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Chemical composition and antioxidative activity of Thai traditional fermented shrimp and krill products

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ABSTRACT

Chemical composition and antioxidative activities of some Thai traditional fermented shrimp and krill products including Jaloo, Koong-Som and Kapi were studied. All products did not contain myosin heavy chain or actin, but contained a large amount of small peptides. Kapi had the highest protein content, whereas carbohydrate content varied with products. Water-soluble fraction from all products possessed DPPH and ABTS radical-scavenging activity, as well as ferric reducing antioxidant power (FRAP) in a concentration-dependent manner. At the same concentration tested, the water-soluble fraction from Kapi exhibited the highest antioxidative activity. Soluble fraction of all products showed high stability over a wide pH range (2–11) and was stable after heating at 40–100 °C for 15–60 min. Fractions from all products heated at 100 °C had increases in FRAP, suggesting the enhancement of antioxidant activity. Therefore, fermented shrimp and krill products could be used as a potential source of nutrients and natural antioxidants.

1. Introduction

Oxidation of biomolecules, including lipid peroxidation, involves a series of free radical-mediated chain reactions and is associated with several types of biological damage (Haliwell, Aeschbach, Löliger, & Aruoma, 1995). Much attention has been focused on the use of antioxidants, especially natural antioxidants, to inhibit lipid peroxidation and to protect biomolecules from damage by free radicals (Haliwell et al., 1995). Proteins and peptides from food have been found to be physiologically active or bioactive. Many peptides that are released *in vitro* or *in vivo* from animal or plant proteins have regulatory functions in the human body, apart from serving as important nutrients. Food-derived peptides exhibit antimicrobial properties, blood-pressure lowering effects, cholesterol-lowering ability, antithrombotic and antioxidant activities (Hartmann & Meisel, 2007).

Fermentation, a common practice in food preservation, plays an important role in improvement of nutritional and functional properties of foods. Cleavage of food proteins by microbial or indigenous proteases yields the bioactive peptides, leading to substantial increases in the biological properties of the food (Steinkraus, 2002). Moreover, fermented food products are a good source of peptides and amino acids (Rajapakse, Mendis, Jung, Je, & Kim, 2005; Sathivel et al., 2003). Among indigenous fermented products, fermented fishery products have been widely consumed in Southeast Asia as main dishes or condiments, due to their delicacy and high nutritional properties. Enzymatic fermentation of small fish and shrimp mediated by indigenous proteases yields short chain peptides and free amino acids, rendering the typical flavour and taste. To prevent putrefaction and food poisoning as well as to yield meaty-savoury flavour, the addition of salt in the range of 2–13% or higher to protein-rich substrates is common practice (Steinkraus, 2002).

Kapi is a typical traditional fermented shrimp paste, generally prepared from the planktonous shrimp or krill (*Acetes vulgaris* or *Mesopodopsis orientalis*), mixed with salt at a ratio of 3–5:1 and sun-dried to decrease the moisture content, then blended thoroughly. The paste is compacted and allowed to ferment for at least 2 months until the desired aroma has developed (Phithakpol, 1993). Jaloo, an indigenous salted krill (*M. orientalis*), is usually produced in communities near the mangrove coastal shore in the south of Thailand. Jaloo is prepared in the same manner as Kapi except that the drying is not required. Fermentation of Jaloo under anaerobic conditions generally takes 2–3 days. Koong-Som is another shrimp product produced by mixing small shrimp (*Acetes* sp.) with salt and palm-sap-sugar concentrate as a source

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of carbohydrate. The mixtures are then left in anaerobic jars at room temperature to develop the fermentation by lactic acid bacteria until a sour taste and the typical flavour of fermented shrimp have developed (TCPS 1032, 2005).

Antioxidative activities associated with enzymatic hydrolysis or fermentation of marine animals have been reported. Fermentation of blue mussel could produce an antioxidative peptide with MW of 962 kDa, exhibiting strong scavenging effects towards superoxide, hydroxyl and DPPH radicals (Rajapakse et al., 2005). Tuna backbone proteins hydrolysed by pepsin contained antioxidant peptide (1519 Da), which had a capability of quenching free radicals, including superoxide, hydroxyl and DPPH radicals (Je, Qian, Byun, & Kim, 2007). Round scad meat hydrolysed by Alcalase or Flavourzyme had DPPH and ABTS radical activity and metal chelating activity; however the activities depended on the degree of hydrolysis and the amount of hydrolysate (Thiansilakul, Benjakul, & Shahidi, 2007). Hydrolysate from silver carp derived using Alcalase or Flavourzyme was reported to show hydroxyl radical-scavenging activity and metal chelating activity (Dong et al., 2008). Antioxidative activity of shrimp (*Acetes chinensis*) products was enhanced when hydrolysed by crude protease from *Bacillus* sp. (He, Chen, Sun, Zhang, & Gao, 2006). Additionally, Mungoong, a paste prepared from the cephalothorax of white shrimp, contained peptides which acted as an effective antioxidant with high stability over a wide pH and temperature range (Binsan et al., 2008).

Fermented shrimp/krill products have been consumed widely in southeastern Asian countries as a condiment or main dish. Apart from serving as a good source of proteins, those products might possess bioactivities, especially natural antioxidants, which provide health benefits. Accordingly, these products could be marketed as health foods with high market value. However, no information regarding the bioactivity of fermented fishery products, especially from shrimps or krill have been reported. Therefore, the objective of this study was to determine the chemical composition and antioxidative activities of fermented shrimp and krill products produced in Thailand, including Kapi, Koong-Som and Jaloo.

2. Materials and methods

2.1. Chemicals

Ethanol, methanol and trichloroacetic acid were obtained from Merck (Darmstadt, Germany). 2,2-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,4,6-trinitrobenzenesulphonic acid (TNBS) were purchased from Sigma Chemical Co. (St. Louis, MO). 2,4,6-Tripyridyl-*s*-triazine (TPTZ), ferric chloride hexahydrate, potassium persulphate, acrylamide, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) and bis-acrylamide were procured from Fluka Chemical Co. (Buchs, Swizerland). Sodium sulphite and ammonium thiocyanate were obtained from Riedel-de Haen (Seelze, Germany).

2.2. Samples

Five samples of Kapi (KP1, KP2, KP3, KP4, KP5) produced from planktonous shrimp (*A. vulgaris*), 2 samples of Koong-Som (KS1, KS2) produced from small shrimps (*Acetes* sp.) (180–200 shrimps/kg) and 2 samples of Jaloo (JL1, JL2) produced from krill (*M. orientalis*) were purchased from different producers in local markets in Songkhla and Trang provinces, Thailand. For each sample (JL1, JL2, KS1, KS2, KP1, KP2, KP3, KP4 and KP5), three different lots were used. For each lot, three samples were purchased and pooled as the composite sample. All samples were packaged in polyethylene bags, stored at 4 °C until use and the storage time was not greater than 1 month.

2.3. Proximate analysis and pH determination of fermented shrimp/krill

Moisture, ash, fat, protein and salt contents of Kapi, Koong-Som and Jaloo were determined according to AOAC methods (1999) with the analytical No. of 35.1.13, 35.1.14, 35.1.25, 35.1.15 and 35.1.18, respectively. Sample pH was determined by a pH meter (Sartorius, Goetingen, Germany), as described by Benjakul, Seymour, Morrissey, and An (1997).

2.4. Determination of free amino group content and degree of hydrolysis (DH) of fermented shrimp/krill

DH of sample was determined according to the method of Benjakul and Morrissey (1997). The sample (1 g) was mixed with 9 ml of 70 mM SDS. The mixture was homogenised at a speed of 11,000 rpm for 1 min and was heated at 85 °C for 30 min. The mixture was then subjected to centrifugation at 10,000*g* for 15 min at room temperature using a Sorvall Model RC-5B Plus refrigerated centrifuge (Newtown, CT). To the supernatant obtained (125 μl), 2.0 ml of 0.2 M phosphate buffer (pH 8.2) and 1.0 ml of 3.41 mM TNBS solution were added. The solution was mixed thoroughly and placed in a temperature-controlled water bath (Memmert, Schwabach, Germany) at 50 °C for 30 min in the dark. The reaction was terminated by adding 2.0 ml of 0.1 M sodium sulphite. The mixture was then cooled at room temperature for 15 min. The absorbance was read at 420 nm and free amino group content was expressed in terms of L-leucine. Degree of hydrolysis (*DH*) was calculated following the method of Benjakul and Morrissey (1997) with a slight modification:

$$\%DH = (L/L_{max}) \times 100$$

where L is the amount of free amino group in the product and L_{max} is the total free amino group after acid hydrolysis (6 M HCl at 100 °C for 24 h).

2.5. SDS–polyacrylamide gel electrophoresis of fermented shrimp/krill

Protein patterns of Kapi, Koong-Som and Jaloo were determined by SDS–PAGE using 4% stacking gel and 12.5% running gel, according to the method of Laemmli (1970). Samples (3 g) were solubilised in 27 ml of 0.17 M SDS (85 °C). The mixture was homogenised for 1 min at a speed of 13,000 rpm using an IKA homogeniser and incubated at 85 °C for 1 h to dissolve total proteins. Proteins (15 μg) determined by the Biuret method (Robinson & Hodgen, 1940) were loaded onto the gel and subjected to electrophoresis at a constant current of 15 mA per gel using a Mini-Protean II unit (Bio-Rad Laboratories, Inc., Richmond, CA, USA). After separation, the proteins were stained with 0.25 mM Coomassie Brilliant Blue R-250 in 12.3 M methanol and 1.25 M acetic acid and destained with 12.3 M methanol and 1.25 M acetic acid for 15 min, followed by 1.23 M methanol and 1.25 M acetic acid for 3 h.

2.6. Preparation of soluble fraction from fermented shrimp/krill

Jaloo (2 g) or Koong-Som (5 g) or Kapi (2 g) was mixed with distilled water (100 ml) and the mixture was homogenised at a speed of 10,000 rpm for 3 min. The homogenate was stirred at room temperature for 30 min. The mixture was then centrifuged at 3000*g* for 10 min at room temperature using a Sorvall Model RC-5B Plus refrigerated centrifuge to remove undissolved debris. The supernatant was used for determination of antioxidative activity.

2.7. Determination of antioxidative activity

2.7.1. DPPH radical-scavenging activity

DPPH radical-scavenging activity was determined by DPPH assay, as described by Binsan et al. (2008) with a slight modification. Sample (1.5 ml) was added to 1.5 ml of 0.15 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) in ethanol. The mixture was mixed vigorously and allowed to stand at room temperature in the dark for 30 min. The absorbance of the resulting solution was measured at 517 nm using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). The blank was prepared in the same manner, except that distilled water was used instead of the sample. A standard curve was prepared using Trolox in the range of 10–60 μM. The activity was expressed as μmol Trolox equivalents (TE)/g sample.

2.7.2. ABTS radical-scavenging activity

ABTS radical-scavenging activity was determined by ABTS assay, as per the method of Arnao, Cano, and Acosta (2001) with a slight modification. The stock solutions were 7.4 mM ABTS solution and 2.6 mM potassium persulphate solution. The working solution was prepared by mixing the two stock solutions in equal quantities. The mixture was allowed to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 ml of ABTS solution with 50 ml of methanol, in order to obtain an absorbance of 1.1 ± 0.02 units at 734 nm using a UV-1601 spectrophotometer. Fresh ABTS solution was prepared for each assay. Sample (150 μl) was mixed with 2850 μl of ABTS solution and the mixture was left at room temperature for 2 h in dark. The absorbance was then measured at 734 nm using a spectrophotometer. A standard curve of Trolox ranging from 50 to 600 μM was prepared. The activity was expressed as μmol TE/g sample.

2.7.3. FRAP (ferric reducing antioxidant power)

FRAP was assayed as per the method of Benzie and Strain (1996). Stock solutions included 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3{\cdot}6H_2O$. A working solution was prepared freshly by mixing 25 ml of acetate buffer, 2.5 ml of TPTZ solution and 2.5 ml of $FeCl_3{\cdot}6H_2O$ solution. The mixed solution was incubated at 37 °C for 30 min and was referred to as FRAP solution. A sample (150 μl) was mixed with 2850 μl of FRAP solution and kept for 30 min in the dark. The ferrous tripyridyltriazine complex (coloured product) was measured by reading the absorbance at 593 nm. The standard curve was prepared using Trolox ranging from 50 to 600 μM. The activity was expressed as μmol TE/g sample.

2.8. Statistical analysis

All analyses were conducted in triplicate. The data were subjected to analysis of variance (ANOVA) and the differences between means were evaluated by Duncan's Multiple Range Test (Steel & Torrie, 1980). SPSS Version 14 (SPSS Inc., Chicago, IL) was used for data analysis.

3. Results and discussion

3.1. Chemical composition of fermented shrimp/krill products

Proximate compositions of Jaloo, Koong-Som and Kapi are shown in Table 1. Jaloo, the salted krill, contained 70.53–77.63% moisture content and 9.82–14.23% protein content. It contained 4.55–5.43% salt and had a neutral pH ranging from 7.24 to 7.38. Jaloo had 3.22–4.26% carbohydrate content with a low fat content. In general, Jaloo has been produced from krill with salt added at high content to prevent spoilage without the addition of any carbon source. For Koong-Som, a higher moisture content (73.35–80.71%) with lower protein content (6.38–7.21%) and salt content (2.74–3.81%) was obtained, in comparison with Jaloo. Koong-Som had a fat content of less than 0.55%, while carbohydrate contents ranged from 6.93% to 15.75%. Sugar concentrate added for lactic acid bacteria fermentation was the major source of carbohydrate in this product. Koong-Som is generally produced by mixing small shrimps with salt and sugar concentrate from palm sap (Phithakpol, 1993). Koong-Som had acidic pH, 3.71–3.89, which was mainly due to lactic acid generated during fermentation.

Kapi, the fermented shrimp paste with brown-reddish colour and meaty-savoury flavour, had a moisture content in the range of 36.78–49.93% and protein contents of 20.14–25.12%. Kapi contained a high salt content, ranging from 19.91% to 24.73%. This was mainly due to a large amount of salt added during processing and the drying during fermentation. High salt content was in accordance with high ash content (24.13–28.99%). All Kapi samples had a pH range of 7.44–7.66. The formation of volatile base compounds such as ammonia, the degradation products generated

Table 1
Chemical compositions and pH of fermented shrimp/krill products.

Sample	% Moisture	% Ash	% Protein	% Fat	% Carbohydrate	% Salt	pH
Jaloo 1	70.53 ± 0.01^{d}	11.28 ± 0.01^{f} (38.28 ± 0.05)	14.23 ± 0.73^{d} (48.29 ± 2.46)	0.74 ± 0.02^{c} (2.5 ± 0.06)	3.22 ± 0.01^{f} (10.94 ± 2.40)	5.43 ± 0.08^{d}	7.38 ± 0.03^{d}
Jaloo 2	77.63 ± 0.05^{b}	7.74 ± 0.01^{g} (34.58 ± 0.03)	9.82 ± 0.06^{e} (43.90 ± 0.27)	0.55 ± 0.13^{c} (2.46 ± 0.60)	4.26 ± 0.01^{d} (19.04 ± 0.33)	4.55 ± 0.09^{de}	7.24 ± 0.02^{e}
Koong-Som 1	73.35 ± 0.15^{c}	4.41 ± 0.18^{i} (16.56 ± 0.68)	6.38 ± 0.09^{f} (23.94 ± 0.33)	0.11 ± 0.01^{d} (0.40 ± 0.04)	15.75 ± 0.18^{a} (59.11 ± 0.37)	2.81 ± 0.04^{f}	3.89 ± 0.02^{f}
Koong-Som 2	80.71 ± 0.09^{a}	5.00 ± 0.03^{h} (25.94 ± 0.13)	7.21 ± 0.03^{f} (37.37 ± 0.16)	0.15 ± 0.04^{d} (0.76 ± 0.19)	6.93 ± 0.03^{c} (35.96 ± 0.35)	3.74 ± 0.00^{ef}	3.71 ± 0.01^{g}
Kapi 1	40.83 ± 0.15^{g}	28.99 ± 0.16^{a} (49.00 ± 0.28)	25.12 ± 0.21^{a} (42.46 ± 0.35)	2.22 ± 0.30^{a} (3.76 ± 0.50)	2.84 ± 0.16^{g} (4.79 ± 0.16)	24.73 ± 0.64^{a}	7.65 ± 0.01^{a}
Kapi 2	49.93 ± 0.23^{e}	24.13 ± 0.42^{e} (48.19 ± 0.83)	21.59 ± 0.02^{b} (43.12 ± 0.04)	2.05 ± 0.11^{a} (4.10 ± 0.22)	2.30 ± 0.42^{g} (4.58 ± 0.18)	19.29 ± 0.41^{c}	7.59 ± 0.02^{b}
Kapi 3	36.78 ± 0.22^{i}	25.94 ± 0.16^{c} (41.04 ± 0.25)	20.14 ± 0.21^{c} (31.85 ± 0.33)	1.53 ± 0.02^{b} (2.42 ± 0.02)	15.61 ± 0.16^{a} (24.70 ± 0.35)	21.18 ± 1.40^{b}	7.59 ± 0.01^{b}
Kapi 4	38.78 ± 0.62^{h}	25.35 ± 0.39^{d} (41.42 ± 0.64)	21.27 ± 0.93^{b} (34.74 ± 1.51)	1.55 ± 0.06^{b} (2.52 ± 0.10)	13.05 ± 0.39^{b} (21.33 ± 1.41)	19.91 ± 0.41^{c}	7.66 ± 0.01^{a}
Kapi 5	47.71 ± 0.30^{f}	26.63 ± 0.10^{b} (50.93 ± 2.10)	20.25 ± 0.28^{c} (38.73 ± 0.54)	1.78 ± 0.04^{b} (3.40 ± 0.08)	3.63 ± 0.10^{e} (6.95 ± 0.45)	21.74 ± 1.54^{b}	7.44 ± 0.01^{c}

Values in parentheses indicate the content based on dry basis.
Mean ± SD from triplicate determinations.
Different superscripts in the same column indicate the significant difference ($p < 0.05$).

during fermentation, might contribute to slightly basic pH. Fat contents of Kapi were from 1.53% to 2.22%. Moreover, a wide range of carbohydrate content (2.30–13.05%) was noticeable. Some producers might add some roots or flour to increase the yield of Kapi and to lower the production cost. The chemical compositions in Kapi were similar to those previously reported by Phithakpol (1993) who noted that pH, moisture, protein, fat and salt contents of Kapi from Thailand were 5.5–6.4, 37.1–51.4%, 7.7–24.0%, 0.4–32.7% and 13.6–21.2%, respectively. Therefore, the composition found in samples varied with types of product, producers, ingredients and processes used. The result indicated that all fermented products, including Jaloo, Koong-Som and Kapi, could be an important source of protein and minerals.

3.2. Free amino group content and DH of fermented shrimp/krill products

Free amino group contents of fermented shrimp/krill products are shown in Fig. 1. Jaloo, Koong-Som and Kapi contained varying free amino group contents (Fig. 1a). The result suggested that partial proteolysis took place during fermentation in all products. For the same product, differences in free amino group contents were obtained, possibly due to the different raw materials, processes as well as fermentation time used. Enzymatic fermentation of small shrimp mediated by indigenous proteinases yields the short chain peptide and free amino acid (Steinkraus, 2002). Additionally, proteinases from lactic acid bacteria (LAB) might contribute to protein degradation of fermented products. The higher free amino group content indicates the greater degradation in Som-fug, a fermented fish paste (Riebroy, Benjakul, & Visessanguan, 2008). Proteolysis of muscle protein during fermentation with LAB could lead to the accumulation of free amino acids (Yin, Pan, & Jiang, 2002). During hydrolysis, a wide variety of smaller peptides and free amino acids was generated, depending on enzyme specificity. Kapi contained a lower free amino group content than that of Jaloo or Koong-Som (Fig. 1a). Since free amino groups could be used by microorganisms or decomposed *via* deamidation during prolonged fermentation, the decreases in free amino group content in Kapi were noticeable.

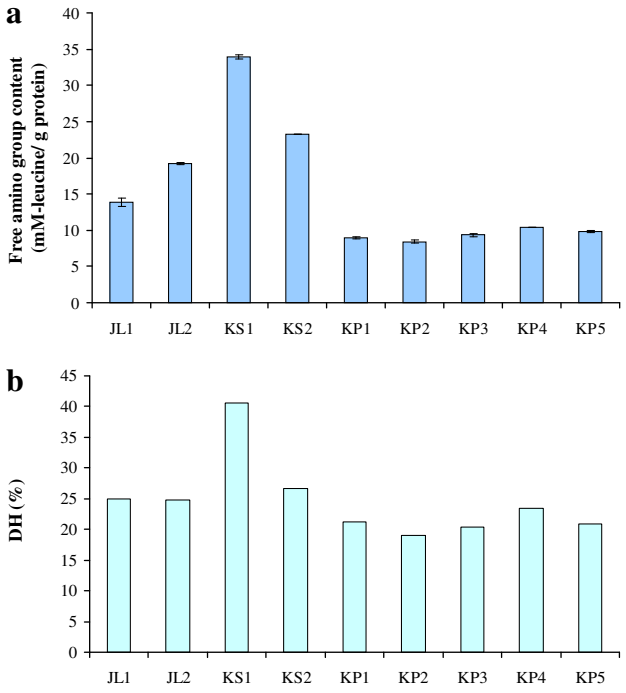

Fig. 1. Free amino group content (a) and degree of hydrolysis (b) of fermented shrimp/krill products, JL: Jaloo, KS: Koong-Som, KP: Kapi. Bars represent the standard deviations from triplicate determinations.

DH of Jaloo, Koong-Som and Kapi are shown in Fig. 1b. *DH* is a measure of the extent of cleavage of peptide linkages. Protein completely hydrolysed to free amino acids has *DH* close to 100% (Panyam & Kilara, 1996). In general, *DH* in all samples had the same trend as free amino acid group content (Fig. 1a). For the same product, *DH* varied with different producers, indicating the different degrees of protein cleavage. Since different raw materials were used for production of different products, the different types or levels of proteolytic enzymes were presumed. This could determine the different reactivity in peptide cleavage, in which degree of hydrolysis could vary. Additionally, different hydrolysis products might be associated with the varying characteristics, taste, flavour and other attributes of resulting fermented products.

3.3. Protein patterns of fermented shrimp/krill products

For all samples, the disappearance of both myosin heavy chain (MHC) and actin was noticeable (data not shown). This suggested that MHC and actin underwent degradation completely through proteolysis during fermentation in all samples. Due to the high salt content, halophilic bacteria have been known to produce salt-tolerant proteases, which contributed to degradation in addition to indigenous proteases in shrimp/krill. For Koong-Som, the low pH might favour the hydrolysis of myofibrillar proteins (Visessanguan, Benjakul, Riebroy, & Thepkasikul, 2004). Complete hydrolysis of MHC and actin was in accordance with the formation of free amino groups (Fig. 1a). MHC was more susceptible to hydrolysis, compared with actin (Riebroy et al., 2008). From the result, proteins or peptides with low molecular weight including 50–54, 30–35 and 18–22 kDa were found in Jaloo and Koong-Som, while those proteins or peptides were not found in Kapi. With a longer period of fermentation of Kapi (3–6 months), proteins were degraded

Table 2
Antioxidative activities of soluble fractions from fermented shrimp/krill products determined by different assays.

Fermented shrimp products	DPPH (μmol TE/g protein)	ABTS (μmol TE/g protein)	FRAP (μmol TE/g protein)
Jaloo 1	4.01 ± 0.10^{g}	111 ± 0.05^{f}	19.1 ± 0.14^{f}
Jaloo 2	7.05 ± 0.03^{e}	152 ± 0.08^{d}	24.9 ± 1.28^{d}
Koong-Som 1	8.58 ± 0.12^{d}	67.9 ± 0.15^{g}	24.8 ± 0.48^{d}
Koong-Som 2	5.84 ± 0.10^{f}	57.6 ± 1.07^{h}	17.0 ± 0.68^{g}
Kapi 1	10.6 ± 0.54^{c}	180 ± 0.69^{c}	31.9 ± 0.82^{c}
Kapi 2	12.1 ± 0.39^{b}	376 ± 7.03^{a}	39.4 ± 0.45^{b}
Kapi 3	4.34 ± 0.35^{g}	128 ± 0.49^{e}	19.5 ± 0.23^{f}
Kapi 4	5.84 ± 0.28^{f}	124 ± 0.31^{e}	21.2 ± 0.47^{e}
Kapi 5	15.5 ± 0.32^{a}	345 ± 7.76^{b}	48.2 ± 0.84^{a}

TE: Trolox equivalents.
Mean ± SD from triplicate determinations.
Different superscripts in the same column indicate significant differences ($p < 0.05$).

intensively. As a consequence, free amino acids or low molecular weight peptides were produced.

3.4. Antioxidative activities of soluble fractions from fermented shrimp/krill products

The antioxidative activities of the water-soluble fractions of Jaloo, Koong-Som and Kapi are shown in Table 2. Water-soluble fractions from all samples possessed the ability to quench DPPH and ABTS radicals. DPPH is a stable free radical that shows a maximum absorbance at 517 nm in ethanol. When DPPH encounters a proton-donating substance such as an antioxidant, the radical would be scavenged and the absorbance is reduced (Shimada, Fujikawa, Yahara, & Nakamura, 1992). Based on this principle, the antioxidant activity of the substance can be expressed as its ability in scavenging the DPPH radical. Jaloo and Koong-Som had DPPH radical-scavenging activity of 3.34–5.30 and 2.72–3.02 μmol TE/g sample, respectively. Kapi showed DPPH radical-scavenging activity in the range of 2.14–7.05 μmol TE/g sample. The lowest DPPH radical-scavenging activity was found in Koong-Som samples, in comparison with Jaloo and Kapi. The differences in the activity among samples. This indicated that peptides or free amino acids in the fermented products possessed the ability to donate the hydrogen atom to free radicals, in which the propagation process could be retarded.

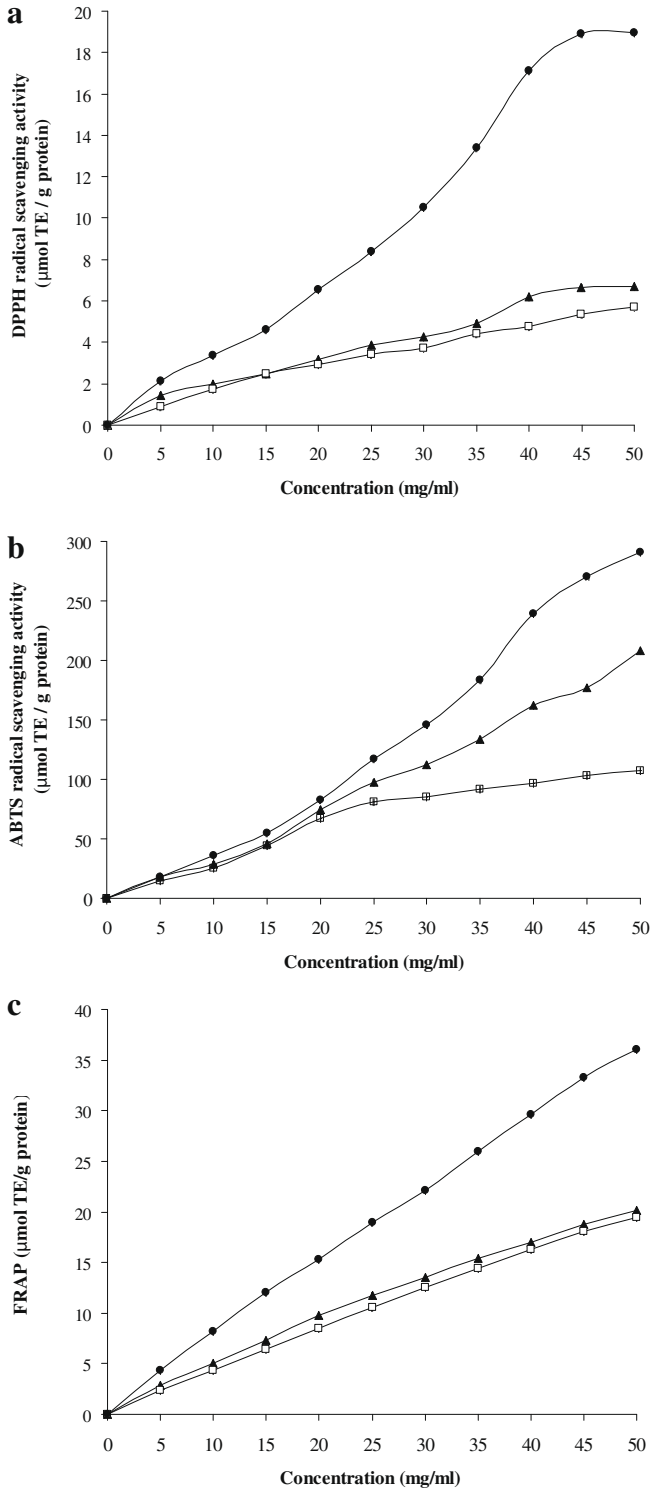

Fig. 2. DPPH radical-scavenging activity (a) ABTS radical-scavenging activity (b) and FRAP (c) of water-soluble fractions from fermented shrimp/krill products at different concentrations, Jaloo (▲), Koong-Som (□) and Kapi (●).

Results of ABTS radical-scavenging activity of soluble fractions from Jaloo, Koong-Som and Kapi were generally similar to those observed for DPPH radical-scavenging activity. The reactions of ABTS with free radical scavengers present in the tested sample occur rapidly and can be assessed by following the decrease in the sample absorbance at 734 nm of ABTS radical. This method can determine both hydrophilic and lipophilic antioxidants in the same sample (Sun & Tanumihardjo, 2007). Koong-Som showed the lowest ABTS radical-scavenging activity, compared with Jaloo and Kapi. This might be associated with the lower protein content as influenced by dilution effect of carbohydrate added into the products.

Varying FRAP was found in different products. In general, Koong-Som showed the lowest FRAP, in comparison with Jaloo and Kapi. FRAP assay measures the reducing capability of tested sample by increasing sample absorbance based on the ferrous ions released (Prior, Wu, & Schaich, 2005). FRAP found in Jaloo, Koong-Som and Kapi suggested their capability of providing the electron. Binsan et al. (2008) reported that the water-soluble fraction from Mungoong, a paste made from the cephalothoraxes of white shrimp exhibited a high antioxidative activity.

Many peptides that are released *in vivo* from animal protein are bioactive and have regulatory function as antioxidants (Hartmann & Meisel, 2007). Muscle proteins were hydrolysed by proteases from LAB into low molecular weight water-soluble polypeptides and further hydrolysed into peptides and free amino acids (Yin, Tong, & Jiang, 2005). Fermented food products are a good source of peptides and amino acids, which possess antioxidant properties (Sathivel et al., 2003). From the results, Kapi and Jaloo had a higher antioxidative activity than Koong-Som. This was in agreement with the higher degradation found in Kapi and Jaloo (Fig. 2). Antioxidative activities of all fermented products were not correlated with *DH* (Fig. 1b). Thus, the radical-scavenging activity of fermented products was most likely governed by the type and amino acid composition of resulting peptides. Antioxidative activity was dependent on certain fermented product types (Virtanen, Pihlanto, Akkanen, & Korhonen, 2006). Size, level and composition of free amino acids and small peptides affect the antioxidative activity (Wu, Chen, & Shiau, 2003). The results indicated that Jaloo, Koong-Som and Kapi contained substances which were electron donors and could react with free radicals to convert them to more stable products and terminate the radical chain reaction.

3.5. Effect of concentrations of water-soluble fraction from fermented shrimp/krill products on antioxidative activity and the correlation among different assays tested

For each fermented product, only one sample with the highest antioxidative activities was selected for study (Table 2). When

DPPH, ABTS radical-scavenging activities and FRAP of soluble fractions were tested, the activities of the water-soluble fraction from Jaloo, Koong-Som and Kapi increased as the concentrations increased up to 50 mg/ml (Fig. 2). At the same concentration tested, Kapi exhibited the highest antioxidative activities, followed by Jaloo and Koong-Som. The results suggested that antioxidative compounds in the fraction tested showed higher radical-scavenging activity and reducing power when higher concentrations were used. This result was in accordance with Je et al. (2007), who reported that the antioxidant peptide from tuna backbone protein can quench three different free radical species in a dose-dependent manner. Hydroxyl radical-scavenging activity of silver carp protein hydrolysates was concentration-dependent (Dong et al., 2008). Protein hydrolysates from aquatic species contain both antioxidative and prooxidative components, and their final effect depends on their concentration (Pokorny & Korezak, 2001). The differences in antioxidative activities of water-soluble fractions from different products were probably due to the differences in peptides produced during fermentation in terms of amino acid sequences, composition as well as the chain length of peptides. The result reconfirmed that *DH* or free amino group content was not the major factor affecting antioxidative activity. Virtanen et al. (2006) reported that the *DH* did not directly relate with antioxidant activity of milk whey during fermentation with lactic acid bacteria but most likely depended on specific characteristics of the bacterial enzyme.

The good correlations between antioxidative activities determined by different assays and expressed as Trolox equivalent antioxidant capacity (TEAC) were observed as depicted in Fig. 3. ABTS and DPPH radical-scavenging activities correlated very well (r^2 = 0.9085 for Jaloo, r^2 = 0.9412 for Koong-Som and r^2 = 0.99 for Kapi) as well as did ABTS radical-scavenging activities and FRAP (r^2 = 0.9598 for Jaloo, r^2 = 0.943 for Koong-Som and r^2 = 0.949 for Kapi). FRAP correlated with DPPH radical-scavenging activities (r^2 = 0.9758 for Jaloo, r^2 = 0.9925 for Koong-Som and r^2 = 0.9635 for Kapi). Binsan et al. (2008) reported the correlation between

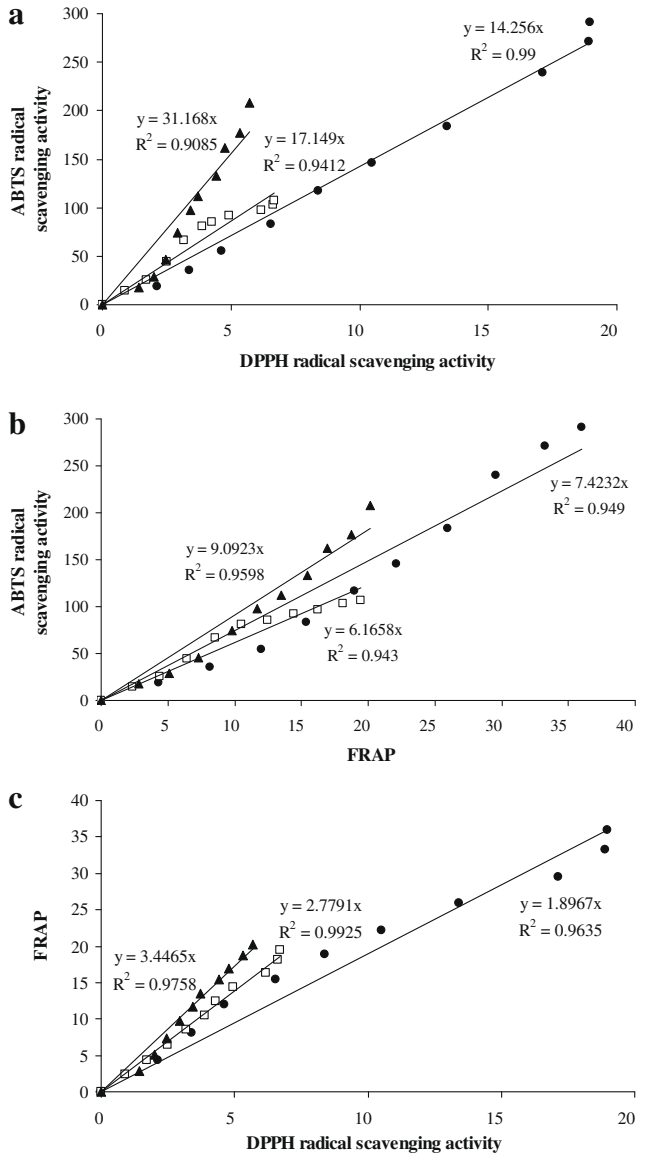

Fig. 3. Correlation between (a) ABTS and DPPH radical-scavenging activity, (b) FRAP and ABTS radical-scavenging activity, and (c) FRAP and DPPH radical-scavenging activity, of the water-soluble fractions from fermented shrimp/krill products, Jaloo (▲), Koong-Som (□) and Kapi (●). Bars represent the standard deviations from triplicate determinations.

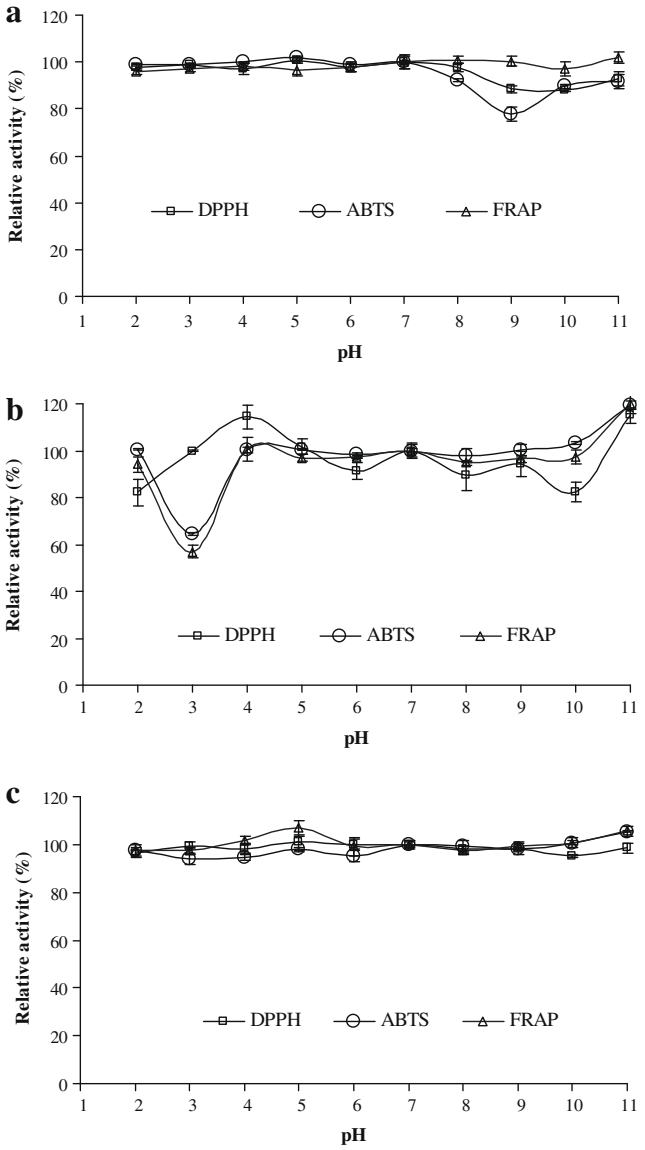

Fig. 4. pH stability of the soluble fraction from fermented shrimp/krill products, Jaloo (a), Koong-Som (b) and Kapi (c). Bars represent the standard deviations from triplicate determinations.

Table 3
Thermal stability of the soluble fraction of fermented shrimp/krill products subjected to heating at various temperatures and times as determined by FRAP.

Temperature (°C)	Time (min)	Relative activity (%)		
		Jaloo	Koong-Som	Kapi
40	15	97.9 ± 1.0 b	102.2 ± 0.9 e	101.1 ± 2.1 ef
	30	100.5 ± 2.4 b	99.8 ± 0.6 e	102.4 ± 0.6 ef
	45	102.3 ± 1.7 b	103.2 ± 5.4 e	103.3 ± 0.2 ef
	60	107.1 ± 3.6 b	106.4 ± 6.1 de	105.3 ± 0.6 de
60	15	107.3 ± 1.2 b	106.6 ± 7.7 de	103.2 ± 2.4 ef
	30	105.0 ± 5.4 b	102.0 ± 8.9 e	99.3 ± 1.2 f
	45	102.9 ± 3.3 b	106.1 ± 2.8 de	101.1 ± 1.7 ef
	60	109.3 ± 1.5 b	107.8 ± 1.5 de	99.0 ± 0.2 f
80	15	109.8 ± 6.1 b	106.2 ± 4.6 e	102.9 ± 2.4 ef
	30	112.2 ± 1.7 b	117.5 ± 5.8 cd	103.5 ± 3.5 ef
	45	107.4 ± 4.7 b	120.4 ± 11.3 c	108.8 ± 4.2 d
	60	118.0 ± 5.7 b	117.1 ± 6.4 cd	100.6 ± 4.4 ef
100	15	120.6 ± 13.7 b	150.2 ± 6.0 a	142.6 ± 2.9 c
	30	122.1 ± 1.4 b	137.6 ± 0.2 b	164.2 ± 2.2 a
	45	155.0 ± 18.8 a	125.2 ± 8.7 c	149.1 ± 3.1 b
	60	160.6 ± 12.5 a	145.2 ± 6.7 ab	144.4 ± 3.7 c

Mean ± SD from triplicate determinations.
Different letters in the same column indicate significant differences ($p < 0.05$).

the antioxidant capacity of Mungoong determined by DPPH and ABTS radical-scavenging assays and FRAP.

3.6. pH stability of water-soluble fraction from fermented shrimp/krill products

The influences of pH on the antioxidant stability of the water-soluble fractions from Jaloo, Koong-Som and Kapi are shown in Fig. 4. DPPH and ABTS radical-scavenging activities and FRAP of the water-soluble fraction of Kapi remained constant when subjected to a pH range of 2–11. For Jaloo, ABTS and DPPH radical-scavenging activities slightly decreased at pH above 8. However, no changes in FRAP were noticeable across the whole pH range tested. Antioxidative activities of Koong-Som tested by all assays were stable in the pH range of 5–9. ABTS radical-scavenging activities and FRAP markedly decreased at pH 3 and DPPH radical-scavenging activity decreased at pH 10. Furthermore, those activities slightly increased at pH 11. These results were in accordance with Binsan et al. (2008) who reported that ABTS radical-scavenging activity of Mungoong, was activated at alkaline pH. Thus, the antioxidative activities from soluble fraction of Jaloo, Koong-Som and Kapi were stable over a wide pH range. This suggested that antioxidative peptides were probably stable in both the stomach and intestine, which have acidic and alkaline pH, respectively. Additionally, they showed potential stability when applied in any food system with a harsh pH.

3.7. Thermal stability of water-soluble fraction from fermented shrimp/krill products

Antioxidant activity of the water-soluble fractions from Jaloo, Koong-Som and Kapi after heating at different temperatures for various times as monitored by FRAP is shown in Table 3. The marked increases in FRAP were noticeable when subjected to higher temperature, especially at 100 °C with increasing heating time (45–60 min). The result indicated that peptides with low molecular weight were most likely stable after heat treatment. In general, proteins were vulnerable to heat treatment, leading to the aggregation of protein and exposure of hydrophobic domain (Sikorski & Naczk, 1981). The increases in antioxidative activity of Kapi extract heated at 100 °C were probably due to the exposure of the hydrophobic domain. Peptides derived from many protein sources with increased hydrophobicity have been reported to correlate with antioxidative properties (Chen, Muramoto, Yamauchi, & Nokihara, 1996). As a consequence, hydrophobic amino acids in the sequences of the peptides were associated with radical-scavenging properties (Rajapakse et al., 2005). Water-soluble fractions from Jaloo, Koong-Som and Kapi exhibited thermal stability as monitored by DPPH and ABTS radical-scavenging activities (data not shown). DPPH radical-scavenging activity of water-soluble fraction from all samples was stable, regardless of heating temperature and time. Activity higher than 90% was retained after all heat treatments. ABTS radical-scavenging activity of Jaloo and Koong-Som was stable when heated up to 100 °C, irrespective of heating times. However, slight decrease in ABTS radical-scavenging activity of Kapi extract was noticeable when heated at 100 °C ($p < 0.05$). Similarly, Binsan et al. (2008) reported that antioxidative activities of Mungoong slightly decreased with prolonged heating time. The denaturation of antioxidative compounds might be enhanced when heated for a longer time and higher temperature. Accumulated energy or enthalpy might be sufficient for antioxidative compounds to undergo denaturation and loss of their activities (Pokorny & Schmidt, 2001). This result suggested that the peptides in Kapi extract with ABTS radical-scavenging activity might be more prone to thermal denaturation.

4. Conclusion

Jaloo, Koong-Som and Kapi, Thai traditional fermented shrimp products exhibited antioxidant activity, which were stable to pH and heating. Therefore, fermented shrimp/krill products can be an important source of natural antioxidants, as well as nutrients. However, further study on isolation and characterisation of antioxidative components in these fermented products should be carried out.

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