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# Chemical composition and antioxidant activity of Klongkone shrimp paste

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# Abstract

Klongkone shrimp paste is a very well known Thai fermented shrimp paste product from Samut Songkram province, Thailand. It is highly accepted by consumers due to its distinct aroma and taste. Chemical composition and antioxidant activity of fourteen samples of shrimp paste from local Klongkone producers were analyzed. It was revealed that the samples contained 37.36-46.85 % moisture, 20.95-30.86% ash, 18.95-25.14 % protein, 0.69-2.05 % lipid, 4.27-17.96 % carbohydrate and 19.78-22.96 % salt. Water activity of the samples were in the range of 0.70-0.74. The antioxidant activity against 2, 2 diphenyl-1-picrylhydrazyl (DPPH) of the samples were ranging from 4.12 to 14.5 TE/g protein. The results indicated that Thai fermented shrimp paste products from Klongkone were good sources of nutrients as well as natural antioxidants. Moreover, the products are safe from food pathogens and can be kept at ambient temperatures.

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Keywords: chemical composition; antioxidant activity; Klongkone; shrimp paste; Thailand

# 1. Introduction

Fermentation is a traditional common method used for fish preservation due to its easy techniques and low cost equipments. During fermentation, hydrolysis of food proteins by either microbial enzymes or endogenous enzymes yield simpler compounds such as peptides, amino acids, and other nitrogenous substances. These peptides and

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amino acids are important contributors to the typical flavor and aroma of fermented products, and they also exhibit antioxidant activities (Rajapakse, Mendis, Jung, Je, & Kim, 2005; Benjakul, Binsan, Visessaguan, Osako, & Tanaka, 2009). Thai fermented shrimp paste or Kapi is widely used as a condiment or an essential ingredient in many Southeast Asian dishes due to its flavor and nutritional properties. Shrimp paste products are commonly made by fishing families in coastal village of Thailand. Shrimp paste product from Klongkone village Samut Songkram province is very well known and highly accepted by consumers due to its excellent sensory properties such as flavor, aroma and texture. It is generally prepared by mixing the small shrimp (Acetes vulgaris) with salt at a ratio of 10:1(w/w). The mixture is sundried for 3 days to less the moisture content and then thoroughly ground before being compacted in a container. The paste is allowed to ferment under ambient conditions until the desired aroma had developed. Klongkone shrimp paste is commercially fermented at 1-3 months before being sold to consumers. Recently, it had been reported that antioxidant activity and amino nitrogen content of Klongkone shrimp paste could be increased through prolonged fermentation for 9 months. However, fermentation time over 5 months brought not only a slight decrease in amino nitrogen but also an increase in undesirable level of ammonia (Prapasuwannakul, Suwannahong, & Saksri, 2014). Faithong, Benjakul, Phatcharat, & Binsan (2010) had previously revealed that shrimp paste products from southern provinces of Thailand had high protein content and exhibited strong antioxidant activities. However, the variation of raw materials, amount of salt, processing conditions and fermentation time could affect the quality of the products. Therefore, the objective of this study was to determine the chemical composition and antioxidant activity of fermented shrimp pastes from Klongkone village, Thailand. The information obtained could be used to market the product as health foods with high market price.

#### 2. Materials and Methods

#### 2.1. Samples

Fourteen samples of fermented shrimp paste produced from planktonous shrimp (*Acetes vulgaris*) were purchased from difference local producers in Klongkone district, Samut Songkram province, Thailand. For each sample, three different lots were used. For each lot, three samples were purchased and pooled as the composite sample. All samples were packed in plastic bag and stored at -20 °C prior to analyses.

#### 2.2. Proximate analysis, pH and water activity determination of shrimp paste

Moisture, protein, ash, fat and salt contents of shrimp paste were determined according to AOAC (1999) with the analytical number of 35.1.13, 35.1.14, 35.1.15, 35.1.25 and 35.1.18 respectively. Sample pH was determined using a pH meter (Metrohm, Switzerland) following the procedure described by Benjakul, Seymour, Morrissey, & An (1997). Sample water activity (a<sub>w</sub>) was measured using a water activity meter (Aqua Lab, WA, USA.). All reagents used to perform analyses were supplied by Sigma (Sigma-Aldrich, USA).

# 2.3. Determination of antioxidant activity of shrimp paste

#### 2.3.1. Preparation of soluble fraction

A preparation of soluble fraction was performed following the same procedure described by Faithong and Benjakul (2012). A one gram of each sample was mixed with 100 ml of distilled water and the mixture was homogenized at a speed of 10,000 rpm for 2 min. using a homogenizer. The homogenate was stirred at room temperature for 30 min. The mixture was then centrifuge at 3000g for 10 min at room temperature using a Tomy LC 230 bench top centrifuge (Tomy, Japan) to remove undissolved debris. The supernatant was used for determination of antioxidant activity.

#### 2.3.2. DPPH radical scavenging activity

DPPH radical scavenging activity was determined following the same DPPH assay described by Faithong and Benjakul (2012). Soluble fraction (1.5 ml) was added to 1.5 ml of 0.15 mM 2, 2 diphenyl-1-picrylhydrazyl (DPPH) in absolute ethanol. The mixture was mixed vigorously and allowed to stand at room temperature in the dark for 30 min. The absorbance of the solution was measured at 517 nm using a V630 UV-Vis spectrophotometer (Jasco, Japan). The blank was prepared in the same procedure, except the distilled water was used instead of the sample. A standard curve was prepared using 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) in the range of 10-60  $\mu$ M. The scavenging activity was expressed as  $\mu$ mol Trolox equivalents (TE)/g sample.

# 3. Results and Discussion

#### 3.1. Chemical composition of shrimp paste products

Chemical composition, pH and water activity  $(a_w)$  of shrimp paste samples are presented in Table 1.

Table 1 Chemical composition, pH and water activity (aw) of shrimp paste products.

Sample	Moisture (%)	Protein (%)	Ash (%)	Fat (%)	Carbohydrate (%)	Salt (%)	рН	a <sub>w</sub>
1	38.47±0.25	23.22±0.25 <sup>b</sup>	23.78±0.38 <sup>d</sup>	1.21±0.32 <sup>b</sup>	13.32±0.42 <sup>d</sup>	21.79±0.40 <sup>b</sup>	7.62±0.01 <sup>b</sup>	0.71±0.008
	g	(37.74±0.48)	(38.65±74)	(1.97±0.58)	(21.65±0.36)	(35.41±0.75)		
2	39.37±0.41 <sup>f</sup>	25.14±0.84 <sup>a</sup>	22.53±0.29 <sup>e</sup>	1.99±0.41ª	10.97±0.16 <sup>f</sup>	22.96±0.42ª	7.57±0.02°	$0.70\pm0.008$
		(44.45±1.38)	(37.16±0.47)	(3.28±0.68)	(18.09±0.29)	(37.87±0.64)		
3	44.23±0.26°	21.67±0.15 <sup>d</sup>	20.95±0.45 <sup>f</sup>	$1.05\pm0.12^{b}$	12.1±0.21e	21.21±0.38 <sup>b</sup>	$7.31\pm0.01^{f}$	$0.72 \pm 0.004$
		(38.86±0.29)	(37.56±0.79)	(1.88±0.36)	(21.70±0.35)	(34.98±0.77)		
4	44.74±0.34 <sup>b</sup>	$21.22\pm0.15^{d}$	23.29±0.31 <sup>d</sup>	$1.24 \pm 0.06^{b}$	9.5±0.36 <sup>g</sup>	21.79±0.26 <sup>b</sup>	$7.42 \pm 0.02^{d}$	$0.72 \pm 0.006$
		(38.40±0.18)	(42.15±0.60)	(2.24±0.14)	(17.21±0.36)	(39.43±0.57)		
5	46.68±0.30 <sup>a</sup>	21.5±0.34 <sup>d</sup>	22.18±0.15 <sup>e</sup>	$1.06 \pm 0.08^{b}$	$8.57 \pm 0.48^{h}$	22.38±0.85ª	7.31±0.03 <sup>e</sup>	$0.73 \pm 0.004$
		(40.34±0.52)	(41.60±0.34)	(1.99±0.19)	(16.07±0.86)	(41.97±1.25)		
6	37.62±0.15 <sup>h</sup>	$21.08 \pm 0.08^{d}$	22.33±0.28 <sup>e</sup>	$1.01 \pm 0.04^{b}$	17.96±0.16 <sup>a</sup>	$21.79\pm0.40^{6}$	7.28±0.02 <sup>e</sup>	0.71±0.009
		(33.79±0.16)	(35.80±0.67)	(1.62±0.12)	(28.79±0.34)	(34.55±0.94)		
7	38.62±0.15 <sup>g</sup>	21.74.±0.15 <sup>d</sup>	22.57±0.33e	$0.98 \pm 0.05^{b}$	16.09±0.23 <sup>b</sup>	21.21±0.22 <sup>a</sup>	7.45±0.01 <sup>d</sup>	0.71±0.006
		(35.42±0.29)	(36.77±0.58)	(1.60±0.12)	(26.21±0.55)	(35.05±0.88)		
8	37.95±0.32 <sup>h</sup>	23.23±0.25 <sup>b</sup>	24.50±0.15°	$1.29\pm0.24^{b}$	$13.03 \pm 0.40^{d}$	21.75±0.48 <sup>b</sup>	$7.22 \pm 0.01^{f}$	0.71±0.003
		(37.44±0.41)	(39.48±0.34)	(2.08±0.39)	(21.00±0.38)	(39.12±0.76)		
9	43.05±0.27 <sup>d</sup>	23.18±0.09 <sup>b</sup>	25.21±0.27 <sup>b</sup>	$1.34\pm0.12^{b}$	$7.22\pm0.30^{i}$	22.11±0.54 <sup>a</sup>	7.14±0.01 <sup>g</sup>	$0.72 \pm 0.006$
		(40.70±0.21)	(44.2±0.50)	(2.35±0.27)	(12.68±0.56)	(39.01±0.84)		
10	42.28±0.28 <sup>e</sup>	25.13±0.34ª	25.86±0.32 <sup>b</sup>	$2.05\pm0.06^{a}$	$4.68 \pm 0.34^{j}$	22.58±0.62ª	$7.20\pm0.01^{f}$	0.72±0.005
		(43.54±0.61)	(44.80±0.54)	(3.55±0.11)	(8.11±0.77)	(40.67±0.46)		
11	44.81±0.34 <sup>b</sup>	$18.95 \pm 0.18^{f}$	25.52±0.11 <sup>b</sup>	0.69±0.04°	10.03±0.28 <sup>g</sup>	21.53±0.37 <sup>b</sup>	7.71±0.01 <sup>a</sup>	0.73±0.008
		(34.34±0.19	(46.24±0.25)	(1.25±0.09)	(18.17±0.44)	(34.42±0.63)		
12	44.05±0.52°	$21.74 \pm 0.84^{d}$	30.86±0.16 <sup>a</sup>	$1.08\pm0.13^{b}$	4.27±0.36 <sup>j</sup>	22.45±0.24ª	$7.24\pm0.01^{f}$	$0.72 \pm 0.004$
		(38.86±1.05)	(51.58±0.38)	(1.93±0.27)	(7.63±1.63)	(34.98±0.77)		
13	37.36±0.12 <sup>h</sup>	22.36±0.21 <sup>c</sup>	25.08±0.22 <sup>b</sup>	$1.17 \pm 0.11^{6}$	14.03±0.16 <sup>c</sup>	21.56±0.26 <sup>b</sup>	$7.45 \pm 0.02^{d}$	0.71±0.003
		(35.70±0.26)	(40.04±0.72	(1.87±0.33)	(22.40±0.26)	(34.98±0.77)		
14	46.85±0.30 <sup>a</sup>	21.83±0.45 <sup>d</sup>	22.56±0.34°	$1.04 \pm 0.08^{b}$	$7.72\pm0.10^{i}$	19.78±0.48 <sup>c</sup>	$7.01 \pm 0.01^{h}$	$0.74 \pm 0.002$
		(41.07±0.75	(42.45±0.82)	$(1.96 \pm 0.18)$	$(14.52 \pm 0.44)$	(37.22±0.85)		

Values in brackets indicate the content based on dry basis.

Mean values  $\pm$  standard deviation from triplicate determinations.

Different superscripts in the same column indicate the significant difference (p<0.05).

Moisture, protein, ash, fat, carbohydrate and salt content of the samples were in the range of 37.36-46.85%, 18.95-25.24%, 20.95-28.86%, 0.69-2.05%, 4.27-17.96% and 19.78-22.96% respectively. The samples contained high salt content which might result from the high amount of salt applied during processing and drying during fermentation. The amount of ash content was in accordance with high salt content. The chemical composition of samples were similar to those reported by Faithong et al. (2010), however, it was noticeable that the salt content of

Klongkone shrimp paste were lower than those of shrimp paste from southern provinces. The samples had a pH range of 7.01-7.71. The slightly basic pH of some samples might be affected by volatile basic compounds such as ammonia and other degradation products liberated during fermentation.

Water activity  $(a_w)$  of the shrimp pastes were found between 0.70 and 0.74. The low  $a_w$  of the samples were mainly attributed to the removal of water and the high salt addition during the processing. An  $a_w$  of 0.70-0.75 can limit the growth of food pathogens and preserve the food from microbial spoilage at ambient temperature (Chirife, 1989).

Comparison of total nitrogen content, pH, moisture content and salt content of the samples with those of the Thai Industrial standard of shrimp paste (Office of Thai Industrial Standard, 1992) are shown in figure 1 and figure 2 respectively. Two samples out of fourteen (14%) had total nitrogen content less than the acceptable level (fig.1a). This might due to the addition of flour or other ingredients during processing to increase the yield and reduce the cost. All samples had pH in the range of the standard (fig 1b). Two samples out of fourteen (14%) had high moisture contents greater than the standard allowed. This result indicated that the fermentation time of the sample might be too short since shrimp paste would loss moisture during fermentation (fig 2a). From fig 2b, it could be clearly seen that almost a half of the samples contained less salt than the acceptable level. This result might be due to the intention of the producers to use less salt during processing in order to obtain their desired product flavor. However, a<sub>w</sub> of these non complied samples were still low enough to prevent spoilage and food pathogens.

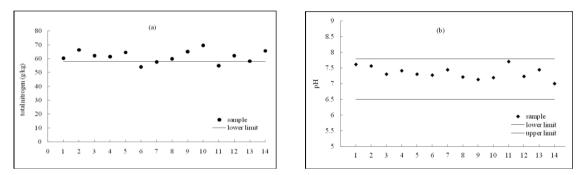


Fig. 1. Total nitrogen content (a) and pH (b) of shrimp paste samples compared with standard limit

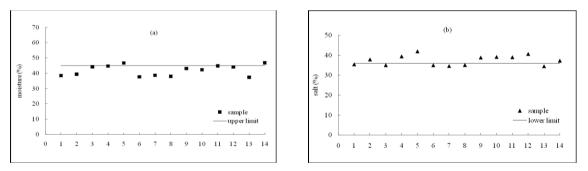


Fig. 2. Moisture content (a) and salt content (b) of shrimp paste samples compared with standard limit

# 3.2. Antioxidant activities of shrimp paste

The antioxidant activities of shrimp pastes are shown in Table 2. DPPH, a stable free radical, can be used to measure the antioxidant activity of the substance because when DPPH encounters with an antioxidant it would be scavenged and the absorbance at 517 nm is reduced. The samples had DPPH radical- scavenging activity in the range of 4.24-14.50 µmol Trolox equivalents/g protein. The result was similar to previous studies reporting that

peptides, amino acids and maillard reaction products in the fermented products possessed the antioxidant activity (Parelta, Hatate, Kawabe, Kuwahara, Wakawatsu, Yuki, & Murata, 2008; Prapasuwannakul, Suwannahong, & Saksri, 2014).

sample	µmol Trolox equivalents/g protein
1	5.75±0.39 <sup>f</sup>
2	5.93±0.51 <sup>f</sup>
3	11.70±0.46 <sup>bc</sup>
4	$10.50{\pm}0.28^{d}$
5	14.31±0.45 <sup>a</sup>
6	$4.24{\pm}0.29^{g}$
7	5.86±0.32 <sup>f</sup>
8	4.34±0.36 <sup>g</sup>
9	11.03±0.57°
10	$10.12 \pm 0.52^{d}$
11	12.1±0.44 <sup>b</sup>
12	9.6±0.38 <sup>e</sup>
13	4.12±0.29 <sup>g</sup>
14	$14.5 \pm 0.54^{a}$

Table 2 Antioxidant activities of soluble fractions from shrimp paste determined by DPPH assay

Mean values  $\pm$  standard deviation from triplicate determinations.

Different superscripts in the same column indicate the significant difference (p<0.05).

#### 4. Conclusion

Fermented shrimp paste products from Klongkone, Thailand are good for health since they are enriched with nutrients as well as natural antioxidants. Their shelf stability at ambient temperatures is attributed mainly to their low water activity.

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