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Contributed Paper

Protein, Calcium and Phosphorus Composition of Fermented Fish in the Lower Mekong Basin

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ABSTRACT

Fermented fish is a well known strategy of food preservations in the Lower Mekong Basin, especially, in Thailand (Pla-ra), Lao PDR (Pa-dag) and Cambodia (Pra-hoc). There are different ways of making fermented fish in different communities, but the preserved fishes obtained have similar properties i.e., high quality nutritional dietaries of protein, calcium and phosphorus. People in each community in the basin are familiar to the taste of various fermented fish which are descended from generations to generations. This study conveys results from laboratory analysis on nutritional values of traditional fermented fish in the three countries. The findings revealed that, though, the content of protein in the fermented fish decreased from in fresh fish, the content of calcium and phosphorus was higher than the ones in fresh fish substantially. Therefore, fermented fish can be another calcium source for people in the region in a substitute for milk consuming promotion which is not the way of life of people in the region.

Keywords: fermented fish, protein, calcium, phosphorus, Lower Mekong Basin

1. INTRODUCTION

Lower Mekong Basin covered the area of Lao PDR, Thailand, Cambodia, Vietnam and it is figurative blood vessel that feeds lives of people in all communities along its watershed and its branches with varieties of freshwater fish in different size and species [1], particularly in the area with no opening to the sea such as in northeast Thailand, in Jampasak province in Lao PDR and in Siem Reap province in Cambodia. Because these areas located adjacent to the largest freshwater in the region, freshwater fish is a source of significant nutrients for everyone in the watershed in the plateau [2]. According to the terrain of the area and the monsoon climate, the amount of fish caught throughout the year was not stable for consuming, so wisdom of fresh fish preservation for protein source had

arisen when the amount of fresh fish was in a high production in a season.

Protein that plays a vital role in producing new cells for body growth is one of six food nutrients (protein, carbohydrate, fat, vitamins, minerals and water). It directly functioned as a substance for repairing wounded parts becoming likewise the original stage. World Health Organization (WHO) recommended a minimum daily intake of 56 g per a day for a 75 kg man and 48 g for a 64 kg woman [3]. Calcium and phosphorus are very important minerals in food for strengthening bones and teeth [4-7]. WHO and FAO (Food and Agriculture Organization) recommended a minimum daily intake of 400-500 mg a day for adult [8].

While the westerners perceived protein, calcium, and phosphorus from milk, easterners in the Lower Mekong Basin contained those essential nutrients primarily from fish, Jensen [9] stated that traditional fish product is like milk for Southeast Asian people. Moreover, in many communities of the area, algae from the Mekong River is also the source of those essentials [10]. One of the traditional fish products which is very well known among people in South East Asia is fermented fish. Fermented fish has been found in all regions of the world, but methods or processes of making it and its local names differ from culture to culture, like Pla-ra in Thailand, Pa-dag in Lao PDR and Pra-hoc in Cambodia [11].

The fermented fish production started from fish mixed with salt or roasted rice or roasted rice bran and then put into a jar with a complete lid. The degrading organic matter by lactic acid bacteria was found and final fermented fish in different colors, odor and flavor were obtained. A unique taste of fermented fish yielded different opinions in each culture, even though this wisdom remained continuously for a long time in the region with evidence of fermented fish production found in the image on the east wall of Bayon sanctuary at Angkor Thom, Siem Reap province in Cambodia [12]. It manipulated the value of fermented fish in each culture.

Fascinated by flavor of fermented fish in their own culture, individual had to produce it by him/herself which caused an inherited wisdom of ingredients, compositions and processes of fermented fish production in his/her own wisdom. It was an interest to study the quality and the level of safety in consuming fermented fish in each community. Moreover, an analyses of protein, calcium and phosphorus content as well as the study of Salmonella sp. and Staphylococcus aureus contamination was administered in the laboratory. An evaluation of acceptance from consumers in terms of colors, odor, flavor, textures and appearance was launched with the likelihood to the fermented fish production in favorite methods of selected community in Thailand, Lao PDR and Cambodia.

2. MATERIAL AND METHODS

2.1 Fermented Fish Production Method

The study was conducted in three countries in the selected communities in which fermented fish was familiar in their daily life.

2.1.1 Thai fermented fish (Pla-ra) production method

The process of the production was from Harnhee village, Don Rat sub district, Ratanaburi district, Surin province, Thailand. Source of fish was from the Moon River.

Jullien's mud carp (*Henicorhynchus siamensis*) were scaled, excrement and the insides of the fish were removed and then washed them thoroughly in clean water. Fish were dry outdoor, then mixed them with rock salt in the ratio of 4:1.5 (fish : salt) and left them in a

basket for one night. Next, salted fish mixed with one part of roasted rice powder and put them into the earthen jar and covered by two-layer of plastic sheet and left for 6 months under the shade.

2.1.2 Lao fermented fish (Pa-dag) production method

The production method was from Taluang village, Muang Pone-thong district, Jampasak province, Lao PDR. Source of fish was from the Kong River.

Jullien's mud carp or Pla soi were scaled; excrement and intestine of the fish were removed and then washed them thoroughly in clean water. Fish were stirred with salt and roasted rice bran. Lao's fermented fish formula was in a ratio of 1:2:3 (rock salt : roasted rice bran : fish). The mixture was put into the earthen jar and covered by plastic sheet or a piece of cloth and left for 6 months under the shade.

2.1.3 Khmer fermented fish (Pra-hoc) production method

The production method of Khmer's fermented fish was from Po village, Kor Krai sub district, Siem Reap district, Siem Reap province, Cambodia. Source of fish was from Tonle Sap.

Gourami *(Trichogaster microlepis)* or "Treykumpley" or "Pla kradee", were scaled; excrement and intestine and also head and bones of the fish were removed, washed them thoroughly in clean water. Fish flesh, then, was soak in water and left over night. Fish flesh was dried under the sun for 6 hours and left in the basket for one night. Next mixed the fish with solar salt in a ratio of 3:1 (fish : salt) and then pounded them lightly in a mortar, after that the mixture was put into the earthen jar and placed them under the sun.

The productions of fermented fish using the above three methods were carried out in three replicates to obtain nine earthen jars. The jars were stored at room temperature. The production process was summarized in Table 1.

2.2 Protein, Calcium and Phosphorus Analysis

The fresh and fermented fishes were analyzed for nitrogen content by kjeldahl nitrogen method [13], calcium content by atomic absorption spectroscopy [14] and phosphorus content by spectrophotometric molybdophosphate method [15].

2.2.1 Protein analysis

The process of protein analysis composed of mixing the sample solid at about 0.7-2.2 g with 7 g of HgO, 15 g of K_2SO_4 and 25 ml of H_2SO_4. The sample was heated for about 30 minutes or more until the solution was clear. The solution was added with 200 ml of distilled water and left to be cool. Then it was mixed with an aliquote of 25 ml of sulfide and left for sedimentation (Hg precipitation) before tilting. The solution again was added with 10 g of NaOH before distillation and after ammonia was evaporated, the titration (using standard NaOH) was processed.

2.2.2 Calcium analysis

A portion of sample (1 g) was processed by dry ashing at 500°C for 2 h and the resulted ash was ground and mixed with nitric acid (3-4 ml). The solution was evaporated before dry ashing again. Ash was resuspended in nitric acid, heated with fume $HClO_4$, and left before added 10 ml HCl (1:1). This sample was processed by wet ashing and 10 ml of HNO_3 was added, add 3 ml of 60% $HClO_4$, heated to evaporate nitric acid and add 10 ml nitric acid before heating (white fume $HClO_4$) and HCl (1:1).

2.2.3 Phosphorus analysis

Sample 10% w/v was mixed with 20 ml molybdovanadate reagent containing 40 g ammonium molybdate.4H_2O, 2 g ammonium metavanadate water and 70% $HClO_4$ before incubated for 10 min and absorbance (400 nm) determining. Percent of P_2O_5 was estimated by 100×(mgP_2O_5 from standard curve/mg of test portion in aliquot).

2.3 Microbiological Analysis

The fermented fish samples were determined for *Salmonella* sp. and *Staphylococcus aureus* by AOAC[14].

2.3.1 *Salmonella* sp. analysis

Place weighted 25 g of sample in sterile lactose broth (225 ml), blended in high speed for 2 min and transferred the homogenate into sterile jar (500 ml), left for 60 min at room temperature, adjusted pH to 6.8±0.2 and incubated (24±2h at 35°C). Sample was transferred 0.1 ml to rappaport-vassiliadis medium before incubation (24±2h at 42± 0.2°C) and transferred 1 ml of sample to 10 ml of tetrathionate broth (10 ml) before incubation (24±2h at 43±0.2°C). Rappaport-vassiliadis was subculture to bismuth sulfite (BS) agar, hektoen enteric (HE) Agar and xylose lysine deoxycholate (XLD) agar before incubation (24±2h at 35°C) repeating with tetrathionate broth incubation. BS plates were examined after 48 h of incubation. *Salmonella* colonies on XLD was pink with/without black centers, may be grossy black, yellow colonies with/without black centers. Colonies on HE have blue-green with or without black centers, large grossy black. Colonies on BS was brown, gray or black sometime with metallic sheen, surrounding medium was brown and turning black when incubation increasing. Some strains were green colonies with little or no dark surrounding medium. Colonies were treated in triple sugar iron (TSI) and lysine iron agar (LIA). *Salmonella* have alkaline (red) and acid (yellow) butt with/ without H_2S (blackening agar) in TSI. In LIA culture have alkaline (purple) in butt considering distinct yellow coloration in butt as an acidic (negative) reaction.

2.3.2 *Staphylococcus aureus* analysis

Place weighted 50 g of sample and mixed with 450 ml of phosphate buffer, homogenized for 2 min at high speed and determined in MPN technique. One milliliter of diluted sample (10^{-2}-10^{-6}) was inoculated in triplicates TSB (10% NaCl + 1% sodium pyruvate) for 48 h at 35°C. One loopful of growth positive sample was transferred to dried Baird Parker medium and mixed. Visible growth on the bottom was streaked to isolate and incubated (48 h at 35-37°C). *S. aureus* colonies were circular, smooth, convex, moist, 2-3 nm in diameter on uncrowned plated, gray-black to jet black, frequently light colored margin, surrounded by opaqued zone and frequently outer clear zone, buttery to gummy colonies when touch with inoculating needle. Occasional nonlipolytics have similarly except surrounding opaque and clear zone were absent. The sample was confirmed by one suspecting *S. aureus* was transferred to BHI broth and TSA incubating (18-24 hr at 35°C). Reconstituted coagulated plasma with EDTA 0.5 ml was mixed thoroughly before incubation (6 h at 35-37°C) for clot formation.

2.4 Sensory Evaluation

The organoleptic properties of fermented fish were studied using 10 untrained panelists from Thai, Lao and Khmer students in Surindra Rajabhat University. Hedonic scale ranged from 1 to 9 was used to indicated the degree of acceptability of each sample (9 = like extremely, 5 = neither like nor dislike, 1 = dislike extremely) [16]. The evaluation

criterian were mainly focused on color, odor, flavor, texture and appearance.

2.5 Data Analysis

The data were analyzed by analysis of variance (ANOVA). Compare of mean was carried out by Duncan' s multiple range test. Statistical analysis was performed using the SPSS statistic program (version 11.0) for Window.

Table 1. Comparative of fermented fish production in Thailand, Lao PDR and Cambodia.

Processing products	Processing details		
	Pla-ra, Thailand	**Pa-dag, Lao PDR**	**Pra-hoc, Cambodia**
1. Fish selection	small-scaled Jullien's mud carp	small-scaledJullien's mud carp	small-scaled Gourami
2. Raw fish preparation	scaled and excrement and intestine removed	scaled and excrement and intestine removed heads and bones removed	scaled and excrement and intestine including
3. Quantification	weight	weight	weight
4. Cleaning	washing	washing in water over night	washing and soaked
5. Dewatering techniques	-	-	drying for 6 hr under sunny condition and left in a basket for over night
6. Preserved ingredient	salt mixing (ratio between fish:salt = 4:1.5)	salt mixing (ratio between fish:salt = 3:1)	salt mixing (ratio between fish:salt = 3:1)
7. Dewatering techniques	over night air drying in basket	-	-
8. Flavored ingredient	roasted rice mixing (ratio between fish: roasted rice = 4:1)	roasted rice bran mixing(ratio between fish: roasted rice bran = 3:2)	-
9. Fermented device utilization	put in to earthen jar	put in to earthen jar	put in to earthen jar
10. Fermenting techniques	covered with 2 layer of plastic sheets	covered with 2 layer of plastic sheets or cloth	covered with 2 layer of plastic sheets
11. Fermenting period	6 months	6 months	6 months
12. Mature stage	Pla-ra	Pa-dag	Pra-hoc
13. Storaging environment	placed in shading	place in shading	placed in sunny

3. RESULTS AND DISCUSSION

3.1 Protein, Calcium and Phosphorus Composition

Fresh and fermented fishes chemical analysis was administered for content of protein, calcium and phosphorus. The results were as follows:

Information in Table 2 indicated that fermented fish in each country obtained less protein than in the fresh fish [17]. It was the result of hydrolyses process, the protein in fresh fish has been changed into amino acid and the acid was used by bacteria in fermented process of growth.

Table 2. Protein content in fresh and fermented fishes.

Samples	Protein content (%)		
	Pla-ra	Pa-dag	Pra-hoc
-Fresh fish	18.35±0.04[a]	18.15±0.05[a]	18.02±0.03[a]
-Fermented fish	12.76±0.03[b]	12.50±0.06[b]	13.73±0.04[a]

Means followed by different letters in the same row are significantly different ($P < 0.05$).

Table 3. Calcium content in fresh and fermented fishes.

Samples	Calcium content (mg/100g)		
	Pla-ra	Pa-dag	Pra-hoc
-Fresh fish	133.89±0.08[a]	127.58±0.04[a]	111.92±0.07[b]
-Fermented fish	837.61±0.07[a]	823.45±0.10[a]	60.94±0.09[b]

Means followed by different letters in the same row were significantly different ($P < 0.05$).

Table 4. Phosphorus content in fresh and fermented fishes.

Samples	Phosphorus content (mg/100g)		
	Pla-ra	Pa-dag	Pra-hoc
-Fresh fish	56.30±0.08[a]	55.12±0.10[a]	50.54±0.12[b]
-Fermented fish	603.90±0.46[a]	598.44±0.87[a]	23.48±0.67[b]

Means followed by different letters in the same row were significantly different ($P < 0.05$).

Information in table 3 and 4 showed in different content of calcium and phosphorus in fresh fish of Thai and Lao, however, it differed from Khmer fresh fish. It might be the result of using different kinds of fish [18]. The fermented fish from three countries contained different chemical content. Thai and Lao fermented fish had higher content of calcium and phosphorus than fresh fish. Content of calcium and phosphorus in fermented fish was greater than in fresh fish because of decomposition process of fish bone and another structures by bacteria lactic acid in the fermented process [19]. On the contrary, there was less calcium in Khmer's fermented fish which was the result of heads and bones of fish were taken off before putting into the production process.

3.2 Microbiological Quality

There was no *Salmonella* sp. and *Staphylococcuss aureus* in Thai, Lao and Khmer

fermented fish of Pla-ra, Pa-dag and Pra-hoc in this study. It was in accordance with the results of the study by Ostergaad et al. [20] who argued that the decrease of pathogens in fermented fish product was the result of the by products of lactic and acetic acid and the decrease of pH in fermented fish.

3.3 General Characteristic of Fermented Fish

From the study, it was found that the color of Thai fermented fish turned into light color, reddish brown in the flesh part of the fish, and was wet and the fish odor was fascinated by roasted rice as shown in Figure 1A. For Lao fermented fish, it was found that the color of Lao fermented fish was in light red brown with moisture and the odor was good by roasted rice bran as shown in Figure 1B. For Khmer fermented fish, it was found that Khmer fermented fish was in light brown with chunk of fish flesh, boneless, and the odor of the fish was quite strong as shown in Figure 1C.

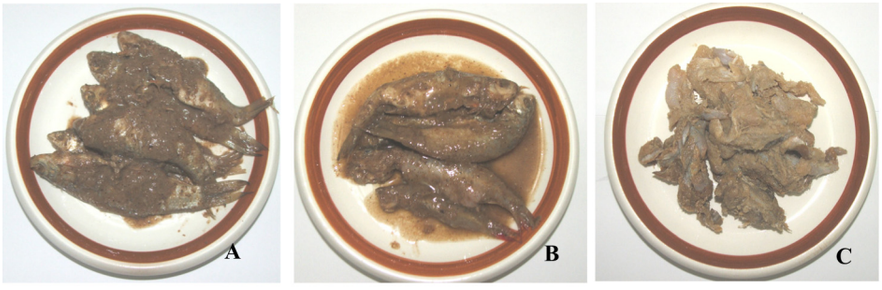

Figure 1. Picture of Pla-ra (A) Pa-dag (B) and Pra-hoc (C).

Table 5. Average sensory scores of fermented fish at 6 months of fermentation.

Sensory properties	Sensory score		
	Pla-ra	Pa-dag	Pra-hoc
color	7.56±0.05[a]	7.60±0.03[a]	7.36±0.03[b]
odor	8.33±0.04[a]	8.16±0.05[a]	7.67±0.08[b]
flavor	8.16±0.02[a]	8.23±0.04[a]	7.73±0.06[b]
texture	8.53±0.04[a]	8.50±0.03[a]	7.83±0.03[b]
appearance	8.13±0.02[a]	8.46±0.02[b]	7.73±0.05[c]

Means followed by different letters in the same row are significantly different ($P < 0.05$).

3.4 Sensory Evaluation

From the analysis of consumers' sensory perceptions in five aspects, the first aspect was about color of Thai, Lao and Khmer fermented fish. It was found that there were differences in color of Thai, Lao and Khmer fermented fish. The most satisfaction was Lao's with the score at 7.60, Thai's was at 7.56 and Khmer's was at 7.36. The second aspect was about odor of the fermented fish. It was found that Thai's was in the highest rank with 8.33 scores, while Lao's was at 8.16 and Khmer's was at 7.67. The third aspect was about flavor. It was found that the highest score was Lao's with 8.23 scores, while Thai's and Khmer's were lower with 8.16 and 7.73 scores. The fourth one was texture, Thai's was in the highest score with 8.53 and the lower

were Lao's and Khmer's with 8.50 and 7.83 scores. The last aspect was appearance of fermented fish, it was found that Lao's was in the highest score at 8.46 and the lower were Thai's and Khmer's with 8.13 and 7.73 scores, as shown in Table 5.

Thai and Lao fermented fish contained fascinated odor due to roasted rice and roasted rice bran mixed with the fish. Roasted rice and rice bran were the source of carbon for bacteria, in addition, it caused brown color from the non-enzyme maillard reaction which was the reaction of amine and amino acid with reduced sugar group [21]. The result was identity in specific odor and flavor of the fermented fish [22].

In Khmer's production process, only salt was added and the fish was soak in water overnight. The protein degradability of fish flesh was in putrefaction reaction in which fish flesh was turned into amino acid, amine, ammonia and hydrogen sulphide. These elements made Khmer's fermented fish in a strong odor and it was in accordance to the research result of Rao [23].

In case of sensory perception evaluation on colors, odor, flavors, texture and appearance, it was found that Khmer fermented fish was the least acceptable and it was statistically significant difference from Thai's and Lao's. It was an important concern that favorite tastes and odors of fermented fish of each consumer in each surrounding and area were different. A quotation from Lao people was that "Pa-dag sap(delicious) tee sood (the most)" while "Pra-hoc ja-ngai (delicious)" for Khmer people.

4. CONCLUSIONS

In conclusion, fermented fish was a kind of high nutrients of protein, calcium and phosphorus and it was safe to consume. It was free from hazardous microorganism. Fermented fish was a favorite disk for every meal and all year round of people in the Lower Mekong Basin. It was an ingredient in both main course and snack. It was Pla-ra was in Thai's way of life, Pa-dag was in Loa's and Pra-hoc was in Khmer's and it was also a food stability of this region. While a health organization in each Mekong sub-region country tried to promote dairy diets to its people as a source of nutrients as it was done in Western countries, why not fermented fish was seriously promoted as a main staple diet for health in the area. Fermented fish was familiar to each community in the region and it was easy to obtain all year round so it is interesting to find out that how we can make it possible for everyone in the region to consume fermented fish as they do to the diary diet in daily life. Fermented fish was accepted as a main staple diet for older generation and how we can make it available for next generation also.

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