutamine and glutamic acid which is known to be the major component of soybean and wheat proteins [43]. These compounds may give an umami taste of the fermented soybean products [44].

The experimental formulas were more accepted by consumers in all attributes than the commercial product ($P \leq 0.05$). Adding 8.26% pineapples aids improving attributes affecting on consumer acceptance, such as color, odor and taste of the imitated fermented fish product made by soybean. This confirmed by PCA illustrations which show that formula adding 8.26% of pineapple cores is highly positive relationship to consumer acceptances. Moreover the formula is highly positive relationship to sensory attributes of fermented odor, sour odor, roasted rice odor, umami and LAB counts. Also, IC_{50} of DPPH presented negative relationship to the formula. This explains that this formula got high consumer acceptances with remarkable feature of fermented odor, sour odor, roasted rice and umami, and had a good performance in DPPH antiradical activity.

In summary, the addition of pineapple cores in fermented soybean, which imitated the fermented fish product produced by soybean, has affected the growth of microorganisms and the change in sensory properties. It promotes the soybean fermentation faster and improved sensory attributes of the products, such as fermented odor, sour odor, roasted rice and umami taste which influenced on consumer acceptances. Also, the addition of pineapple cores in the products gives higher DPPH antiradical activity. These results show that the imitated fermented fish product made by soybean adding 8.26% of pineapple cores could be used for the possible commercial production of functional food to alleviate oxidative stress.

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