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RESEARCH PAPER

Comparative Studies on Autolysis and Antioxidative Properties of Salted Shrimp Paste (Kapi) from *Acetes Vulgaris* and *Macrobrachium Lanchesteri*

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Abstract

Autolysis of *Acetes vulgaris* and *Macrobrachium lanchesteri* was comparatively studied. Maximal autolytic activities were found at pH 7, 55°C for *A. vulgaris* and pH 8, 60°C for *M. lanchesteri*. Both shrimps dominantly contained serine proteases as indicated by the marked inhibition by PMSF and SBTI. When both shrimps were used for *Kapi* production and characterized, differences in characteristics as well as antioxidant activities were obtained. *Kapi* produced from *A. vulgaris* (KA) showed the higher degree of hydrolysis (15.93±0.51%), peroxide values (0.20±0.23 mg cumene peroxide/ kg sample) and TBARS value (0.55±0.16 mg MDA/ kg sample), compared with *Kapi* produced from *M. lanchesteri* (KM), indicating the higher protein degradation and lipid oxidation in this sample. Moreover, KA also possessed the higher antioxidative activities including ferric reducing antioxidant power (FRAP) (14.12±1.34 μmol TE/ g sample), chelating activity (3.21±0.05 μmol EE/ g sample), hydrogen peroxide (30.05±0.49 μmol TE/ g sample) and singlet oxygen scavenging activities (48.46±0.32 μmol EE/ g sample) than KM, except for DPPH and ABTS radical scavenging activities, which showed lower activity ($P<0.05$) and had no difference ($P>0.05$), respectively. Therefore, different levels and types of endogenous proteases in both shrimps determined the autolysis, characteristics and antioxidative properties of resulting *Kapi*.

Keywords: *Acetes vulgaris*, *Macrobrachium lanchesteri*, autolysis, *Kapi*, antioxidative properties.

Introduction

Kapi, a traditional salted shrimp paste, has been consumed widely in Thailand as a condiment to enhance the palatability of foods in various dishes. *Kapi* has a strong typical flavor and its color varies from a pinkish or purplish grey to a dark greyish brown (Pongsetkul *et al.*, 2014). *Kapi* is made by mixing shrimp or krill with solar salt at a ratio of 3-5: 1. After being salted, the mixture is sun-dried, and then it is thoroughly blended or homogenized to produce semi-solid paste. The paste is fermented at room temperature (25-30°C) for at least 1 month or longer until the typical aroma is developed (Faithong *et al.*, 2010). Nowadays, the planktonous krill (*Mesopodopsis orientalis*), which is the traditional raw material for *Kapi* production, has dropped by 3% per year over the last decade (Meland & Willassen, 2007). To conquer the insufficient raw material, the alternative source, especially small shrimp belonging to *Acetes vulgaris*, has become promising for *Kapi* production in the southern part of Thailand, owing to its high availability (Faithong *et al.*, 2010). Recently, another species, *Macrobrachium lanchesteri*, a by-catch from commercial fishing, has been used as an alternative raw material because of its abundance, lower price, and availability throughout the year (Pongsetkul *et al.*, 2016a). For *Kapi* production, differences in raw material, the quantity of salt used, and the treatment of raw materials prior to fermentation result in varying compositions and quality of final products (Peralta *et al.*, 2008). Pongsetkul *et al.* (2015a) found that the delay before salting led to the distorted quality of *Kapi* due to microbial deterioration. It has been known that autolysis, which varies with shrimp species, is a crucial process in converting raw material to final *Kapi*.

Kapi has been reported to contain peptides with bioactivity, especially antioxidant activity (Peralta *et al.*, 2008; Faithong & Benjakul, 2012; Pongsetkul *et al.*, 2014). Cleavage of food proteins by microbial or indigenous proteases yields the bioactive peptides, leading to substantial increases in the biological properties of fermented food products (Faithong *et al.*, 2010). *Kapi* produced from different shrimps, mediated by different indigenous proteases, might have different characteristics and antioxidative activities. Nevertheless, no information on autolysis of shrimp, *A. vulgaris* and *M. lanchesteri*, and

properties of resulting *Kapi* from both species, has been reported. Therefore, the objectives of this investigation were to comparatively study the autolysis of both shrimp species and to characterize some properties and antioxidative activities of *Kapi* produced from both shrimps.

Materials and Methods

Sample Collection

For each lot, 10 kilograms of shrimp *A. vulgaris* (average body length 15.6±1.4 mm, average wet weight 0.0413±0.0098 g, n=20) and *M. lanchesteri* (average body length 27.5±1.9 mm, average wet weight 0.0701±0.0107 g, n=20) were caught from the coast in Ko-yo and The-Pha in Songkhla province, Thailand, respectively. After capture, shrimp were transported in ice with a shrimp/ice ratio of 1:2 (w/w) in a polystyrene container to the Department of Food Technology, Prince of Songkla University, Hat Yai, Thailand, within approximately 2 h.

Study on Autolysis of *A. vulgaris* and *M. lanchesteri*

Effect of Temperature

Whole shrimps with the physiological pH of 7.0-7.4 were ground using a blender. Ground shrimp (3 g) was placed in a 50-mL beaker and covered with aluminium foil. The samples were incubated for 60 min at various temperatures (30-80°C) in a temperature-controlled water bath (Memmert, Schwabach, Germany). Autolytic reaction was terminated by adding 27 mL of cold 5% (w/v) trichloroacetic acid (TCA). The mixture was homogenized using an IKA Labortechnik homogenizer (Selangor, Malaysia) at a speed of 11,000 rpm for 2 min, followed by centrifugation at 8,000×g for 30 min. The amount of the soluble peptide in the supernatant was measured using the Lowry method (Lowry *et al.*, 1951) and expressed as mmol tyrosine equivalent/min/g sample.

Effect of pH

Ground shrimp (3 g) was homogenized at 11,000 rpm for 1 min with 12 mL of different buffers having various pHs (McIlvain's buffer consisting of 0.2 M sodium phosphate and 0.1 M sodium citrate with pH range of 2-7, and 0.1 M glycine-NaOH with pH range of 8-11). The homogenate from each species was incubated at the corresponding temperature. Autolysis was terminated after 60 min of incubation by the addition of 15 mL of cold 9% (w/v) TCA, followed by centrifugation as previously described. Soluble peptides in the supernatant were determined using the Lowry method and expressed as mmol tyrosine equivalent/min/g sample.

Effect of Salt Concentration

The effect of NaCl at various concentrations on autolytic activity was studied. Firstly, the buffer showing the maximal activity was added with NaCl to obtain different final concentrations [0, 5, 10, 15, 20, 25 and 30% (w/w)]. Ground sample (3 g) was mixed with the aforementioned buffers and then incubated at the optimal temperature for 60 min. After incubation, 15 mL of cold 9% (w/v) TCA were added and the mixture was homogenized (Klomklao *et al.*, 2008). Soluble peptides were determined by the Lowry method (Lowry *et al.*, 1951) and expressed as mmol tyrosine equivalent/min/g sample.

Effect of Various Protease Inhibitors

Ground shrimp (0.5 g) was homogenized (11,000 rpm for 1 min) with 1.5 mL of the buffer yielding the maximal activity. The homogenate was mixed with 2 mL of protease inhibitor solution to obtain the final designated concentrations (1 mM pepstatin A, 0.1 mM E-64, 10 mM EDTA, 1 g/L SBTI, 5 mM PMSF, 5 mM TLCK, 5 mM TPCK and 1 mM Iodoacetic acid) (Sriket *et al.*, 2011). The mixtures were allowed to stand in ice for 30 min, followed by incubation at the optimal temperature for 60 min. Autolysis was terminated by addition of 2 mL of of cold 15% (w/v) TCA. The mixture was homogenized and centrifuged as mentioned above. The soluble peptide contents were then determined by the Lowry method (Lowry *et al.*, 1951) and expressed as mmol tyrosine equivalent/min/g sample.

Study on Properties of *Kapi* Produced from *A. vulgaris* and *M. lanchesteri*

Preparation of *Kapi*

Kapi was prepared following the method of Pongsetkul *et al.* (2015a). Shrimps were mixed with salt at the ratio of 5:1 (w/w) and then kept at room temperature (28-32°C) overnight. Subsequently, the samples were drained, mashed, spread out on fiberglass mats and dried with sunlight (38-42°C). After the moisture contents of dried shrimp were in the range of 35-40%, salted shrimps were transferred into earthen jars and covered with plastic bag tightly (close system). After 30 days of fermentation at room temperature (28-32°C), *Kapi* samples were collected and referred to as KA (*Kapi* produced from *A. vulgaris*) and KM (*Kapi* produced from *M. lanchesteri*). The obtained samples were subjected to analyses.

Formal, Ammonia and Amino Nitrogen Contents

Formal nitrogen content was determined by the titration method as described by Thai Industrial Standard (1983). *Kapi* (2 g) was mixed with 10 ml of

distilled water. Then, the mixture was homogenized at a speed of 9500 rpm for 2 min using an IKA Labortechnik homogenizer (Selangor, Malaysia). Ten ml of formalin solution (38%, v/v; pH 9) was added and mixed well. The mixture was titrated with 0.1 N NaOH to obtain pH of 9.0. Formal nitrogen content was calculated and expressed as mg N/g sample using the following equation:

$$\text{Formal nitrogen content (mg N/g)} = \frac{\text{mL of NaOH (pH 7-pH 9)} \times 0.1 \times 14}{\text{Weight of sample (g)}}$$

Ammonia nitrogen content was determined by the titration method as described by Thai Industrial Standard (1983). *Kapi* (2 g) was placed in 400 mL Kjeldahl flask containing 100 mL of distilled water and 3 g of MgO. The mixture was distilled and the distillate was collected in 50 mL of 4% (w/v) boric acid containing the mixed indicators (0.125 g methyl red and 0.082 g bromocresol green in 95% alcohol (100 mL): 0.1% methylene blue in distilled water with ratio of 5:1). The solution was then titrated with 0.05 N H_2SO_4 to reach the end-point. Ammonia nitrogen content was calculated as follows:

$$\text{Ammonia nitrogen content (mg N/g)} = \frac{5.6 \times 0.05 \times \text{mL of } H_2SO_4}{\text{Weight of sample (g)}}$$

Amino nitrogen content was calculated based on the difference between formal and ammonia nitrogen contents (Thai Industrial Standard, 1983) and expressed as mg N/g sample.

Degree of Hydrolysis (DH)

Firstly, the sample (1 g) was mixed with 9 mL of 5% (w/v) SDS. The mixture was homogenized at a speed of 11,000 rpm for 1 min. The homogenate 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 (Model RC-B Plus centrifuge Newtown, CT, USA). Thereafter, 2.0 mL of 0.2 M phosphate buffer (pH 8.2) and 1.0 mL of 0.01% (w/v) TNBS solution was 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 sulfite. 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. DH of *Kapi* was determined according to the method of Benjakul and Morrissey (1997) and expressed as degree of hydrolysis (% DH).

Peroxide Values (PV)

PV was measured following the method of Maqsood and Benjakul (2010). Ground sample (1 g) was homogenized at a speed of 13,500 rpm for 2 min in 11 mL of chloroform/methanol (2:1, v/v). Homogenate was then filtered using a Whatman No. 1 filter paper. Two milliliters of 0.5% NaCl was then added to 7 mL of the filtrate. The mixture was vortexed at a moderate speed for 30 s and then centrifuged at 3000×g for 3 min to separate the sample into two phases. Two milliliters of cold chloroform/methanol (2:1) were added to 3 mL of the lower phase. Twenty-five microliters of ammonium thiocyanate and 25 µL of iron (II) chloride were added to the mixture. Reaction mixture was allowed to stand for 20 min at room temperature prior to reading the absorbance at 500 nm. A standard curve was prepared using cumene hydroperoxide at the concentration range of 0.5-2 ppm. PV was reported as mg cumene preoxide/kg sample.

Thiobarbituric Acid Reactive Substances (TBARS)

TBARS value was determined as per the method of Pongsetkul *et al.* (2015a). Ground sample (1 g) was mixed with 9 mL of 0.25 N HCl solution containing 0.375% TBA and 15% TCA. The mixture was heated in boiling water for 10 min, followed by cooling with the running water. The mixture was centrifuged at 4,000×g for 20 min (MIKRO20, Hettich Zentrifugan, Germany). The supernatant was collected and the absorbance was read at 532 nm using a UV-160 spectrophotometer (Shimadzu, Kyoto, Japan). A standard curve was prepared using malonaldehyde bis (dimethyl acetal) at concentrations ranging from 0 to 2 ppm. TBARS value was expressed as mg malonaldehyde (MDA)/kg sample.

Antioxidant Activities

Preparation of Water Extract

Water extract was prepared as per the method of Pongsetkul *et al.* (2015b). *Kapi* sample (1 g) was mixed with 25 mL of distilled water. The mixtures were homogenized at a speed of 11,000 rpm for 2 min, followed by centrifugation at 8,500×g for 15 min at room temperature. The supernatant was collected and adjusted to 25 mL using distilled water before analyses.

Radical Scavenging Activities and Reducing Power

DPPH and ABTS radical scavenging activities as well as ferric reducing antioxidant power (FRAP) were determined according to the method of Faithong and Benjakul (2012). The activity was expressed as µmol Trolox equivalents (TE)/g sample.

For the determination of DPPH radical scavenging activity, water extract of samples (1.5 mL) was added with 1.5 mL of 0.15 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) in 95% ethanol. The mixture was then mixed vigorously and allowed to stand for

30 min in dark at room temperature. The resulting solution was measured at 517 nm using an UV-1601 spectrophotometer. The blank was prepared in the same manner except that distilled water was used instead of the sample. The standard curve was prepared using Trolox in the range of 10-60 µM.

To examine ABTS radical scavenging activity, the stock solutions including 7.4 mM ABTS solution and 2.6 mM potassium persulphate solution were prepared. The working solution was made by mixing two stock solutions in equal quantities and allowed them to react in the dark for 12 h at room temperature. The solution was then diluted by mixing 1 mL of ABTS solution with 50 mL of methanol to obtain an absorbance of 1.1 (±0.02) at 734 nm using an UV-1601 spectrophotometer. ABTS solution was prepared freshly for each assay. To initiate the reaction, 150 µL of sample were mixed with 2.85 mL of $ABTS^{\bullet+}$ solution. The mixture was incubated at room temperature for 2 h in dark. The absorbance was then read at 734 nm using an UV-1601 spectrophotometer. Distilled water was used instead of the sample and prepared in the same manner to obtain the blank. A Trolox standard curve (50-600 µM) was prepared.

For FRAP, the stock solutions included 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, 20 mM $FeCl_3.6H_2O$ solution and 300 mM acetate buffer (pH 3.6). The working solution was prepared freshly by mixing 25 mL of acetate buffer, 2.5 mL of TPTZ solution and 2.5 mL of $FeCl_3.6H_2O$ solution. The mixture was incubated at 37°C for 30 min and was referred to as FRAP solution. The sample (150 µL) was mixed with 2.85 mL of FRAP solution. The mixture was allowed to stand in dark for 30 min at room temperature. Ferrous tripyridyltraizine complex, coloured product, was measured by reading the absorbance at 593 nm. The blank was prepared in the same manner except that distilled water was used instead of the sample. The standard curve was prepared using Trolox ranging from 50 to 600 µM.

Metal Chelating Activity

Metal chelating activity was assayed according to the method of Pongsetkul *et al.* (2015b). Sample (220 µL) was mixed with 5 µL of 2 mM $FeCl_2$ and 10 µL of 5 mM ferrozine. The mixture was allowed to stand at room temperature for 20 min. Absorbance at 562 nm was read. EDTA with the concentrations of 0-30 µM was used as standard. Metal chelating activity was expressed as µmole EDTA equivalent (EE)/g sample.

Hydrogen Peroxide and Singlet Oxygen Scavenging Activities

Hydrogen peroxide and singlet oxygen scavenging activities were investigated as described by Pongsetkul *et al.* (2015b). The activities were expressed as µmol Trolox equivalents (TE)/g sample.

To determine hydrogen peroxide scavenging activity, the extract (3.4 mL) was mixed with 600 µL of 43 mM hydrogen peroxide in 0.1 M phosphate buffer (pH 7.4). The absorbance at 230 nm of the reaction mixture was recorded after 40 min of reaction at 25°C. For the blank, hydrogen peroxide was omitted and replaced by 0.1 M phosphate buffer (pH 7.4). Trolox (0-10 mM) was used as standard.

For determination of singlet oxygen scavenging activity, the chemical solutions and the extract were prepared in 45 mM sodium phosphate buffer (pH 7.4). The reaction mixture consisted of 0.4 mL of extract, 0.5 mL of 200 µM *N,N*-dimethyl para-nitro-soaniline (DPN), 0.2 mL of 100 mM histidine, 0.2 mL of 100 mM sodium hypochlorite, and 0.2 mL of 100 mM H_2O_2. Thereafter, the total volume was made up to 2 mL with 45 mM sodium phosphate buffer (pH 7.4). The absorbance of the reaction mixture was measured at 440 nm after incubation at room temperature (25°C) for 40 min. Blank was run for each sample in the same manner, except DPN, histidine, and NaOCl solutions were replaced by sodium phosphate buffer. A standard curve of Trolox (0-10 mM) was prepared.

Statistical Analysis

Experiments were run in triplicate using three different lots of samples. Completely randomized design (CRD) was used throughout the study. Data were subjected to analysis of variance (ANOVA), and mean comparisons were carried out by the Duncan's multiple range test (Steel *et al.*, 1980). Analysis were performed using SPSS statistic program (Version 10.0) (SPSS, 1.2, 1998).

Result and Discussion

Autolysis of *A. vulgaris* and *M. lanchesteri*

Effect of Temperature and pH

Autolytic degradation of *A. vulgaris* and *M. lanchesteri* as affected by temperature and pH is shown in Figure. 1A and 1B, respectively. Maximal autolytic activities were found at 55 and 60°C for *A. vulgaris* and *M. lanchesteri*, respectively. At the physiological pH, autolytic activity increased markedly from 30°C and reached the maximum at 55 and 60°C for *A. vulgaris* and *M. lanchesteri*, respectively. With increasing temperatures, the activity was sharply decreased. At high temperature, unfolding or structural changes of proteases in both shrimps might occur, leading to a loss in proteolytic activity (Klomklao *et al.*, 2008). For the pH profile, the optimum pHs of autolysis, determined at the optimal temperature, were 7 for *A. vulgaris* and 8 for *M. lanchesteri*. The activity was drastically decreased when samples were subjected to very acidic or alkaline pHs. The increased repulsion between charged residues of protein molecules, associated with

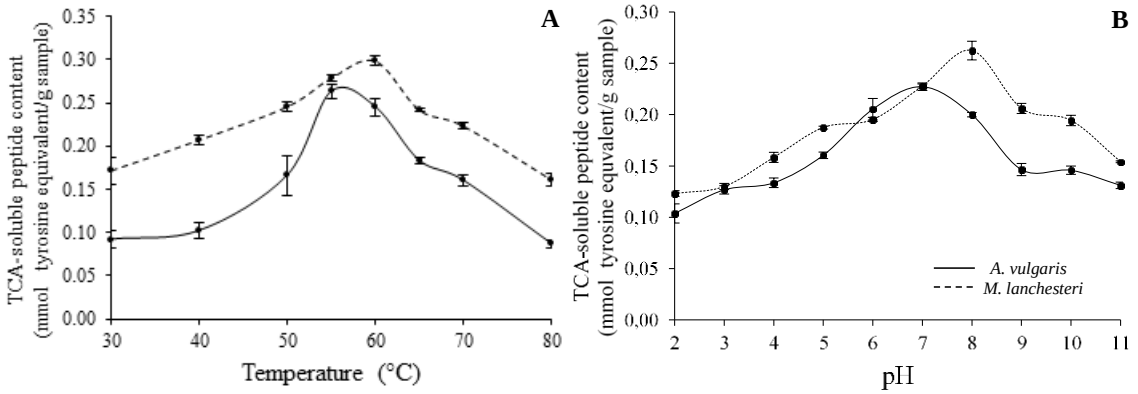

Figure 1. Temperature profile (A) and pH profile of autolysis of *A. vulgaris* and *M. lanchesteri*. Autolytic activity was expressed as mmol tyrosine equivalent/g sample. Bars represent the standard deviation (n=3).

conformational changes, led to the denaturation of proteases under those conditions (Klomklao *et al.*, 2008). For both shrimp species, autolysis was most likely mediated by heat-activated proteases that were active at neutral or slightly alkaline pHs. Optimal temperature and pH for autolysis of both species tested were similar to those of proteases from Antarctic krill (*Euphausia superba*) (pH 8.2, 60°C) (Osnes & Mohr, 1985), North Pacific krill (*Euphausia pacifica*) (pH 8-9, 50°C) (Sun *et al.*, 2014), hepatopancreas of fresh water prawn (*Macrobrachium rosenbergii*) (pH 7, 60°C) (Sriket *et al.*, 2011) or Pacific brown shrimp (*Penaeus californiensis*) (pH 8, 50°C) (Vega-Villasante *et al.*, 1995), etc.

During *Kapi* production, salted shrimps were exposed to varying temperatures throughout the process from approximately 27 to 45°C (data not shown). Endogenous proteases of both *A. vulagris* and *M. lanchesteri* with the optimal temperature around 55-60°C were postulated to play a role in proteolysis at aforementioned temperatures used for *Kapi* production. The differences in pH and temperature profiles of autolysis between both species might determine the degradation rate of proteins during *Kapi* process, in which the formation of degradation products could be varied. As a consequence, autolysis mediated by various proteases in both shrimps might have the impact on the characteristics of resulting *Kapi* differently.

Effect of Salt Concentration

A sharp decrease in autolysis was observed as the concentration of salt increased up to 10% (Figure 2). The loss in activity more than 50% was noticeable when the concentration of NaCl was above 20% (w/v) in both shrimps. The losses in activity occurred as NaCl concentration increased, probably owing to the partial denaturation of proteases caused by the "salting out" effect (Klomklao *et al.*, 2008). The result revealed that NaCl at high concentration (15-25%), which is commonly used for *Kapi* production, could lower autolysis mediated by endogenous proteases in raw material. When NaCl concentration was above 20%, *M. lanchesteri* sample remained the slightly higher activities, compared with *A. vulgaris.* The result suggested that *M. lanchesteri* plausibly showed higher autolysis in the presence of high salt, compared to *A. vulgaris*. Varying degradation of proteins during *Kapi* fermentation, influenced by different raw material and concentration of salt, could led to the different characteristics of final product (Pongsetkul *et al.*, 2015a).

Effect of Various Protease Inhibitors

Various protease inhibitors exhibited the inhibition toward autolysis of *A. vulgaris* and *M. lanchesteri* differently as shown in Figure 3. For autolysis of *A. vulgaris* at pH 7, PMSF and SBTI, the specific inhibitors for serine proteases, showed the highest inhibitory activity (81.63% and 59.46%, respectively). It was noted that TLCK and TPCK, specific inhibitor of trypsin and chymotrypsin, respectively, also showed high inhibitory activity. Furthermore, autolytic activity of *A. vulgaris* was slightly inhibited (less than 25%) by other inhibitors, including aspartic acid protease inhibitor (pepstatin A), metallo-protease inhibitor (EDTA) and cysteine protease inhibitor (E-64, iodoacetic acid). Thus, there were several kinds of proteases present in *A. vulgaris.* Similar inhibition of autolysis in *M. lanchesteri* was observed with those inhibitors. All serine protease inhibitors (SBTI, PMSF, TLCK and TPCK) had high inhibitory effect, compared with others. The result was in agreement with our previous study, in which both shrimps, *A. vulgaris* and *M. lanchesteri*, contained several groups of proteases, but serine proteases were dominant, particularly trypsin or chymotrypsin-like enzymes (Pongsetkul *et al.*, 2016b). Several crustaceans including *Penaeus vannamei* (Garcia-Carreno *et al.*, 2008), *Macrobrachium rosenbergii* (Sriket *et al.*, 2012) and *Penaeus californiensis* (Navarrete-del-Toro *et al.*, 2015), etc. contained serine proteases as the major enzymes. The result indicated that digestive tract or organs in whole shrimp used for *Kapi* production contributed to the proteolysis or autolysis of shrimp

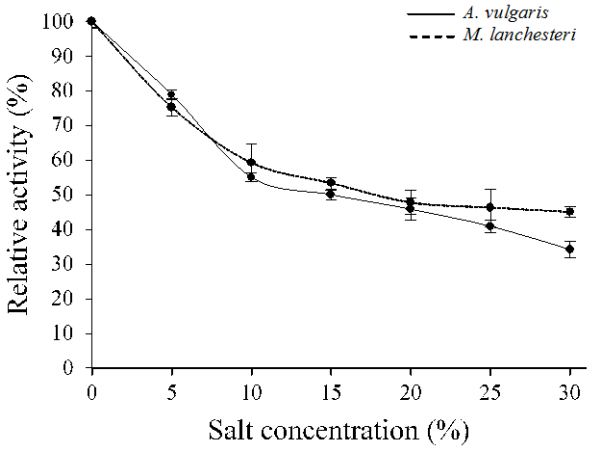

Figure 2. Effect of salt concentrations on autolysis of *A. vulgaris* and *M. lanchesteri*. Autolytic activity was expressed as mmol tyrosine equivalent/g sample. Bars represent the standard deviation (n=3).

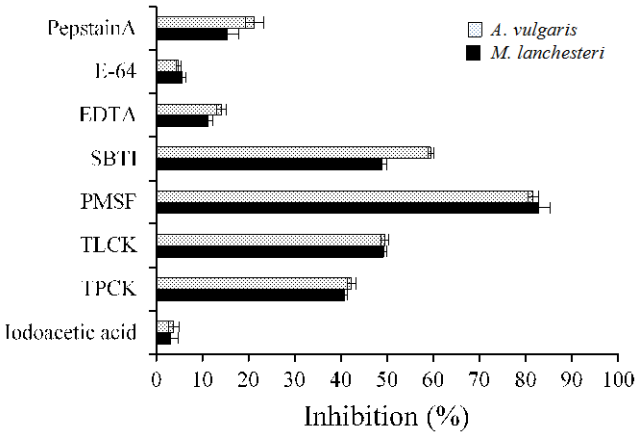

Figure 3. Effect of various inhibitors on autolysis of *A. vulgaris* and *M. lanchesteri*. The autolysis was performed at pH 7, 55°C and pH 8, 60°C for *A. vulgaris* and *M. lanchesteri*, respectively. Bars represent the standard deviation (n=3).

during *Kapi* processing.

Properties of Resulting *Kapi*

Formal, Ammonia and Amino Nitrogen Contents

Formal, ammonia and amino nitrogen contents of KA and KM are shown in Table 1. KA had the higher formal nitrogen content (14.92 mg N/g sample), compared with KM (11.76 mg N/g sample) ($P<0.05$), suggesting the greater cleavage of peptides caused by endogenous or microbial proteases in the former. Generally, the formal nitrogen content has been used to indicate the degree of protein hydrolysis (Klomklao *et al.*, 2006). There was no difference in ammonia nitrogen content between these two samples ($P>0.05$). The ammonia nitrogen content indicates the breakdown of soluble protein and peptides into free amino acid and volatile nitrogen (Pongsetkul *et al.*, 2014). The volatile nitrogenous compounds might contribute to different characteristics of the products, especially flavor. Amino nitrogen content, calculated based on the differences between formal nitrogen content and ammonia nitrogen content, represents the amount of primary amino group of the sample (Klomklao *et al.*, 2006). KA had the higher amino nitrogen content ($P<0.05$). The result reconfirmed that higher cleavage of peptides was obtained in KA. Therefore, different shrimps determined the rate of degradation, more likely associated with different autolytic activities.

Degree of Hydrolysis (DH)

Degree of hydrolysis (DH) is the measure of the extent of cleavage of peptide linkages. DH close to 100% means that all proteins in the sample are completely hydrolyzed to free amino acids (Panyam & Kilara, 1996). As shown in Table 1, DH of KA and KM was 15.93 and 13.23%, respectively. The higher DH of KA correlated well with the higher degradation of proteins as indicated by the higher formal nitrogen content and amino nitrogen content. The result suggested that *A. vulgaris* was more pronounced in protein degradation during *Kapi* fermentation, compared with *M. lanchesteri*. Degradation of

Table 1. Nitrogen contents, degree of hydrolysis and lipid oxidation products of *Kapi* produced from *A. vulgaris* and *M. lanchesteri*

Compositions/Characteristics	KA	KM
Nitrogen content (mg N/g sample)		
Formal nitrogen content	14.92±1.20a	11.76±0.13b
Ammonia nitrogen content	1.10±0.19a	1.03±0.02a
Amino nitrogen content	13.82±1.03a	10.72±0.13b
Degree of hydrolysis (%)	15.93±0.51a	13.23±0.46b
Lipid oxidation products		
PV (mg cumene peroxide/ kg sample)	0.20±0.23a	0.18±0.251a
TBAR (mg MDA/ kg sample)	0.55±0.16a	0.28±0.30b

Values are given as mean ± SD (n = 3).
Different lowercase superscripts in the same row indicate the significant difference (P<0.05).
* KA, KM: *Kapi* produced from *A. vulgaris* and *M. lanchesteri*, respectively.

proteins in shrimp resulted in an increase in peptides and free amino acids and could be related with the development of flavor and odor of *Kapi*. Compounds such as peptides and amino acids contribute to the taste or flavor in a complex manner in the resulting *Kapi* (Visessanguan *et al.*, 2004).

Lipid Oxidation Products (PV and TBARS)

Lipid oxidation of KA and KM was monitored by measuring PV and TBARS values, representing the primary and secondary oxidation products, respectively (Table 1). PV of KA and KM were 0.20 and 0.18 mg cumene/kg sample, respectively, suggesting that lipid oxidation occurred during processing and fermentation of *Kapi* to some extent. There was no difference in PV between these two samples (P>0.05). However, KA had the higher TBARS value (0.55 mg MDA/kg sample), compared with KM (0.28 mg MDA/kg sample) (P<0.05). The higher TBARS value of KA sample might probably due to the greater decomposition of hydroperoxides into the secondary oxidation products, especially aldehydes, in the later stage of lipid oxidation. Furthermore, during fermentation, autolysis or endogenous enzymatic activity might cause the disruption of the organelles associated with the release of pro-oxidants as well as reactants (Takeungwongtrakul & Benjakul, 2013). This resulted in the enhanced lipid oxidation in *Kapi*. Lipid oxidation is one of the deteriorative reactions causing the unacceptability of fish and shrimp product (Nirmal & Benjakul, 2009). Nevertheless, some lipid oxidation products in both KA and KM might contribute to typical odor and flavor of *Kapi*, leading to differences in acceptability of resulting *Kapi* produced from different shrimps.

Antioxidative Activities

Water extracts of both KA and KM showed antioxidative activities differently as shown in Figure 4. Water extract of KM showed the higher DPPH radical scavenging activity (8.82 μmol TE/g sample) than that of KA (8.20 μmol TE/g sample), indicating that peptides or free amino acids in KM had the higher ability in donating hydrogen atom to free radicals, in which the propagation process of oxidation could be retarded (Peralta *et al.*, 2008). There was no difference in ABTS radical scavenging between KA and KM (P>0.05). In general, ABTS assay is an excellent tool for determining both hydrophilic and lipophilic antioxidants for hydrogen donating (scavengers of aqueous phase radicals) and chain breaking (scavenger of lipid peroxyl radicals) (Leong & Shui, 2002). Binsan *et al.*, (2008) reported that antioxidants in salted shrimp paste were more likely water soluble peptides. Furthermore, other antioxidative compounds including MRPs were also present in salted shrimp paste. Those peptides or MRPs were mostly hydrophilic in nature and were extracted into water effectively. However, KA showed the higher antioxidative activities including FRAP, metal chelating activity, hydrogen peroxide scavenging activity and singlet oxygen scavenging activity, compared with KM (P<0.05). FRAP is generally used to measure the capacity of a substance in reducing TPTZ-Fe(III) complex to TPTZ-Fe(II) complex (Faithong & Benjakul, 2012). The higher FRAP activity in KA suggested the higher capability of providing the electron. Metal chelating activity of water extract of KA and KM were 3.21 and 2.93 μmol EE/g sample, respectively. The higher metal chelating activity of water extract of KA demonstrated the higher capacity of iron binding. The extracts could reduce the redox potential, thereby stabilizing the oxidized form of the metal ion (Oktay *et al.*, 2003). Moreover, the higher capacity of scavenging of hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) of water extracts was also found in KA sample (P<0.05). H_2O_2 is a reactive non radical, which can permeate biological membranes and is concerted to more reactive species such as hydroxyl radical and singlet oxygen (Choe & Min, 2005). It is the precursor for the generation of hydroxyl radical, which is a strong initiator of lipid oxidation (Suh *et al.*, 2011). Thus, the removal of H_2O_2 is very important for antioxidant defence in cell or food systems. KA exhibited higher ability for scavenging H_2O_2 than KM (P<0.05). This might be governed by the differences in peptides

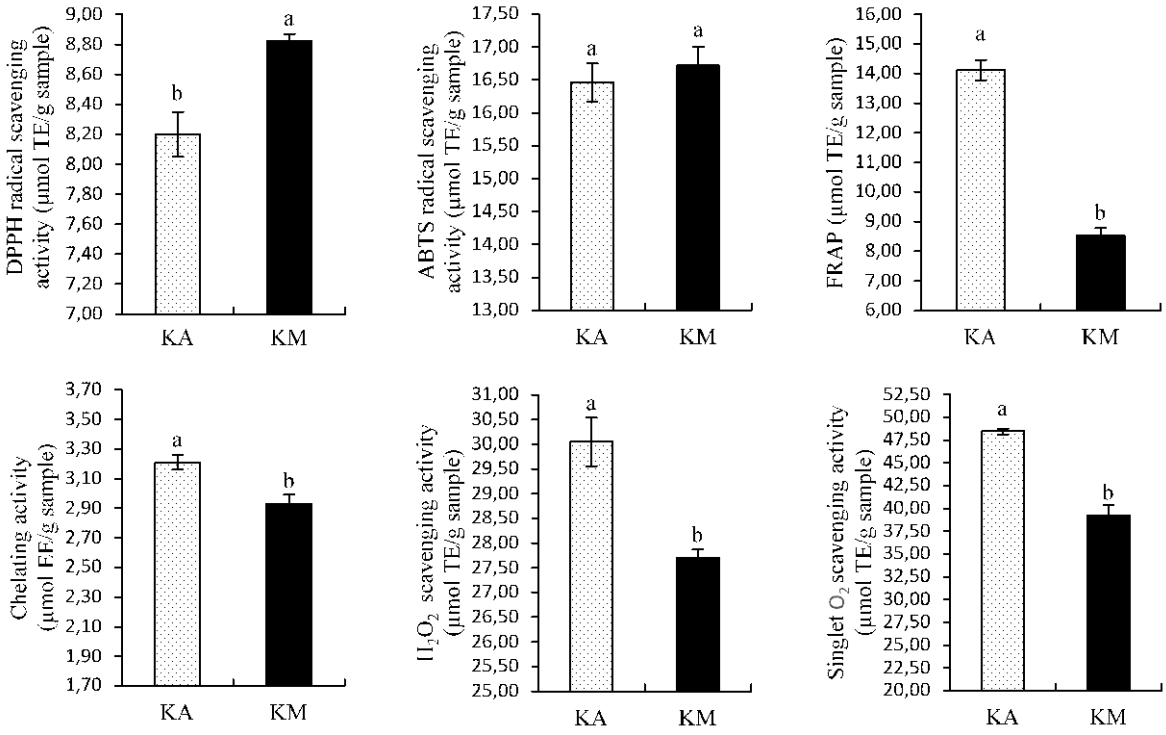

Figure 4. Antioxidative activities of water extract from *Kapi* produced from *A. vulgaris* (KA) and *M. lanchesteri* (KM). Bars represent the standard deviation (n=3). Different lowercase letters on the bars indicate the significant difference (P<0.05).

between both *Kapi* samples. Gou *et al.* (2009) reported that dipeptide containing tyrosine residue at its C-terminus was associated with strong hydrogen peroxide scavenging activity. The higher singlet oxygen scavenging activity was also observed in KA sample (P<0.05). Singlet oxygen, which is a highly reactive, electrophilic and non-radical molecule, can be formed by the reaction between photosensitizers and triplet oxygen in the presence of light (Min & Boff, 2002). Singlet oxygen can directly react with electron-rich double bonds of unsaturated fatty acids without the formation of free-radical intermediates (Choe & Min, 2005).

Overall, both KA and KM might contain various antioxidant peptides with different modes of action. The different raw material might be degraded to varying extents, in which peptides could be produced in different fashions. Pongsetkul *et al.* (2015b) found that different commercial salted shrimp pastes obtained from different parts of Thailand had varying antioxidative activities.

Conclusion

A. vulgaris and *M. lanchesteri* contained serine proteases as the dominant enzymes with optimum pH and temperature of 7, 55°C and 8, 60°C, respectively. Both shrimps could be used as alternative raw material for *Kapi* production because of their high availability throughout the years and lower price, compared with krill (*Mesopodopsis orientalis*), typically used as the raw material. However, the differences in some properties were obtained. *Kapi* produced from *A. vulgaris* generally showed higher protein degradation, lipid oxidation and also possessed the higher antioxidative activities, compared with *Kapi* produced from *M. lanchesteri*. Therefore, different shrimp with different endogenous proteases associated with varying autolysis affected the characteristics and antioxidative properties of *Kapi*.

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