คุณภาพทางเคมี กายภาพ และจุลชีววิทยาของปลาส้มที่จำหน่ายในจังหวัดพระนครศรีอยุธยาต่อการยอมรับของผู้บริโภค

Physical, Chemical and Microbiological Qualities of *Plaa-Som* as Commercialized in Phranakhon Si Ayutthaya Province on Consumer Acceptance

ปวีณา รัตนเสนา และ พิทยา ใจคำ*

Paweena Rattanasena and Pittaya Chaikham*

คณะวิทยาศาสตร์และเทคโนโลยี มหาวิทยาลัยราชภัฏพระนครศรีอยุธยา

Faculty of Science and Technology, Phranakhon Si Ayutthaya Rajabhat University

Received : 16 January 2018

Accepted : 11 June 2018

Published online : 15 June 2018

บทคัดย่อ

ในการศึกษานี้ได้เก็บตัวอย่างปลาส้มจากตลาดในจังหวัดพระนครศรีอยุธยา และทำการวิเคราะห์คุณภาพด้านกายภาพ เคมี จุลชีววิทยา และประสาทสัมผัส โดยพบว่า ตัวอย่างที่เก็บมามีความแตกต่างด้านสี และค่าความแน่นเนื้อ แต่มีค่าความเป็นกรด-ด่าง และปริมาณกรดแลคติกทั้งหมดได้มีค่าใกล้เคียงกัน ในตัวอย่างยังพบสารไบโอเจนิคเอมีน 5 ชนิดหลักในปริมาณที่แตกต่างกัน ซึ่งพบฮิสตามีนสูงที่สุด (118.42-163.92 mg/kg) รองลงมาคือ คาดาวีรีน (69.33-90.44 mg/kg) พิวเทรสซีน (65.92-90.18 mg/kg) ไทรามีน (52.69-97.04 mg/kg) และทริปตามีน (42.15-61.14 mg/kg) นอกจากนั้นยังพบว่า ในตัวอย่างปลาส้มพบเฉพาะแบคทีเรียกรดแลคติคเท่านั้น แต่ไม่พบแบคทีเรียก่อโรค และแบคทีเรียที่ทำให้อาหารเน่าเสีย สำหรับผลการวิเคราะห์ทางประสาทสัมผัสพบว่า ทั้งปลาส้มดิบ และปลาส้มที่ผ่านการทอดได้รับคะแนนการยอมรับค่อนข้างสูงจากผู้บริโภค จากผลการทดลองในงานวิจัยแสดงว่าวัตถุดิบ สูตร และขั้นตอนการผลิตมีผลต่อคุณภาพ และลักษณะที่แตกต่างกันของผลิตภัณฑ์ปลาส้มที่ขายในตลาดของจังหวัดพระนครศรีอยุธยา

คำสำคัญ : ปลาส้ม, คุณภาพด้านกายภาพ, คุณภาพด้านเคมี, การยอมรับของผู้บริโภค

***Corresponding author. E-mail** : pittaya.chaikham@gmail.com

Abstract

In this study, *Plaa-Som* samples were bought from six markets located in Phranakhon Si Ayutthaya province and analyzed for physical, chemical, microbiological and sensory qualities. They were shown to have differences in color parameters and firmness value, whereas \similar levels of pH and total lactic acids were observed. There were five predominant biogenic amines found in *Plaa-Som* samples at different levels, whereby histamine was shown to be the highest (118.42-163.92 mg/kg), followed by cadaverine (69.33-90.44 mg/kg), putrescine (65.92-90.18 mg/kg), tyramine (52.69-97.04 mg/kg) and tryptamine (42.15-61.14 mg/kg). In addition, only lactic acid bacteria were found in *Plaa-Som* samples and the indicator pathogenic and spoilage bacteria remained undetected. For sensory evaluation, both uncooked and fried *Plaa-Som* samples received the highest overall acceptability scores. The results from this research displayed that raw materials, formula and production could result in different qualities and characteristics of *Plaa-Som* products as commercialized in local markets located in Phranakhon Si Ayutthaya province.

Keywords : *Plaa-som*, physical quality, chemical quality, consumer acceptance

Introduction

Plaa-Som is a Thailand's traditional fermented fish product with sour flavor. In the central and north-eastern parts of Thailand, *Plaa-Som* can be produced by fermentation of freshwater fish with salt, boiled rice (or steamed sticky rice) and garlic, but in the southern part, *Plaa-Som* has been made from fish fermented with salt, palm syrup and sometimes roasted rice (Paludan-Müller *et al*., 2002). *Plaa-Som* products are characterized into 4 types according to the appearances, including whole *Plaa-Som*, pieces of *Plaa-Som*, shredded *Plaa-Som*, and *Nham Plaa-Som* (processed *Plaa-Som* that is wrapped in banana leaves). The levels of salt (1-10%, w/w) applied in *Plaa-Som* product have been shown to affect the number of microbes, rate of fermentation, sensory quality and also safety of the product. The major microbial populations in *Plaa-Som* during fermentation are lactic acid bacteria (LAB), yeasts and molds, including *Pediococcus pentosaceus*, *Lactobacillus alimentarius/farciminis*, *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactococcus garvieae*, *Weisella confusa*, *Weisella cibaria*, *Streptococcus bovis* and *Zygosaccharomyces rouxii* (Paludan-Müller *et al*., 2002; Kopermsub and Yunchalard, 2010; Pringsulaka *et al*., 2012). During early fermentation, LAB may be less than 10 CFU/g but they can grow rapidly to the final populations of approximately 10^7 CFU/g. The report of Kopermsub and Yunchalard (2010) showed that even though the predominant LAB populations during the early stage of *Plaa-Som* fermentation were *L. garvieae*, *S. bovis* and *W. cibaria*, the succession of *W. cibaria, P. pentosaceus* and *L. plantarum* was found during the later stage (after 48 h of incubation), whereby *L. plantarum* was the major species. Interestingly, *Som-Fug*, an another fish product of which ingredients were similar to *Plaa-Som*, including minced fish fillet, salt (2-5%, w/w), ground boiled rice (2-

12%, w/w) and minced garlic (4%, w/w), was found to contain early populations of LAB, including *Lactobacillus lactis* sp. *lactis*, *Leuconostoc citreum*, *Lactobacillus paracasei* sp. *paracasei*, *W. confusa*, *L. plantarum*, *Lactobacillus pentosus* and *P. pentosaceus*, whereby *L. plantarum/pentosus* was found to be the dominant species in the later stage of fermentation (Paludan-Müller *et al*., 1999). For another similar product, *Pa-Som*, which is a product from Laos and made from fish or fish fillet, salt, garlic and cooked sticky or non-sticky rice, has been shown to contain *Lactococcus* sp., *Weissella* sp., *Enterococcus* sp. and *Pediococcus* sp. (Marui *et al*., 2014). Development of aroma, flavor and color of fermented fish products was found to be mainly due to coagulase-negative staphylococci and yeasts (Riebroy *et al*., 2004). In addition, during the fermentation of fish, proteolytic activities of microbial enzymes were shown to be the key biochemical changes that resulting in formation of peptides, amino acids, aldehydes, organic acids and amines, all of which greatly affected both texture and flavor of the fermented products (Roseiro *et al*., 2008). LAB populations have also been shown to play important roles on suppression of potentially pathogenic bacteria in fermented fish products by releasing lactic acid (to pH approximately 4.5), enzymes, as well as bacteriocins. Indeed, bacteriocin-producing LAB, particularly *L. plantarum*, have been considered as Generally Recognized as Safe (GRAS) microorganisms and their bacteriocins were emphasized as natural food bio-preservatives (Pringsulaka *et al*., 2012).

This may suggest that different recipes could result in many differences of *Plaa-Som* characteristics. Hence, there should be exploration and evaluation for commercially available fermented fish products. For example, six brands of "*Suanyu*", which is Chinese traditional fermented whole fish product, have been assessed and found that they have similar microbial quality with the dominant populations of LAB, *Staphylococcus* and yeasts, and low numbers of *Enterobacteria* and *Pseudomonas*. However, the chemical characteristics of "*Suanyu*" samples were somewhat different due to the influences of materials, ingredients and fermentation processes and conditions (Zeng *et al*., 2013).

Nowadays, *Plaa-Som* has been widely produced at both household and industrial scales, and Phranakhon Si Ayutthaya is one of the major producers of *Plaa-Som* due to its location that has Chao Phraya and Pa Sak rivers run through. Most of *Plaa-Som* commercially sold in Phranakhon Si Ayutthaya is the whole *Plaa-Som*. Therefore, in this study, six samples of *Plaa-Som* were collected from the markets located in Phranakhon Si Ayutthaya province and the differences of *Plaa-Som* qualities, including physical, chemical and microbiological characteristics as well as the sensory attributes, were investigated.

Methods

1. Product sampling

Six samples of whole *Plaa-Som,* which were produced from silver carp (*Barbonymus gonionotus*), were chosen from the major producers located in different areas of Phranakhon Si Ayutthaya province, including Uthai

(UT, No. 14), Sena (SN, No. 12), Phachee (PC, No. 9), Bangpahan (BPH, No. 7), Bangpa-in (BPI, No. 6) and Phranakhon Si Ayutthaya (PSA, No. 1) districts, as shown in Figure 1. The samples were placed on ice and transported to the Faculty of Science and Technology, Phranakhon Si Ayutthaya Rajabhat University within 2 h after purchasing. Then, *Plaa-Som* samples were analyzed for their physical and microbiological characteristics, and the rest of them were kept at -25°C until required for chemical analysis. In this case, *Plaa-Som* samples were found to have different levels of salt, moisture, protein, fat and ash, of which accounting for 3.11-4.05%, 70.83-76.74%, 15.28-17.01%, 1.68-1.94% and 4.06-4.63%, respectively.

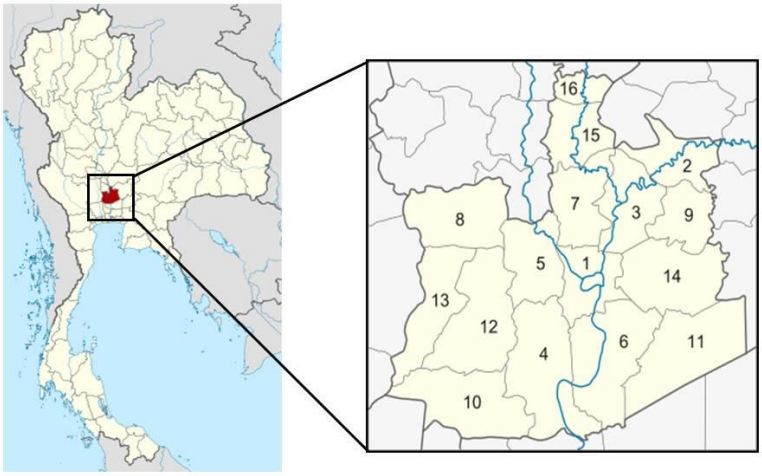

Figure 1 *Plaa-som* from different markets in Phranakhon Si Ayutthaya area; Uthai (14), Sena (12), Phachee (9), Bangpahan (7), Bangpa-in (6) and Phranakhon Si Ayutthaya (1) districts

2. Color parameter and textural measurements

Color parameters viz. CIE *L* (lightness), *a** (redness) and *b** (yellowness) of uncooked *Plaa-Som* were measured using a Minolta Chroma Meter CR-300 colorimeter (Minolta, Japan). For textural analysis, the firmness of samples was conducted using a texture analyzer (CT3, Brookfield, USA) with test conditions: test probe is TA5, test speed 1 mm/s, trigger load 2 g and test target distance 3 mm.

3. pH and total lactic acid

The samples were prepared following the method of AOAC (2000) with some modifications and the pH was measured using a pH meter (Sartorius PB-20, Germany). Total lactic acid were quantified using the UV spectrophotometric method at λ_{max} 340 nm (Perkin Elmer UV WINLAB, Perkin Elmer, USA) according to the manufacturer's instructions (R-Biopharm AG, Germany).

4. Determination of biogenic amines

Biogenic amine compounds in *Plaa-Som* were extracted and determined following the methods as described by Riebroy *et al.* (2004) with some modifications. Briefly, 10 g of *Plaa-Som* meat was blended for 2 min before mixing with 50 ml of 10% trichloroacetic acid (TCA) solution using a stomacher at a speed of 250 rpm for 10 min. The mixture was centrifuged at 8,000*g* for 20 min at 4°C. One milliliter of its supernatant was well-mixed with 0.2 ml of 2 M sodium hydroxide and 0.3 ml of saturated sodium bicarbonate, and then allowed to incubate at room temperature for 30 min. Then, 2 ml of dansyl chloride solution (10 mg/ml) were added to the reaction mixture and incubated at 40°C for 45 min. The reaction was stopped by adding 0.1 µl of 25% ammonia. After centrifugation at 3,000*g* for 30 min, the supernatant was filtered through a 0.20-µm nylon membrane filter (Vertical, Thailand) and 20 µl of the filtrate were injected into a High Performance Liquid Chromatography (HPLC) system (Shimadzu CL-10 ADVP, Shimadzu, Japan). Separation of biogenic amines was achieved using a C18 column (YMC-Pack ODS-AM, 5 µm, 4.6 mm ID × 250 mm; YMC, Japan). The mobile phase was a mixture of 0.1% acetic acid (A) and 0.1% acetic acid in acetonitrile (B) with a flow rate of 1 ml/min. The gradient system of the mobile phase started at 50% A and 50% B (0 min) and then solvent B was raised to 90% within 25 min; after that, the gradient was switched to 50% A and 50% B within 10 min and held for 5 min. The column temperature was set at 40°C and the UV detection wavelength was fixed at 254 nm by photodiode array detector (SPD-M20A; Shimadzu). Peak areas were determined and converted to the contents of biogenic amine in *Plaa-Som* samples.

5. Microbiological analysis

The assessments of total lactic acid bacteria, *Salmonella* sp., *Clostridium perfringens*, *Escherichia coli*, yeasts and molds in uncooked *Plaa-Som* were investigated according to the Bacteriological Analytical Manual (BAM) as described by the US Food and Drug Administration (2001).

6. Sensory evaluation

Sensorial attributes of uncooked and fried *Plaa-Som* were evaluated for appearance, texture, color, odor/sourness and overall acceptance. The samples were tasted by 50 volunteers who gave the scores according to 9-point hedonic scale, of which 9 = most favorable, 5 = neither like nor dislike and 1 = least favorable. All samples were identified using 3-digit random numbers and placed on plastic dishes before serving. The texture attributes of uncooked *Plaa-Som* were tested by press the forefinger on the sample's top. For fried *Plaa-Som*, the participants were instructed to rinse their mouths with warmed water every time before tasting each sample.

7. Experimental designs and data analysis

Completely randomized design (CRD) was used for assessments the samples in this study and randomized complete block design (RCBD) was used for sensory evaluation. Analysis of variance (ANOVA) was carried out using a SPSS software version 17 (SPSS Inc., USA). Differences among the treatment means were compared by Duncan's multiple range tests ($P < 0.05$).

Results and Discussion

Physical qualities of *Plaa-Som* samples

In this study, six *Plaa-Som* samples were bought from the markets in different Phranakhon Si Ayutthaya areas: Uthai, Sena, Phachee, Bangpahan, Bangpa-in and Phranakhon Si Ayutthaya districts. There was a study that suggesting the color of *Plaa-Som* as one of the important sensory qualities which could affect the consumer decision on purchasing the fish product (Zhou *et al*., 2014).

Table 1 Color parameters and firmness values of *Plaa-Som* samples as commercialized in Phranakhon Si Ayutthaya province.

Sample codes	Color parameters			Firmness
	*L**	*a**	*b**	(g)
UT	65.33 ± 0.39^{b}	1.38 ± 0.06^{b}	8.33 ± 0.51^{b}	16.63 ± 1.04^{a}
SN	68.49 ± 1.05^{a}	1.12 ± 0.09^{a}	8.74 ± 0.27^{a}	15.42 ± 0.96^{ab}
PC	60.81 ± 0.64^{b}	2.03 ± 0.13^{b}	8.61 ± 0.44^{b}	16.03 ± 1.13^{a}
BPH	64.18 ± 0.60^{a}	1.60 ± 0.07^{a}	9.06 ± 0.41^{a}	14.19 ± 0.57^{b}
BPI	67.67 ± 0.72^{b}	1.93 ± 0.09^{b}	8.75 ± 0.56^{b}	15.08 ± 0.85^{ab}
PSA	65.05 ± 0.75^{a}	1.15 ± 0.11^{a}	9.18 ± 0.28^{a}	12.64 ± 0.80^{c}

Data were presented as mean ± S.D. of six replicates.

$^{a-b}$ Data followed by the different letters in the same column were significantly different ($P < 0.05$).

^{ns} means were not significantly different at $P > 0.05$.

Changes of color parameters during fermentation, distribution and storage have been considered as defects of *Plaa-Som* products. The color parameters *viz*. *L** (brightness), *a** (reddish) and *b** (yellowish) of purchased *Plaa-Som* samples are illustrated in Table 1. *Plaa-Som* samples were found to have different levels of *L**, *a** and *b**, ranging from 60.81 to 68.49, 1.12 to 2.03 and 8.33 to 9.18, respectively, all of which indicated that *Plaa-Som* samples were in bright, white color with little tinges of redness and yellowness. Accordingly, the report of Saithong *et al.* (2010) elucidated that increases of lightness and yellowness were appeared to be the most informative parameters for color changes in *Plaa-Som* products, whereas the redness was found to decline during the fermentation process. This report also showed that the color intensities of fermented whole fish depended on fish species, salt contents, rice cultivars and other ingredients, as well as production steps/techniques. In addition, pH, low-temperature storage and packaging also influence the concentration and chemical properties of pigments and subsequently *Plaa-Som* color parameters. Moreover, proteolysis by microbial enzymatic reaction is one of the significant physicochemical changes (i.e. color, texture, flavor, etc.) during the ripening of fermented meats (Zeng

et al., 2013). Generally, LAB and molds in *Plaa-Som* have been found to be able to produce the metabolites such as organic acids, which including acetic and citric acids (Riebroy *et al*., 2004). These acids could induce the degradation of heme pigments (myoglobin and hemoglobin) which are responsible for the color changes of fermented meats (Schwartz *et al*., 2008). The decrease of redness and increase of brightness in meats during the fermentation can be associated with microbial conversion of myoglobin. Indeed, various species of LAB have been found to be able to reduce ferrimyoglobin [Mb(Fe^{3+})] to ferrous myoglobin [Mb(Fe^{2+})] and change the muscle color from brown to bright red or pink color (Zhang *et al*., 2007). Arihara *et al*. (1993) found that *Kurthia* sp. and *L. fermentum* JCM1173 were capable of converting Mb(Fe^{3+}) to NO-Mb(Fe^{2+}) which was the cured meat pigment.

Besides color parameters, *Plaa-Som* samples were evaluated for their firmness, which ranging between 12.64 and 16.63 g. PSA was found to have firmness significantly lesser than the other samples ($P < 0.05$). This suggested that PSA was softer than the others, perhaps due to improper storage or longer durations of fermentation and being put on sale. Firmness is also the key sensory characteristic for *Plaa-Som*. This parameter was shown to have positive relationship with fish's collagen contents (myofibrillar proteins), which being influenced by collagen cross-linking (Listrat *et al*., 2016). In addition, Sato *et al.* (1986) reported that some factors, such as species, size and age had positive correlations with muscular collagen contents, tenderness and elasticity of the fish flesh. Fish with firm flesh was found to exhibit strongly dense network of collagen fibers in the endomysium, whereas this network was much looser in the less firm flesh (Ando *et al*., 1992). Sato *et al*. (1986) and Khairina *et al*. (2016) suggested that fish collagen did not maintain its structural properties during cooking and fermentation. In this study, an increase of acidity (Table 2) in *Plaa-Som* provided the coagulation of myofibrillar proteins, which may result in the decrease of firmness of the final product. Differences in firmness among the samples might be due to the different network structures induced by organic acids. In addition, different bonding and extension of aggregation was also postulated, which leading to the varying textural properties (Riebroy *et al*., 2005). Zayas (1997) revealed that textural properties of meat products were affected by fat, salt (sodium chloride) and pH.

Chemical qualities of *Plaa-Som* samples

Accordingly, all six *Plaa-Som* samples had similarity in the levels of pH and total lactic acid ($P > 0.05$), as illustrated in Table 2. In general, the pH of *Plaa-Som* mixture was around 6.30-6.31. The reduction of pH values in the fermented samples can be caused by formations of nitrogen-based compounds and organic acids during the fermentation process and putting on sale (Khairina *et al*., 2016). The differences of ingredients, such as the ratio of salt and rice used for the producing *Plaa-Som*, could also affect this parameter. On the contrary, some fermented fish products were found to have significant increase of pH values during fermentation (Babu *et al*., 2005; Taorem & Sarojnalini, 2012). This may be due to proteolytic activity of the microorganisms that increase the amount of non-protein nitrogen compounds (Khairina *et al*., 2016). Similar to our study, Riebroy *et al.* (2004) found that lactic acid

was the major organic acid in *Som-Fug* (a Thai fermented fish fillet), and its level at approximately ~2.5% was the most acceptable with highest sensory score for flavor and taste attributes. Visessanguan *et al*. (2004) reported that lactic and acetic acids played an important role in imparting the tangy acidic character and may enhance saltiness, and they were also responsible for the flavor and consumer acceptability of *Nham* (a Thai fermented pork). Furthermore, Dave & Ghaly (2011) found that lactic acid could suppress the growth of some pathogenic microorganisms, such as *Clostridium botulinum*, because of its abilities to reduce pH level, exert feedback inhibition and interfere with proton transfer across cell membranes. However, lactic acid contents of some samples did not correlate well with the decrease of pH, probably due to the differences in buffering capacity of muscle proteins and/or different indigenous microflora in the ingredients (Riebroy *et al*., 2004).

Table 2 Acidity of *Plaa-Som* samples as commercialized in Phranakhon Si Ayutthaya province.

Sample codes	pH^{ns}	Total lactic acid^{ns} (%)
UT	4.53 ± 0.05	2.41 ± 0.03
SN	4.49 ± 0.03	2.38 ± 0.05
PC	4.50 ± 0.04	2.40 ± 0.04
BPH	4.47 ± 0.04	2.40 ± 0.02
BPI	4.50 ± 0.02	2.42 ± 0.06
PSA	4.48 ± 0.03	2.37 ± 0.04

Data were presented as mean ± S.D. of six replicates. ^{ns} means were not significantly different at $P > 0.05$.

Table 3 Concentrations of predominant biogenic amines in *Plaa-Som* samples as commercialized in Phranakhon Si Ayutthaya province.

Sample codes	Levels of biogenic amines (mg/kg)				
	Tryptamine	Putrescine	Cadaverine	Histamine	Tyramine
UT	49.67 ± 2.12^{d}	90.18 ± 3.17^{a}	74.01 ± 5.33^{c}	263.92 ± 1.69^{b}	62.25 ± 5.73^{d}
SN	55.40 ± 1.63^{b}	73.42 ± 4.01^{c}	70.63 ± 3.14^{cd}	218.42 ± 3.05^{e}	76.06 ± 2.48^{c}
PC	61.14 ± 3.95^{a}	69.54 ± 2.53^{cd}	81.78 ± 2.00^{b}	246.03 ± 4.18^{c}	80.87 ± 3.90^{bc}
BPH	48.02 ± 1.50^{d}	83.16 ± 1.67^{b}	90.44 ± 0.94^{a}	224.16 ± 5.02^{de}	97.04 ± 4.47^{a}
BPI	52.96 ± 2.81^{c}	80.42 ± 1.80^{b}	69.33 ± 3.58^{d}	270.05 ± 4.63^{a}	85.63 ± 4.05^{b}
PSA	42.15 ± 2.57^{e}	65.92 ± 2.11^{d}	75.92 ± 2.40^{c}	228.34 ± 7.31^{d}	52.69 ± 3.77^{e}

Data were presented as mean ± S.D. of six replicates.

$^{a-e}$ Data followed by the different letters in the same column were significantly different ($P < 0.05$).

Biogenic amine contents in *Plaa-Som* samples

There were different levels of five biogenic amines found in *Plaa-Som* samples (Table 3) , including tryptamine (42.15-61.14 mg/kg), putrescine (65.92-90.18 mg/kg), cadaverine (69.33-90.44 mg/kg), histamine (118.42-163.92 mg/kg) and tyramine (52.69-97.04 mg/kg). The study of Riebroy *et al*. (2004) indicated that biogenic amines, such as histamine, putrescine and cadaverine, found in fermented fish could be used as important indicators of bacterial contamination. This could reflect the proper and hygienic production according to the standard of *Good Manufacturing Practice* (GMP). In this study, the levels of histamine were found to be very high in all *Plaa-Som* samples. Janeway *et al*. (1999) revealed that histamine has been found to cause allergic reactions, headache, nausea, stomach cramp and diarrhea. There were attempts to use modified LAB as an alternative starter culture for reducing the accumulation of biogenic amines in fermented fish products. Zhang *et al.* (2013) and Nie *et al*. (2014) exhibited the great reduction of putrescine and cadaverine in silver carp sausage fermentation by adding amine-negative mixed starter culture (*L. plantarum* ZY40 and *Saccharomyces cerevisiae* JM19). However, amine-negative mixed starter culture could not decrease the levels of spermidine and histamine, the most toxic amine, and their levels merely marginally varied during the fermentation. Also, Halasz *et al.* (1994) reported that enterobacteriacece, *Pseudomonas* sp., micrococcaceae, enterococci and LAB were the microbial groups that could produce biogenic amines in fermented meat/fish products. Therefore, this suggested that higher or lower contents of such biogenic amines in the samples could primarily due to the contamination levels of spoilage microorganisms and/or LAB.

Table 4 Quantities of lactic acid bacteria and indicator microorganisms in *Plaa-Som* samples as commercialized in Phranakhon Si Ayutthaya province.

Sample codes	Number of microorganisms (log CFU/g)				
	Lactic acid bacteria	*Salmonella* sp.^{ns}	*C. perfringens*^{ns}	*E. coli*^{ns}	Yeasts and molds^{ns}
UT	8.96 ± 0.43^{ab}	< 1	< 1	< 1	< 1
SN	9.16 ± 0.37^{a}	< 1	< 1	< 1	< 1
PC	8.60 ± 0.44^{b}	< 1	< 1	< 1	< 1
BPH	9.78 ± 0.67^{a}	< 1	< 1	< 1	< 1
BPI	9.23 ± 0.25^{a}	< 1	< 1	< 1	< 1
PSA	8.75 ± 0.39^{ab}	< 1	< 1	< 1	< 1

Data were presented as mean ± S.D. of six replicates.

$^{a-b}$ Data followed by the different letters in the same column were significantly different ($P < 0.05$).

^{ns} means were not significantly different at $P > 0.05$.

Microbiological qualities of *Plaa-Som* samples

Table 4 shows the microbiological qualities of *Plaa-Som* samples. None of the indicating bacteria, including *Salmonella* sp., *C. perfringens* and *E. coli*, and also yeasts and molds, were found in 1 g of each *Plaa-Som* sample. The guidelines of Thai Community Product Standard for *Plaa-Som* (TCPS. 26/2014) suggested that there should be no *Salmonella* sp. per 25 g of sample, less than 3 log CFU of *C. perfringens* per 1 g sample, less than 3 MPN of *E. coli* per 1 g sample, and less than 3 log CFU of yeasts and molds per 1 g sample (Thai Industrial Standard Institute, 2014). Moreover, there were 8.60 to 9.78 log CFU of lactic acid bacteria counts per 1 g of *Plaa-Som* samples. These bacteria were the major microbes that affecting the quality, odor, flavor and safety of *Plaa-Som* and other Thai fermented meat products (Riebroy *et al*., 2004, Visessanguan *et al*., 2004). In general, different levels of salt applied in *Plaa-Som* products could also influence their qualities and microbial populations. For instance, Paludan-Müller *et al*. (2002) prepared low- and high-salt batches of *Plaa-Som*, containing 6-7% and 9-11% salt, respectively, and found that low-salt batches had rapid decrease of pH from 6 to 4.5, but high-salt batches had no changes of pH, which was most likely due to inhibition of LAB growth by high levels of salt. Indeed, all batches were shown to have the number of LAB counts at approximately 8-9 log CFU/g and yeast counts at 7 log CFU/g, with the exception of 11% salt batch, of which counts were 1-2 log lower (Paludan-Müller *et al*., 2002). Furthermore, addition of garlic as carbohydrate source was shown to be beneficial for fermentation of fish products. For example, when *Som-Fug*, which is a fish product that contains ingredients similar to *Plaa-Som*, was added with both garlic-fermenting *L. plantarum* and *P. pentosaceus* and starch-fermenting *L. lactis* ssp. *lactis* and *L. paracasei* ssp. *paracasei*, its fermentation was successful in 2 days with the production of 2.5% acid and the decrease of pH to 4.5 (Paludan-Müller *et al*., 1999). This may suggest the usefulness of LAB optimization for fermenting the fish products. Nevertheless, selection of the appropriate LAB strains based only on their abilities to inhibit pathogenic bacteria may not be useful. Hwanhlem *et al.* (2011) showed that *Streptococcus salivarius* and *Enterococcus faecalis* were LAB isolated from *Plaa-Som* and could effectively inhibit the foodborne pathogens (*Salmonella* sp., *Staphylococcus aureus* and *E. coli*) due to their acidifying activity; nonetheless, *Plaa-Som* products fermented by these LAB were not different from that produced by non-starter cultured, back-slopping and starter cultured processes in regards of color, smell, taste, sour, texture and overall acceptance of *Plaa-Som* (Hwanhlem *et al*., 2011). In addition, the use of mixed starter culture for fermentation of *Plaa-Som* has been accomplished. Saithong *et al*. (2010) prepared *Plaa-Som* by co-culturing between *L. plantarum* IFRPD P15 and *Lactobacillus reuteri* IFRPD P17, whereby *L. plantarum* IFRPD P15 was the major lactic acid producer and *L. reuteri* IFRPD P17 could effectively inhibit the pathogenic *E. coli* within 24 h. However, when either *L. plantarum* IFRPD P15 or *L. reuteri* IFRPD P17 was used individually, incomplete inhibition of *E. coli* was observed.

Sensory scores for uncooked and fried *Plaa-Som* samples

The uncooked *Plaa-Som* samples were evaluated for sensory attributes (Table 5). The results suggested that acceptability scores of appearance, color and odor were ranging from 7.85 to 8.23, 7.65 to 7.93 and 7.49 to 8.04, respectively, all of which were not significantly different ($P > 0.05$). Furthermore, for *Plaa-Som* sample purchased from PSA market, the scores of texture and overall acceptability were found to be significantly lower than the rest of samples ($P < 0.05$). In addition, during the evaluation for sensory attributes, the volunteers were suggested to touched *Plaa-Som* samples for testing the samples' firmness and they ranged the level of samples' firmness in consistent with the results in Table 1.

Table 5 Sensory attributes of uncooked *Plaa-Som* samples as commercialized in Phranakhon Si Ayutthaya province.

Sample codes	Average liking scores				
	Appearance^{ns}	Texture	Color^{ns}	Odor^{ns}	Overall acceptance
UT	8.03 ± 0.75	7.82 ± 0.65^{a}	7.90 ± 0.55	7.80 ± 0.60	7.67 ± 0.45^{ab}
SN	7.91 ± 1.03	7.75 ± 0.58^{a}	7.82 ± 0.76	8.04 ± 0.57	7.93 ± 0.26^{a}
PC	7.85 ± 0.87	7.80 ± 0.45^{a}	7.74 ± 0.50	7.96 ± 0.40	7.98 ± 0.30^{a}
BPH	8.23 ± 1.24	7.63 ± 0.50^{a}	7.93 ± 0.92	7.95 ± 0.55	7.80 ± 0.64^{a}
BPI	8.17 ± 0.74	7.85 ± 0.37^{a}	7.65 ± 0.61	7.87 ± 0.57	7.56 ± 0.50^{ab}
PSA	8.00 ± 0.95	6.80 ± 0.69^{b}	7.65 ± 0.53	7.49 ± 0.68	7.29 ± 0.61^{b}

$^{a-b}$ Data followed by the different letters in the same column were significantly different ($P < 0.05$).

^{ns} means were not significantly different at $P > 0$.

Table 6 Sensory attributes of fried *Plaa-Som* samples as commercialized in Phranakhon Si Ayutthaya province.

Sample codes	Average liking scores				
	Appearance^{ns}	Texture	Color^{ns}	Sourness^{ns}	Overall acceptance
UT	7.63 ± 0.65	7.89 ± 0.38^{a}	7.61 ± 0.78	7.45 ± 0.41	7.59 ± 0.61^{ab}
SN	7.51 ± 0.54	8.15 ± 0.44^{a}	7.63 ± 0.62	7.39 ± 0.50	7.63 ± 0.52^{a}
PC	7.45 ± 0.63	8.20 ± 0.40^{a}	7.59 ± 0.57	7.40 ± 0.47	7.76 ± 0.40^{a}
BPH	7.56 ± 0.51	7.98 ± 0.56^{a}	7.50 ± 0.50	7.46 ± 0.45	7.60 ± 0.49^{a}
BPI	7.52 ± 0.57	8.12 ± 0.41^{a}	7.67 ± 0.64	7.45 ± 0.58	7.59 ± 0.43^{ab}
PSA	7.50 ± 0.60	7.03 ± 0.43^{b}	7.63 ± 0.44	7.54 ± 0.64	7.40 ± 0.83^{b}

$^{a-b}$ Data followed by the different letters in the same column were significantly different ($P < 0.05$).

^{ns} means were not significantly different at $P > 0$.

For fried *Plaa-Som* samples as shown in Table 6, the acceptability scores of appearance, flavor and sourness were not significantly different ($P > 0.05$). Fried PSA sample was found to have significantly low scores for texture and overall acceptability ($P < 0.05$), which were in agreement with the results of its uncooked sample. Differences in sensorial characteristics among fermented samples could be influenced by the differences in ingredients, fermentation method and storage conditions (Riebroy *et al*., 2004). Chemical compositions and acidity also contributed to the consumer acceptability (Khairina *et al*., 2016). It is well known that elastic texture is a good indicator of *Plaa-Som* quality. Hence, *Plaa-Som* from difference areas were shown to receive different acceptability levels, which assuredly were associated with textural properties of the products. In this case, color parameters, chemical and microbiological qualities had no effect on consumer acceptance.

Conclusions

The results from this study showed that six samples of *Plaa-Som* had different values of color parameters (L^*, a^* and b^*) and firmness. However, they shared similarity in the levels of pH and total lactic acid. Histamine was the major biogenic amines found in *Plaa-Som* samples, followed by tryptamine, putrescine, cadaverine and tyramine. In summary, the differences in raw materials, recipes and processes of production could result in *Plaa-Som* products with different in qualities and characteristics. Consumer acceptability of *Plaa-Som* related with only firmness values, while other qualities had no effect on this term.

Acknowledgements

This research was financially supported by the Office of the Higher Education Commission.

References

Ando, M., Toyohara, H. & Sakaguchi, M. (1992). Three-dimensional structure of collagen fibrillar network of pericellular connective tissue in association with firmness of fish muscle. *Nippon Suisan Gakkaishi*, *58*, 1361-1364.

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

Arihara, K., Kushida, H., Kondo, Y., Itoh, M., Luchansky, J.B. & Cassens, R.G. (1993). Conversion of metmyoglobin to bright red myolobin derivatives by *Chromobacterium violaceum*, *Kurthia* sp., and *Lactobacillus fermentum* JCM1173. *Journal of Food Science*, *58*, 38-42.

Babu, U.S.J., Rao, B.M., Khasim, D.I. & Nair, K.G.R. (2005). Biochemical and microbiological quality of formic acid. *Fishery Technology*, *42*, 163-170.

Dave, D. & Ghaly, A.E. (2011). Meat spoilage mechanisms and preservation techniques: A critical review. *American Journal of Agricultural and Biological Sciences*, *6*, 486-510.

Halasz, A., Barath, A., Simon-Sarkadi, L. & Holzapfel, W. (1994). Biogenic amines and their production by microorganisms in food. *Trends in Food Science and Technology*, *5*, 42-50

Hwanhlem, N., Buradaleng, S., Wattanachant, S., Benjakul, S., Tani, A. & Maneerat, S. (2011). Isolation and screening of lactic acid bacteria from Thai traditional fermented fish (*Plasom*) and production of *Plasom* from selected strains. *Food Control*, *22*, 401-407.

Janeway, C.A, Travers, P., Walport, M. & Capra, J.D. (1999). *Immunobiology: The immune system in health and disease* (4th ed.). London: Current Biology Publication.

Khairina, R., Fitrial, Y., Satrio, H. & Rahmi, N. (2016). Physical, chemical, and microbiological properties of "*Ronto*" a traditional fermented shrimp from South Borneo, Indonesia. *Aquatic Procedia*, *7*, 214-220.

Kopermsub, P. & Yunchalard, S. (2010). Identification of lactic acid bacteria associated with the production of *plaa-som*, a traditional fermented fish product of Thailand. *International Journal of Food Microbiology*, *138*, 200-204.

Listrat, A., Lebret, B., Louveau, I., Astruc, T., Bonnet, M., Lefaucheur, L., Picard, B. & Bugeon, J. (2016). How muscle structure and composition influence meat and flesh quality. *The Scientific World Journal*, http://dx.doi.org/10.1155/2016/3182746.

Marui, J., Boulom, S., Panthavee, W., Momma, M., Kusumoto, K., Nakahara, K. & Saito, M. 2014. Culture-independent analysis of the bacterial community during fermentation of *pa-som*, a traditional fermented fish product in Laos. *Fisheries Science*, *80*, 1109-1115.

Nie, X., Zhang, Q. & Lin, S. (2014). Biogenic amine accumulation in silver carp sausage inoculated with *Lactobacillus plantarum* plus *Saccharomyces cerevisiae*. *Food Chemistry*, *153*, 432-436.

Paludan-Müller, C., Huss, H.H. & Gram, L. (1999). Characterization of lactic acid bacteria isolated from a Thai low-salt fermented fish product and the role of garlic as substrate for fermentation. *International Journal of Food Microbiology*, *46*, 219-229.

Paludan-Müller, C., Madsen, M., Sophanodora, P., Gram, L. & Møller, P.L. (2002). Fermentation and microflora of *plaa-som*, a Thai fermented fish product prepared with different salt concentrations. *International Journal of Food Microbiology*, *73*, 61-70.

Pringsulaka, O., Thongngam, N., Suwannasai, N., Atthakor, W., Pothivejkul, K. & Rangsiruji, A. (2012). Partial characterisation of bacteriocins produced by lactic acid bacteria isolated from Thai fermented meat and fish products. *Food Control*, *23*, 547-551.

Riebroy, S., Benjakul, S., Visessanguan, W. & Tanaka, M. (2005). Physical properties and microstructure of commercial *Som-fug*, a fermented fish sausage. *Europian Food Research and Technology*, *220*, 520-525.

Riebroy, S., Benjakul, S., Visessanguan, W., Kijrongrojana, K. & Tanaka, M. (2004). Some characteristics of commercial *Som-fug* produced in Thailand. *Food Chemistry*, *88*, 527-535.

Roseiro, L., Santos, C., Sol, M., Borges, M., Anjos, M., Gonalves, H. & Carvalho, A.S. (2008). Proteolysis in *Painho de Portalegre* dry fermented sausage in relation to ripening time and salt content. *Meat Science*, *79*, 784-794.

Saithong, P., Panthavee, W., Boonyaratanakornkit, M. & Sikkhamondhol, C. (2010). Use of a starter culture of lactic acid bacteria in *plaa-som*, a Thai fermented fish. *Journal of Bioscience and Bioengineering*, *110*, 553-557.

Sato, K., Yoshinaka, R., Sato, M. & Shimizu, Y. (1986). Collagen content in the muscle of fishes in association with their swimming movement and meat texture. *Bulletin of the Japanese Society for the Science of Fish*, *52*, 1595-1600.

Schwartz, S.J., von Elbe, J.H. & Giusti, M.M. (2008). *Colorants*, in Damodaran, S., Parkin, K.L., & Fennema, O.R. (Eds.), Fennema's Food Chemistry (4th ed.). Boca Raton, FL: CRC Press.

Taorem, S. & Sarojnalini, C.H. (2012). Effect of temperature on biochemical and microbiological quality of *Ngari*. *Nature and Science*, *10*, 32-40.

Thai Industrial Standard Institute. (2014). Thai Community Product Standard (TCPS) No. 27/2014: *Plaa-Som*. Bangkok, Thailand: Ministry of Industry.

U.S. Food and Drug Administration. (2001). *Bacteriological analytical manual (BAM)*. New Hampshire Avenue, Washington, DC: Department of Health and Human Services.

Visessanguan, W., Benjakul, S., Riebroy, S. & Thepkasikul, P. (2004). Changes in composition and functional properties of proteins and their contributions to *Nham* characteristics. *Meat Science*, *64*, 579-588.

Zayas, J.F (Ed.). (1997). *Water holding capacity of proteins*, in Functionalily of proteins in food (pp 76-133). Germany: Springer-Verlag, Heidelberg.

Zeng, X., Xia, W., Jiang, Q. & Yang, F. (2013). Chemical and microbial properties of Chinese traditional low-salt fermented whole fish product *Suanyu*. *Food Control*, *30*, 590-595.

Zhang, Q., Lin, S. & Nie, X. (2013). Reduction of biogenic amine accumulation in silver carp sausage by an amine-negative *Lactobacillus plantarum*. *Food Control*, *32*, 496-500.

Zhang, X., Kong, B. & Xiong, Y.L. (2007). Production of cured meat color in nitrite-free Harbin red sausage by *Lactobacillus fermentum* fermentation. *Meat Science*, *77*, 593-598.

Zhou, C., Tan, S., Li, J., Chu, X. & Cai, K. (2014). A novel method to stabilize meat colour: Ligand coordinating with hemin. *Journal of Food Science and Technology*, *51*, 1213-1217.