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Chemical and physical properties of Thai traditional shrimp paste (*Ka-pi*)

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ABSTRACT

Thai traditional fermented shrimp paste (*Ka-pi*) is widely consumed as condiment and seasoning ingredient in Thailand. Chemical and physical properties of thirteen shrimp paste samples were examined. The water content and water activity (A_w) of the products ranged from 33.95-52.19 % and 0.64-0.72, respectively while salt concentration was 7.00 to 10.85 %. The nitrogen content varied from 2.87 to 6.85 % (w/w). KS1 showed the highest protein content (42.8 %) followed by SP1 and SP3 (42.4 and 41.3 %), respectively. The predominant amino acids were glutamic acid (70.1-593.9 µg/g), lysine (112.7-546.3 µg/g) and leucine (29.5-544.9 µg/g). Total bacterial cell count in the samples ranged from $1.3x10^3$ - $2.9x10^5$ cfu g^{-1} while lactic acid bacteria (LAB) were not detected. This study will be provided the information for improving the quality of products in manufacture to meet the demand of consumer.

INTRODUCTION

The traditional fermented shrimp paste (*Ka-pi*), a purple-pink to dark brown color, strong odor and paste-like consistency is widely consumed as seasoning ingredient in Thailand. In Southeast Asia, there are the distinguished names of this fermented product such as *bagoong* (Philippines), *shiokara* (Japan), *mam ruoc* (Vietnam), *terasi* (Indonesia), *ngapi* (Myanmar) and *belacan* (Malaysia) (Wittanalai *et al*., 2011; Chaijan and Panpipat, 2012). *Ka-pi* is made from small sized shrimps (*Acetes* spp.) (Hajeb and Jinap, 2012). It depends on the types of shrimps available in each country and then mixed with salt and sun dried for 2 days. These dried shrimps are then ground into a fine paste and fermented 2 days, and put into the jar before fermenting for at least 2 months (Wittanalai *et al*., 2011; Chuon *et al*., 2014). During fermentation, the protein hydrolysis of shrimp paste is mediated by the action of indigenous and microbial proteases yield short chain peptides and free amino acids which enhance the flavor and taste (Chaijan and Panpipat, 2012; Hajeb and Jinap, 2012). Therefore, the shrimp paste is a good source of peptide and amino acids. In Malaysia, *belacan* contains free glutamic acid more than 4,200 mg/100 g (Hajeb and Jinap, 2012). However, a little information regarding the chemical and physical properties of Thai shrimp paste (*Ka-pi*) has been reported. Thus, the objective of this study was to determine the chemical and physical properties including the microbiological property of shrimp paste collected from Nakhon Sri Thammarat province in Thailand.

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MATERIALS AND METHODS

Sample collection

Two and eleven shrimp paste (*Ka-pi*) samples were collected from Songkhla and Nakhon Sri Thammarat provinces in Thailand, respectively. These samples were directly obtained from the market and supermarket which packed in plastic bag at room temperature, and they were maintained at 4 °C until analysed.

Microbial and chemical analyses were immediately performed after the samples were brought to the laboratory (within several days). Fermentation period, sampling location and other information are provided in Table 1. Ten samples of *Kapi* (indicated as SP) were collected from the markets and three samples (indicated as KS) were collected from the supermarkets.

Proximate analyses

Shrimp paste samples (5 g) were homogenized thoroughly with 25 ml of distilled water (w/v) prior to determine salt concentration and pH. Salt concentration was measured by handy salt concentration meter (APAL-ES2, AS ONE) and pH was measured by pH meter (C-62, AS ONE). Water content was determined by using moisture analyzer (AND MX-50 using manual) while water activity (A_w) of each sample was measured at 25 °C using a water activity analyzer (Aw SPRINT TH-500, Novasina AG, Switzerland). Nitrogen content (N-content) analysis was performed by using an automated nitrogen and carbon analyzer (Sumigraph NC-220F, Sumika Chemical Analysis Service, Ltd.). Protein was calculated by 6.25 x N-content.

LC/MS measurement of free amino acids

In brief, free amino acids were extracted from shrimp paste. Samples were diluted 1:100 with pure water and centrifuged for 15 min at 15,000 rpm and 4 ºC. The supernatant was passed through a 0.20 µm filter (Kurabo, Osaka, Japan); the filtrate was injected directly into an Accurate-Mass Q-TOF LC/MS with an Agilent 6530 (Agilent Technologies, Santa Clara, CA, USA) coupled to an Agilent 1260 Infinity HPLC (Santa Clara, CA, USA). To identify the Amino Acid Standard (Agilent Technologies, Logistics Center - USA). The accurate mass data was detected at high resolution and high mass accuracy (± 2 ppm), which was essential for accurate chemical formula assignment.

Total ion spectra were collected over a mass range of m/z 60‑400 in positive mode at an acquisition rate of 5.0 spectra/s. The drying gas temperatures and flow rate were 300 ºC and 6.0 L/min, respectively. The sheath gas temperature and flow rate were 350 °C and 12.0 L/min, respectively. The nebulizer gas pressure, skimmer voltage, octopole RF, and fragmentor voltage were 50 psi, 60 V, 750 V, and 110 V, respectively. The capillary voltage was 3.5 kV. Continuous internal calibration was performed during analysis to achieve the desired mass accuracy of recorded ions with the ions of m/z of 112.9855. Chromatographic separation of the analytes was done using an Intrada Amino Acid (50 x 3 mm i.d., particle size 3.0 µm) (Imtakt Corpration, Kyoto) using 100 mM ammonium formate (A) and acetonitrile/formic acid 0.1% (v/v) (B) as eluent. A gradient was delivered at 600 µl/min starting from 86% phase B; it was maintained for 3 min at 86% phase B and then raised to 100% in 10 min; and then the column was equilibrated back to the initial condition in 4 min. Thus, the total analysis time was 14 min, including column washing and re-equilibration. All data, acquired in the positive ion mode, were collected and processed using MassHunter Workstation Software Quantitative Analysis ver. B.07.00 for TOF software (Agilent Technologies).

Measurement of color

Color of shrimp paste samples were measured by spectrophotometer CM-5 (Konika Minolta) and reported in the CIE system. L^* (lightness), a^* (redness/greenness), and b^* (yellowness/blueness).

Histamine analysis

Histamine concentration of shrimp paste samples was determined by colorimetric enzymatic assay (Kikoman Biochemifa Company, Japan, 2014). The colored tetrazolium salt was created in the presence of 1-methoxy-5-methylphenazinium methylsulfate (1-methoxy PMS) and measured at 470 nm.

Bacterial cell count

A 1 g of sample stored in a refrigerator was homogenized with 9 ml normal saline containing 8.5 g kg^{-1} NaCl. Ten-fold dilutions of homogenates (100 µl) were spread on standard agar medium (composed of 5.0 g peptone, 2.0 g yeast extract, 1.0 g glucose and 15.0 g agar in 1 L distilled water) for general bacteria and de Man, Rogosa, and Sharpe (MRS) agar medium for lactic acid bacteria (LAB), and incubated at 30 ºC for 2-7 days. Viable counts (colony-forming unit; cfu) were reported.

Table 1: Location and fermentation period of Thai traditional shrimp paste (*Ka-pi*).

Sample source	Sample no.	Sampling location	Fermentation period
Local market	SP1	Amphoe Meuang, Nakhon Sri Thammarat province	<2 months
	SP2	Amphoe Khanom, Nakhon Sri Thammarat province	<2 months
	SP3	Tambon Banglaung, Amphoe Meuang, Nakhon Sri Thammarat province	<1 month
	SP4	Amphoe Tha sala, Nakhon Sri Thammarat province	1 month
	SP5	Amphoe Satingpra, Songkhla province	<2 months
	SP6	Amphoe Pak Phanang, Nakhon Sri Thammarat province	<2 months
	SP7	Amphoe Hua Sai, Nakhon Sri Thammarat province	<3 months
	SP8	Amphoe Sichon, Nakhon Sri Thammarat province	3 months
	SP9	Tambon Pak Nakhon , Amphoe Meuang, Nakorn Sri Thammarat province	2 months
	SP10	Songkhla province	Several months
Supermarket	KS1	M-Peaw Brand, Kapi Chumchon Bangyai, Tambon Bangchak, Amphoe Meuang, Nakhon Sri Thammarat province	Several months
	KS2	Tonmai and Konmek shop, Amphoe Ronpiboon, Nakhon Sri Thammarat province	3 months
	KS3	Kapi Namprikkungtadum brand, Tambon Pakpoon, Amphoe Meuang, Nakhon Sri Thammarat province	Several months

Table 2: Proximate composition of Thai shrimp paste (*Ka-pi*).

Sample no.	Water* content (%)	Water activity* (A_w)	Salt Concentration* (%)	pH*	N-content* (%)	Protein* (%)
SP1	46.32	0.65±0.001	7.15	7.67	6.79	42.4
SP2	44.64	0.66±0.005	7.00	6.96	5.55	34.7
SP3	36.63	0.65±0.001	8.90	8.31	6.61	41.3
SP4	33.95	0.64±0.003	8.65	8.11	6.23	38.9
SP5	42.52	0.68±0.001	8.90	7.81	4.75	29.7
SP6	45.49	0.69±0.001	8.90	7.04	2.87	17.9
SP7	45.74	0.68±0.002	9.05	6.94	3.19	19.9
SP8	52.19	0.72±0.001	7.54	7.02	4.76	29.8
SP9	48.53	0.70±0.001	8.60	7.05	3.83	23.9
SP10	46.72	0.68±0.002	9.50	7.84	6.15	38.4
KS1	44.54	0.66±0.006	10.85	7.72	6.85	42.8
KS2	48.95	0.69±0.001	10.15	7.73	4.47	27.9
KS3	47.69	0.68±0.000	8.25	7.35	4.68	29.2

*Standard variation value < 0.01

RESULTS AND DISCUSSION

Samples collection

Ten and three samples of shrimp paste were collected from the local market and the supermarket in Thailand, respectively. Sample number, SP1, SP2, SP3, SP4, SP6, SP7, SP8, SP9, KS1, KS2 and KS3 were collected from Nakhon Sri Thammarat province while SP5 and SP10 were collected from Songkhla province (Table 1).

Proximate analyses

The water content and water activity (A_w) of the shrimp paste samples collected from the local market were 33.95-52.19 % and 0.64-0.72 while the samples from supermarket were 44.54-48.95 % and 0.66-0.69, respectively (Table 2). *Aw* of local products has wide range compared with those from supermarket. Local products were homemade or small lot, therefore some ingredients were different among the samples. A_w can be related to the consistency of shrimp paste which varied from soft and pasty to dry and hard. A_w of the final shrimp paste product depend on period which the sample was dried under the sun (Pongsetkul *et al*., 2014). The shrimp paste can be classified as an intermediate moisture food, with an A_w of about 0.7 (Fennema, 1996). The low A_w of shrimp paste products could be associated with increase the shelf-life and preserve the product from microbial spoilage at ambient temperature (Goulas and Kontominas, 2005; Prapasuwannakul and Suwannahong, 2015). In addition, the low A_w would prevent rancidity of the product and limited the growth of food pathogens (Hajeb and Jinap, 2012). Thirteen shrimp paste samples contained salt which is needed for preservation. Salt concentration ranged from 7.00 to 10.85 %. Moreover, the salt content would enhance both the shelf-life and the flavor of shrimp paste products. All shrimp paste samples had a neutral to slightly alkaline pH ranging from 7.02-8.31. The slightly alkaline might have been caused by the formation of volatile base compounds such as ammonia or other degradation products, the degradation products generated during fermentation. The shrimp paste collected from the local market contained 2.87-6.79 % of N-content and 23.9-42.4 % protein while the local market contained 4.47-6.85 % of N-content and 27.9-42.8 % protein.

The samples contained nitrogen content varied from 2.87 to 6.85%. Generally, the nitrogen content has been used to indicate the degree of protein hydrolysis. Thus, nitrogen content can be the indicator for the level of the cleavage of peptides (Angeles and Garcia-Carreno, 2002). The highest nitrogen contents were 6.85, 6.79 and 6.61 % in KS1, SP1 and SP3 samples, respectively. This was higher than the nitrogen contents found in fish sauce which ranged from 0.3 to 3.0 % (w/v) (Park *et al.*, 2001; Tungkawachara *et al.*, 2003; Xu *et al.*, 2008). In addition, the nitrogen content in soy sauce was 3 to 10 % (dried; w/w). Therefore, nitrogen content of the samples was in the range of liquid seasoning. The protein content ranged from 17.9-42.8 %. The sample KS1 showed the highest protein content (42.8 %) followed by 42.4 and 41.3 %, in SP1 and SP3, respectively. So, the protein content could be indicated the good source of proteins.

Free amino acid content

The amino acid contents of the shrimp paste collected from the local market and the supermarket were variable (Table 3). The predominant amino acids of thirteen shrimp paste products were glutamic acid (70.1-593.9 µg/g), lysine (112.7-546.3 µg/g) and leucine (29.5-544.9 µg/g) (Table 3), compared to the content of glutamic acid in shrimp paste and fish sauce which ranged from 3.1-7.0 % (w/w) and 0.5-1.5 % (w/v), respectively (Kim *et al*., 2014; Park *et al*., 2001) while to the Cambodian traditional fermented fish products which contained 4.9 g kg^{-1} glutamic acid as reported by Chuon *et al*. (2014). SP4 was showed highest glutamic acid followed by KS1 and SP2 that agreed with our study. The high amounts of glutamic acid were indicated the rich of umami taste.

In 1990, Kim and Rhee reported that arginine, aspartate, isoleucine, lysine, proline, serine, threonine and valine were related to taste and flavor. Some amino acids, alanine, glycine, serine, threonine and valine were related to sweet taste while arginine, histidine, isoleucine, leucine, methionine, phenylalanine and tryptophan are related to bitter taste. In addition, γ-aminobutyric acid (GABA) was detected in all samples (0.7-2.6 µg/g). The GABA containing in food has reported to play role as health benefits in many kinds of fermented products (Dhakal *et al*., 2012).

Table 3: Amino acid contents (µg/g) of Thai shrimp paste (*Ka-pi*).

Sample no.	Phe	Leu	Ile	Met	Pro	Val	Ala	Glu	Gly	Gln	Asp	His	Lys	Arg	GABA
SP1	112.1	296.1	197.9	97.3	99.0	161.4	173.9	83.2	137.6	16.2	212.4	27.5	311.9	41.5	1.9
SP2	118.7	252.3	158.0	66.1	74.6	142.9	147.2	524.8	98.4	14.6	129.2	15.8	196.6	30.8	1.9
SP 3	131.6	324.6	183.0	95.6	71.8	160.5	162.8	399.1	187.9	5.2	151.6	23.7	301.4	22.0	2.1
SP 4	164.7	454.8	264.5	124.2	93.8	212.3	229.2	593.9	220.3	9.4	221.7	32.1	458.0	30.5	2.6
SP 5	56.8	135.4	82.7	38.7	64.6	90.2	118.7	298.3	94.9	5.8	139.8	11.3	165.6	24.9	1.5
SP 6	79.8	166.4	84.4	44.7	48.5	93.4	101.5	70.1	80.6	14.4	96.5	12.1	143.1	50.2	0.7
SP 7	121.2	301.5	200.7	86.2	68.3	149.7	126.9	310.7	89.3	2.2	174.4	11.3	196.7	10.0	1.1
SP 8	179.9	383.6	237.2	113.6	33.8	204.8	174.5	427.5	133.0	11.3	209.8	27.8	315.0	28.9	2.0
SP 9	123.1	246.5	140.5	76.4	66.3	143.2	120.5	253.9	97.1	6.2	155.1	12.9	195.7	18.2	1.0
SP 10	255.8	544.9	364.3	162.9	135.4	269.7	245.0	260.8	206.6	46.0	281.7	37.6	546.3	224.4	2.6
KS1	153.0	277.9	139.1	77.2	107.3	205.5	182.3	591.3	136.1	18.3	274.7	41.3	376.7	56.1	2.6
KS2	114.6	297.1	168.1	70.4	70.2	128.3	140.5	450.9	109.0	29.0	169.5	31.5	229.6	138.2	1.4
KS3	13.4	29.5	18.5	8.2	28.3	29.5	90.1	151.9	93.4	7.4	124.2	11.1	112.7	33.8	1.3

Phe, Phenylalanine; Leu, Leucine; Ile, Isoleucine; Met, Methionine; Pro, Proline; Val, Valine; Ala, Alanine; Glu, Glutamic acid; Gly, Glycine; Gln, Glutamine ; Asp, Aspartic acid; His, Histidine; Lys, Lysine; Arg, Arginine; GABA, gamma-Aminobutyric acid

From our results, the high nitrogen content of the samples indicated the cleavage of peptides to high amount of free amino acids (Angeles and Garcia-Carreno, 2002). However, only samples SP1 and SP6 that showed low glutamic acid content compared to the other samples.

Measurement of color

The different shrimp paste products had different colors, L^*(lightness), a^*(redness) and b^*(yellowness) ranged from 29.6-39.48, 6.01-9.15 and 8.33-17.91, respectively. These samples were purple-brown to dark brown in color. The difference in color might be caused by the difference pigment contents in raw materials, process as well as ingredients added. The carbonyl groups of aldehydes and ketone, the oxidation products, could react with amino groups of free amino acids or peptides generated during hydrolysis, leading to yellow or brown color development (Yarnpakdee *et al*., 2014). Brown development of shrimp paste products may be occurred by enzymatic and non-enzymatic reactions such as active polyphenoloxidase or Maillard reaction. Moreover, the oxidation of free astaxanthin resulted in the pale discoloration of products (Chaijan and Pannipat, 2012).

Table 4: CIELAB colorimetric values of Thai shrimp paste (*Ka-pi*).

Sample no.	L^*	a^*	b^*
SP1	39.22	6.78	14.62
SP2	35.51	8.5	12.67
SP3	34.37	6.01	9.23
SP4	32.6	6.34	8.33
SP5	35.87	6.74	12.5
SP6	29.6	8.28	10.8
SP7	30.61	7.03	9.04
SP8	32.93	9.51	9.58
SP9	29.78	9.2	11.07
SP10	39.48	7.91	10.4
KS1	36.76	6.4	12.39
KS2	32.89	8.66	10.36
KS3	28.56	8.29	17.91

L^*, lightness; a^*, redness-greenness; b^*, yellowness-blueness Standard variation value < 0.01

Histamine analysis

The histamine concentration of the shrimp paste collected from the local market and the supermarket ranged from <20-46.70 and 25.11-53.85 ppm, respectively (Table 5). The histamine detected was below the limit of 200 ppm according to Codex Alimentarius (Vallejos *et al*., 2012) that would not exhibited the toxicity. Low histamine content here supported the procedure used for shrimp paste production as the shrimps were immediately mixed with salt at the time that the raw materials reached the shore because histamine associated with incorrect handling and the storage of raw materials (Pilapil *et al*., 2015). The level of histamine of all samples were differed which can be attributed to differences in quality of the raw materials, endogenous microflora, improper handling and environment during the production and the free amino acids (histidine) presented as a precursors (Singh *et al*., 2012).

Table 5: Histamine and bacterial numbers of Thai shrimp paste (*Ka-pi*).

Sample no.	Histamine conc. (ppm)	Bacterial cell number (cfu/g)
SP1	46.70	2.9×10^4
SP2	30.73	1.7×10^4
SP3	29.19	1.3×10^3
SP4	31.06	2.1×10^3
SP5	32.60	3.4×10^4
SP6	<20	8.2×10^4
SP7	26.65	4.3×10^4
SP8	<20	6.1×10^4
SP9	24.45	6.8×10^4
SP10	29.19	1.3×10^5
KS1	30.29	2.9×10^5
KS2	53.85	7.0×10^4
KS3	25.11	1.1×10^5

LAB were not detected.

Bacterial cell count

Total bacterial cell count of the samples varied from 1.3×10^3 to 2.9×10^5 cfu g^{-1} while the number of lactic acid bacteria (LAB) in thirteen shrimp paste products was not detected (Table 5). Based on A_w, bacteria distributed as the rest cell or spore. Therefore, these shrimp pastes are stable for long period though the maximum bacterial number was 10^5 order.

As mentioned above, the water content, water activity (A_w), N-content, protein, amino acids content and histamine concentration of the shrimp paste samples collected from the local market and the supermarket were variable based on the raw

materials that may due to the different sources upon factories. Based on our data, quality of shrimp paste may be controlled by salt concentration, water content and fermentation period. Therefore, the analysis of chemical properties of *Ka-pi* from the different factories and provinces used should be further more studied in the view point of application.

CONCLUSION

The differences of shrimp paste samples were variable in compositions. They contained major protein component that indicated the good sources of proteins and amino acids. In addition, the low A_w and low histamine concentration but the high salt content, therefore, these products would become stable for a long period. In this study, the insights on Thai shrimp paste products acquired will help to both establish the basic scientific information of these products and also the future of manufacturing.

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