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EFFECT OF POST-MORTEM STORAGE PRIOR TO SALTING ON QUALITY OF SALTED SHRIMP PASTE (*KAPI*) PRODUCED FROM *MACROBRACHIUM LANCHESTERI*

Jaksuma Pongsetkul[1], Soottawat Benjakul[1*], Punnanee Sumpavapol[1], Kazufumi Osako[2] and Nandhsha Faithong[1]

[1]Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla, Thailand, 90112;
[2]Department of Food Science and Technology, Tokyo University of Marine Science and Technology, 5-7 Konan 4, Minato-ku, Tokyo 108-8477, Japan;
*Corresponding author:*soottawat.b@psu.ac.th*

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ABSTRACT

Effect of post-mortem storage time of shrimp (*Macrobrachium lanchesteri*) on quality of shrimp and the resulting *Kapi*, salted shrimp paste, was investigated. Shrimp underwent deterioration when stored at room temperature (28-30°C) up to 18 h as indicated by the increases in pH, total volatile base (TVB), trimethylamine (TMA) contents, thiobarbituric acid reactive substances (TBARS) and total viable count (TVC). Protein degradation was more pronounced as evidenced by the decrease in band intensity of myosin heavy chain with coincidental increase in TCA soluble peptides. Post-mortem storage time of shrimp prior to salting had impact on the quality of resulting *Kapi*. With increasing storage time, *Kapi* became browner with higher antioxidative activity. Also, volatile compounds including aldehydes, ketones, alcohols and pyrazines increased continuously. The highest and lowest overall likeness scores were obtained for *Kapi* prepared from shrimp stored for 6 and 18 h, respectively. Therefore, post-mortem storage time of shrimp used as raw material had the marked influence on quality of resulting *Kapi*.

1. Introduction

Kapi, a Thai traditional fermented shrimp paste, is widely consumed as a condiment. In general, small shrimp or krill have been used as the main raw materials to produce *Kapi*. Shrimp or krill tissues undergo enzymatic breakdown during the fermentation and bacterial action assists in proteolysis and flavour development (Hajeb and Jinap, 2015). Peptides in salted shrimp paste possessed bioactive activities, especially antioxidant activities (Peralta et al., 2008; Faithong and Benjakul, 2012; Pongsetkul et al., 2014; Pongsetkul et al., 2015)

Kapi is made by mixing shrimp or krill with salt at a ratio of 3-5:1, followed by sun-drying. Sun-dried salted shrimp ithoroughly ground before being compacted in a container, usually earthen jar and allowed to ferment for at least 1 month or longer until the typical aroma is developed (Pongsetkul et al., 2014). Salting and drying processes increase the shelf-life and flavour intensity of the product. Putrefactive microorganisms are inhibited by salt at concentrations above 6 to 8% (Phithakpol, 1993). The delay in salting might contribute to the differences in quality of resulting *Kapi.*

However, the planktonous shrimp or krill (*Mesopodopsis orientalis*), which is the traditional raw material for *Kapi* production, have dropped by 3% per year since 1990

(Meland and Willassen, 2007). Therefore, the alternative raw material for *Kapi* production has been searched. Small shrimp (*Macrobrachium lanchesteri*) is generally by-products from commercial fishing and it usually founds in southern part of Thailand throughout the year. This species seem to be a potential alternative source for making *Kapi* because of its availability and low price. Generally, species of shrimp, the quantity of salt used, and the treatment of raw materials prior to fermentation, can be varied, leading to the different characteristics, especially flavour and taste (Phithakpol, 1993).

Nevertheless, no information regarding the characteristics and quality of *Kapi* from shrimp (*M. lanchesteri*) as influenced by post-mortem storage before salting has been reported. Thus, the aim of this study was to investigate the effect of different post-mortem storage time of shrimp (*M. lanchesteri*) prior to salting on the characteristics and some

2. Materials and methods

2.1. Sample collection

Live shrimp (*M. lanchesteri*) were purchased from a village in The-Pha, Songkhla, Thailand. 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. Whole shrimp had 80.04% moisture, 16.31% protein, 0.58% lipid, 2.26% ash and 0.81% carbohydrate as determined by AOAC method (AOAC, 2000).

2.2. Quality changes of shrimp during post-mortem storage

Upon arrival, shrimp were placed in the basket and stored at room temperature (28-30°C). Shrimp were periodically taken at 0, 3, 6, 9, 12, 15 and 18 h. The collected samples were pooled and blended using a blender (National, Tokyo, Japan) prior to analysis.

2.2.1. Chemical analysis

2.2.1.1. pH

The pH was measured according to the method of Nirmal and Benjakul (2009).

2.2.1.2. Total volatile base (TVB) and trimethylamine (TMA) contents

TVB and TMA contents were determined using the Conway micro-diffusion method (Conway and Byrne, 1936). The amounts of TVB and TMA were calculated and expressed as mg N/ g sample.

2.2.1.3. Thiobarbituric acid reactive substances (TBARS)

TBARS were determined as described by Nirmal and Benjakul (2009) with some modifications. A standard curve was prepared using 1,1,3,3-tetramethoxy propane at concentrations ranging from 0 to 2 ppm. TBARS value was calculated and expressed as mg malonaldehyde/ kg sample.

2.2.1.4. TCA-soluble peptide content

Oligopeptide content of samples was determined according to the method of Sriket et al. (2012). Soluble oligopeptide content in the supernatant was measured according to the Lowry method (Lowry et al., 1951) and expressed as μmol tyrosine equivalent/ g sample.

2.2.1.5. Protein patterns

Protein patterns were determined by SDS-PAGE using 4% stacking gel and 10% running gel according to the method of Laemmli (1970). High and low molecular weight protein markers (GE Healthcare UK Limited, Buckinghamshire, UK) were used to estimate the molecular weight of proteins.

2.2.2. Microbiological analysis

Shrimp (25 g) were transferred into a stomacher bag containing 225 ml of peptone water. Blending was performed in a Stomacher 400 Lab Blender (Seward Ltd., Worthing, UK) at high speed for 3 min. Peptone water was used for diluting the samples. Thereafter, the sample diluted in serial 10-fold steps was used for analysis by the spread plate technique on plate count agar (PCA-Merck). The plates were incubated at 35°C for 48 h. Total viable count

(TVC) was recorded and expressed as log CFU/ g sample (BAM, 2001).

2.3. Effect of post-mortem storage time on characteristics and properties of *Kapi*

2.3.1. Preparation of Kapi

Shrimp with different post-mortem storage times (0, 6, 12 and 18 h) were used as raw material. Shrimp were mixed with salt at a ratio of 5:1 (w/w) and transferred into the basket and covered with cheesecloth. Salted shrimp were made as per the method of Pongsetkul et al. (2015). After 30 days, the obtained *Kapi* samples were taken for analyses.

2.3.2. Characterization of Kapi

2.3.2.1. pH

pH of samples was determined as previously described.

2.3.2.2. Formal, ammonia and amino nitrogen contents

Formal, ammonia and amino nitrogen contents were determined by the titration method as described by Pongsetkul et al. (2014). The results were calculated and expressed as mg N/ g sample.

2.3.2.3. Colour

Colour of samples was determined using a colourimeter (ColourFlex, Hunter Lab Reston, VA, USA) and reported in the CIE system. L^* (lightness), a^* (redness/ greenness), and b^* (yellowness/blueness) were recorded. Additionally, ΔE^* (total difference of colour) and ΔC^* (the difference in chroma) were calculated.

2.3.2.4. Browning index, Maillard reaction product and antioxidative activity

- Preparation of water extract

Water extract was prepared according to the method of Peralta et al. (2008) with a slight modification. *Kapi* (2 g) was mixed with 50 ml of distilled water. The mixtures were homogenised at a speed of 10,000 ×g for 2 min. The homogenates were then subjected to centrifugation at 13,000 ×g for 15 min at room temperature. The supernatant was collected. The pellet was re-extracted in the same manner. The supernatants were combined and adjusted to 50 ml using distilled water. These extracts were then analysed as follows:

- Measurement of browning intensity

The browning intensity of the extract was measured as per the method of Pongsetkul et al. (2014). Appropriate dilution was made using distilled water and the absorbance was measured at 420 nm using the UV-1601 spectrometer (Shimadzu, Kyoto, Japan).

- Measurement of Maillard reaction products

Fluorescent intermediate products from Maillard reaction in the extract were determined as described by Pongsetkul et al. (2014). A_{280} and A_{295} of the extracts were determined also determined.

2.3.2.5. Antioxidative activities

Water extracts prepared as mentioned above were determined for antioxidative activities. Prior to assay, the extracts were approximately diluted using distilled water.

- DPPH radical scavenging activity

DPPH radical scavenging activity was determined according to the method of Faithong and Benjakul (2012) with a slight modification. The standard curve was prepared using Trolox in the range of 10-60 μM. The activity was expressed as μmol Trolox equivalents (TE)/ g sample.

- ABTS radical scavenging activity

ABTS radical scavenging activity was determined as described by Pongsetkul et al. (2014) with a slight modification. A Trolox standard curve (50-600 μM) was prepared and ABTS radical scavenging activity was expressed as μmol Trolox equivalents (TE)/ g sample.

- Ferric reducing antioxidant power (FRAP)

FRAP was evaluated by the method of Faithong and Benjakul (2012). The standard curve was prepared using Trolox ranging from 50 to 600 μM. The activity was expressed as μmol Trolox equivalents (TE)/ g sample.

2.3.2.6. Volatile compounds

Volatile compounds of *Kapi* samples were determined using a solid-phase microextraction gas chromatography mass spectrometry (SPME

GC-MS) following the method of Pongsetkul et al. (2014). Volatile compounds were presented in term of abundance.

2.3.2.7. Sensory properties

Kapi samples were evaluated by 50 untrained panellists, who consumed *Kapi* regularly. The samples were cut to obtain a thickness of 1 cm. The sample (2×2 cm^2) was wrapped with aluminum foil. Thereafter, it was heated in a hot air oven at 60°C for 30 min. Panellists were instructed to rinse their mouths with water or cucumber between different samples. The panellists were asked to assess samples for appearance, colour, odour, flavour, texture and overall-liking using a 9-point hedonic scale (Mellgard et al., 2007).

2.4. Statistical analysis

All experiments were conducted in triplicate. Statistical analysis was performed using one-way analysis of variance (ANOVA). Mean comparison was carried out using Duncan's multiple range test (Steel et al., 1980). SPSS statistic program (Version 10.0) (SPSS, 1.2, 1998) was used for data analysis.

3. Results and discussions

3.1. Quality changes of shrimp during post-mortem storage

3.1.1. Changes in pH

A slight increase in pH of shrimp (*M. lanchesteri*) from 7.07 to 7.42 was observed as the post-mortem time increased (Fig.1A). The increase of pH might be associated with the production of volatile basic components, such as ammonia, trimethylamine, etc. by some spoilage bacteria (Pongsetkul et al., 2014). However, the different buffering capacity of muscle proteins from different species plausibly contributed to varying rate of pH changes (Riebroy et al., 2008). In the present study, shrimp were stored at room temperature, in which the deterioration could take place to a faster rate, compared with the storage in ice or refrigerated condition. After capture, shrimp were placed in container without icing before off-loading. This could induce the spoilage, particularly with the longer storage or handling time.

3.1.2. Changes in total volatile base (TVB) and trimethylamine (TMA) contents

Fig.1B and Fig.1C show the changes in TVB and TMA contents of shrimp during 18 h of post-mortem storage at room temperature. TVB and TMA contents of fresh shrimp (0 h) was 6.99 mg N/ g sample and 4.70 mg N/ g sample, respectively. As the post-mortem time increased, both TVB and TMA content continuously increased up to the end of storage (18 h) (P<0.05). After 18 h, the highest TVB (81.55 mg N/ g sample) and TMA content (14.49 mg N/ g sample) were obtained. This was more likely the results of the deterioration of nitrogenous compounds. Trimethylamine oxide (TMAO), a non-volatile and non-odouriferous compound, could be reduced to trimethylamine (TMA) as mediated by spoilage microorganisms (Dissaraphong et al., 2006). In general, the increases in both TVB and TMA contents were in accordance with the increase in pH (Fig.1A). The result suggested that the spoilage caused by bacteria occurred to a higher extent in shrimp, particularly when the storage time increased.

3.1.3. Changes in thiobarbituric acid reactive substances (TBARS)

Changes in TBARS value of shrimp during 18 h of post-mortem storage at room temperature are presented in Fig.1D. TBARS value of fresh shrimp was 0.60 mg MDA/ kg sample. It was increased when post-mortem time increased (P<0.05) and the highest TBARS value (0.97 mg MDA/ kg sample) was found in shrimp with post-mortem time of 18 h (P<0.05). The result suggested that lipid oxidation took place during the extended storage. Shrimp or krill lipids have been known to contain high content of polyunsaturated fatty acid (PUFA) (Takeungwongtrakul et al., 2012). Those PUFA are prone to oxidation as indicated by the presence of TBARS in the samples. Furthermore, autolysis caused by endogenous proteases might lead to the

disruption of the organelles, thereby facilitating the release of pro-oxidants as well as reactants (Dissaraphong et al., 2006). This led to the enhanced lipid oxidation in the sample.

3.1.4. Changes in TCA-soluble peptide

TCA-soluble peptide content of fresh shrimp (0 h) was 63.75 mmol/ g sample (Fig.1E). It was suggested that protein of shrimp rapidly degraded into small peptide or free amino acids after death, particularly during transportation. As the post-mortem time increased, a continuous increase in oligopeptides was observed (P<0.05). After 18 h, TCA-soluble peptide content of shrimp was 77.26 mmol/ g sample. Whole shrimp contained the cephalothorax, where hepatopancreas and other digestive organs were located. Hepatopancreas of Pacific white shrimp was rich in trypsin (Senphan and Benjakul, 2014). Therefore, degradation caused by both indigenous and microbial proteases could be enhanced when shrimp were stored at room temperature for an extended time as evidenced by the higher TCA-soluble peptide contents.

3.1.5. Protein patterns

Fig.2 shows protein patterns of whole shrimp with various post-mortem storage times. Fresh shrimp contained myosin heavy chain (MHC) as the most dominant protein. The band intensity of MHC was gradually decreased when post-mortem storage time increased. MHC still remained at 10-15%, compared to that found in fresh shrimp (0 h), after storage time for 18 h. Sriket et al. (2012) reported that MHC is susceptible to proteolytic degradation than other muscle proteins such as actin, troponin and tropomyosin. For actin (MW of 45 kDa), it was found at lower extent, compared with MHC. It was noted that band intensity of actin remained constant throughout 18 h of storage. Additionally, proteins or peptides with MW about 100 kDa continuously decreased, whilst proteins or peptides with MW about 20 kDa increased with increasing storage time. The result suggested that proteins in shrimp underwent degradation drastically during post-mortem storage. Shrimp cephalothorax containing hepatopancreas has been known to be the major source of proteases, mainly serine protease and metalloprotease (Sriket et al., 2012). Those indigenous proteases along with bacterial proteases played a role in hydrolysis of proteins in shrimp. The formation of low molecular weight peptides was in accordance with the increasing TCA-soluble peptide contents (Fig.1E).

3.1.6 Change in total viable count (TVC)

Fresh shrimp showed initially TVC of 3.22 log colony forming units (CFU)/ g sample (Fig.1F). Microbiological counts of shrimp increased as post-mortem time increased (P<0.05). After 18 h of storage at room temperature, the highest TVC (5.75 log CFU/ g sample) was found. In general, TVC increased markedly when the sample was stored at room temperature, in which mesophiles could grow rapidly. Vanderzant et al. (1973) reported that warm water marine shrimp often showed total aerobic counts of 10^6 CFU/ g sample when captured, but after cleaning process and storage at low temperature, microbial count can be lowered. Those microorganisms might contribute to the final quality of the resulting *Kapi* when used as raw material.

3.2. Effect of post-mortem storage time on characteristics and properties of *Kapi*

3.2.1. pH

pHs of *Kapi* produced from shrimp with different post-mortem storage times are shown in Table1. pH of all samples was in ranges of 7.28-7.54. There were no differences in pH (P>0.05) amongst *Kapi* samples produced from shrimp stored for up to 12 h. Nevertheless, *Kapi* prepared from shrimp stored for 18 h had the highest pH (7.54) (P<0.05). Higher pH of *Kapi* correlated with the increasing pH of shrimp used as raw material, particularly those having the longer post-mortem storage time. This was also in accordance with increasing TVB content in raw material (Fig.1B).

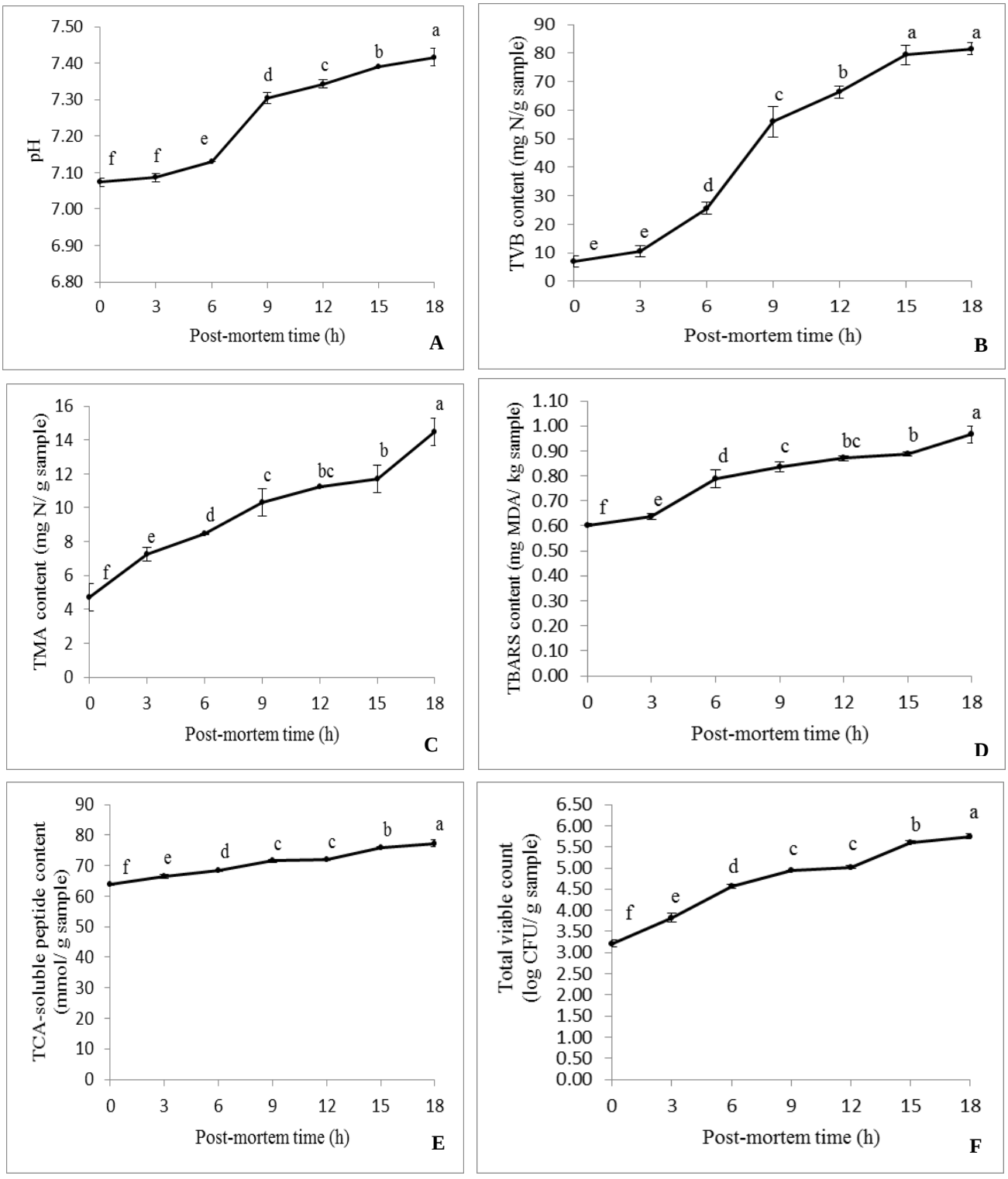

Figure 1. pH (A), total volatile base (TVB) (B), trimethylamine (TMA) contents (C), thiobarbituric acid reactive substances (TBARS) (D), TCA-soluble peptide contents (E) and total viable count (TVC) (F) of shrimp (*M. lanchesteri*) during post-mortem storage at room temperature. Different lowercase letters on the bars indicate the significant difference (P<0.05).

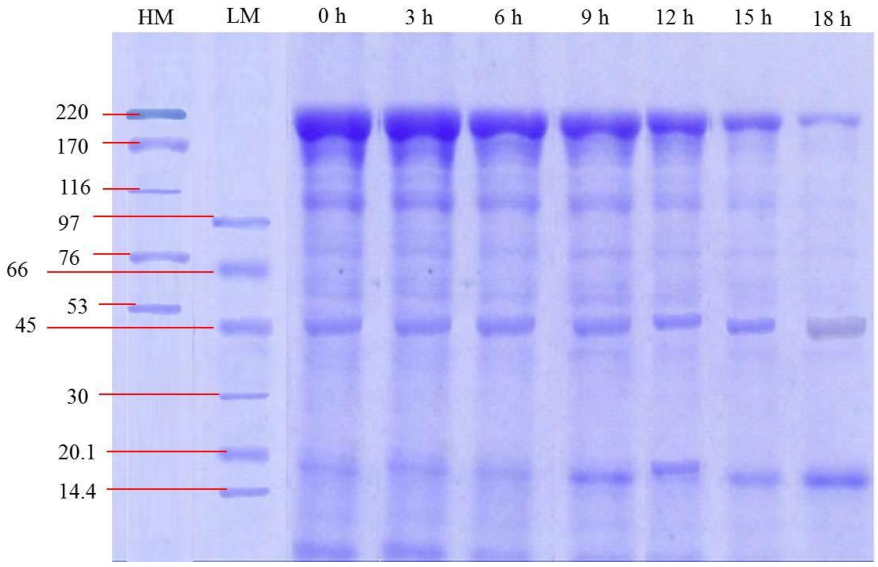

Figure 2. Protein patterns of shrimp (*M. lanchesteri*) during post-mortem storage at room temperature, HM: high molecular weight marker, LM: low molecular weight marker.

Table 1. pH, colour, browning intensity and antioxidative properties of *Kapi* produced from shrimp with different post-mortem times.

Properties	Post-mortem time			
	0 h	6 h	12 h	18 h
pH	7.28±0.53^{b}	7.31±0.10^{b}	7.30±0.35^{b}	7.54±0.46^{a}
*L**	49.76±0.30^{a}	45.95±1.01^{b}	42.94±0.46^{c}	41.76±1.07^{d}
*a**	6.53±0.27^{c}	6.89±0.30^{b}	7.67±0.18^{a}	7.67±0.93^{a}
*b**	13.54±0.49^{a}	11.88±0.29^{b}	11.41±0.17^{c}	9.36±0.45^{d}
Δ*E**	46.32±0.41^{c}	49.58±0.91^{b}	52.50±0.43^{a}	53.25±1.14^{a}
Δ*C**	14.12±0.53^{a}	12.82±0.22^{b}	12.83±0.06^{b}	11.19±0.50^{c}
A_{280}	0.64±0.02^{c}	1.04±0.07^{a}	0.92±0.05^{b}	1.06±0.12^{a}
A_{295}	0.78±0.01^{c}	0.77±0.19^{d}	0.84±0.11^{b}	0.87±0.06^{a}
Browning intensity (A_{420})	0.15±0.03^{d}	0.22±0.04^{c}	0.35±0.02^{b}	0.56±0.01^{a}
Fluorescence intensity	392.67±8.47^{b}	399.34±6.66^{b}	397.22±6.76^{b}	416.85±1.90^{a}
DPPH radical scavenging activity (μmol TE/ g sample)	1.05±0.19^{d}	1.65±0.19^{c}	2.14±0.14^{b}	2.38±0.19^{a}
ABTS radical scavenging activity (μmol TE/ g sample)	10.22±0.13^{d}	12.06±0.17^{c}	13.69±0.13^{b}	15.62±0.21^{a}
FRAP (μmol TE/ g sample)	7.59±0.31^{d}	10.61±0.25^{c}	11.85±0.18^{b}	12.89±0.24^{a}

Mean ± SD from triplicate determinations. Values in parentheses indicate the content expressed, based on dry weight. Different lowercase superscripts in the same row indicate the significant difference (P<0.05).

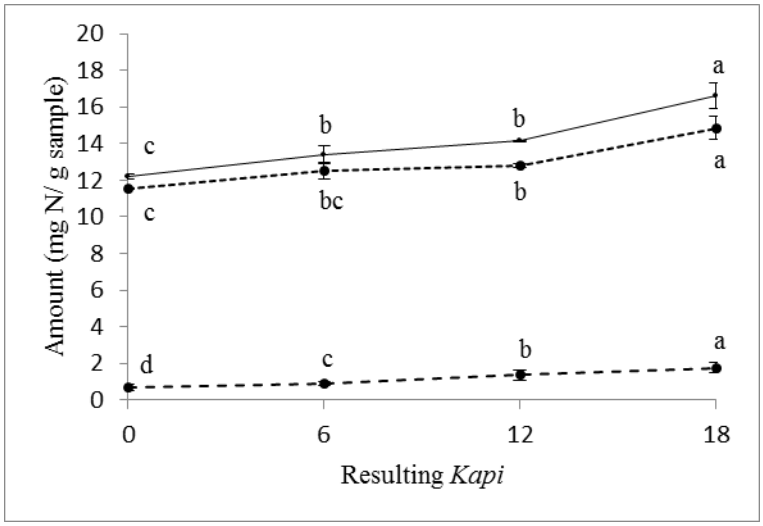

Figure 3. Formal nitrogen content (____), ammonia nitrogen content (.....) and amino nitrogen content (_ _ _ _) of K*api* produced from shrimp with different post-mortem times. Different lowercase letters on the bars within the same parameter indicate the significant difference (P<0.05). Bars represent the standard deviation (n = 3).

3.2.2. Nitrogen content

Formal, ammonia and amino nitrogen contents of resulting *Kapi* are depicted in Fig.3. Formal nitrogen content increased from 12.21 to 16.61 mg N/ g sample as post-mortem time of shrimp used as raw material increased from 0 to 18 h. In general, formal nitrogen content has been used to measure the degree of protein hydrolysis (Faithong and Benjakul, 2012). The results indicated that shrimp having the longer post-mortem storage time yielded *Kapi* with the greater degradation of protein as evidenced by greater formal nitrogen content. The varying degradation of proteins might contribute to characteristics of *Kapi* differently.

Ammonia nitrogen content of all *Kapi* samples increased when the post-mortem time of shrimp used as raw material increased (P<0.05) as shown in Fig.3. *Kapi* produced from shrimp with 18 h of post-mortem time showed the highest ammonia nitrogen content (1.75 mg N/ g sample. The ammonia nitrogen content indicates the breakdown of soluble protein and peptides into free amino acid and volatile nitrogen (Faithong and Benjakul, 2012). Higher degradation of protein might favour the subsequent deamination of proteins, as indicated by higher ammonia nitrogen content. Amino nitrogen contents in *Kapi* using shrimp having various post-mortem times as raw material are depicted in Fig.3. Amino nitrogen content represents the amount of primary amino group of the sample. An increase in amino nitrogen content is related to the degradation of polypeptide (Pongsetkul et al., 2014). A similar trend was found to that of formal and ammonia nitrogen contents. The results suggested that the longer post-mortem storage times of raw material resulted in considerable increases in free amino acids. Those free amino acids might serve as the nutrient for microorganisms. Also they could contribute to the taste or flavour of *Kapi.*

3.2.3. Colour

Kapi produced from shrimp with different post-mortem storage times had differences in colour as shown in Table1. L^* (lightness), a^* (redness), b^* (yellowness), ΔE^* (total difference in colour) and ΔC^* (difference in chroma) were in the range of 41.76-59.76,

6.53-7.67, 9.36-13.54, 46.32-53.25 and 11.19-14.12, respectively. *L**, *b** and Δ*C**-value of *Kapi* decreased when the post-mortem time of shrimp used as raw material increased, whereas *a**, Δ*E**-value of *Kapi* sample slightly increased with increasing post-mortem times of shrimp prior to salting ($P<0.05$). The results indicated that *Kapi* became darker when unfresh shrimp were used as raw material. *Kapi* produced from shrimp with 18 h of post-mortem time had the highest *a**-value, suggesting that the pronounced autolysis might cause the increased release of carotenoids from carotenoprotein during extended post-mortem times of shrimp. Astaxanthin, has a red-orange in colour, especially when it was separated from protein moiety (Faithong and Benjakul, 2012). With the delay in salting, biochemical change, especially enzymatic reactions e.g. polyphenoloxidase (PPO) occurred (Nirmal and Benjakul, 2009). This more likely contributed to the darker colour of resulting *Kapi*

3.2.4. Browning and Maillard reaction product

Browning intensity and Maillard reaction product of water extract of *Kapi* produced from shrimp with different post-mortem storage times are shown in Table1. Browning intensity (A_{420}) of resulting *Kapi* was observed when there was the delay in salting of shrimp ($P<0.05$). During extended post-mortem times, enzymatic browning reactions induced by polyphenoloxidase (PPO) occurred (Pongsetkul et al., 2014). PPO has been known to induce the hydroxylation of phenols with subsequent polymerisation, in which melanin is formed (Nirmal and Benjakul, 2009). Therefore, prolonged post-mortem times prior to salting might result in substantial increases in browning intensity. This coincided with the decreases in *L**-value (Table1).

A_{280} and A_{295} of resulting *Kapi* slightly increased when the post-mortem time of shrimp used as raw material increased ($P<0.05$). Generally, A_{280} and A_{295} have been used to determine the formation of non-fluorescent intermediate compounds of the Maillard reaction (Ajandouz et al., 2001). With larger amount of free amino group in shrimp stored for a longer time, the Maillard reaction could take place during fermentation of 30 days to a higher degree. This was evidenced by higher A_{280} and A_{295}.

No differences in fluorescence intensity among *Kapi* produced from shrimp stored for 0, 6 and 12 h ($P>0.05$). Nevertheless, the increase in fluorescence intensity was noticeable in *Kapi* produced from 18 h-stored shrimp. Fluorescence intensity has been used to monitor the occurrence of intermediate products, which subsequently undergo polymerisation to form the brown pigments (Ajandouz et al., 2001). With higher Maillard reaction intermediates, *Kapi* produced from shrimp with 18 h of post-mortem time could further undergo browning reaction to a higher extent. As a result, *Kapi* was browner in colour.

3.2.5. Antioxidative activities

Antioxidant activities of water extract of *Kapi* produced from shrimp with different post-mortem times as tested by DPPH, ABTS radical scavenging activity and FRAP are presented in Table1. DPPH, ABTS radical scavenging activity and FRAP of water extracts of *Kapi* were in range of 1.05-2.38, 10.22-15.62 and 7.59-12.89 μmol TE/ g sample, respectively.

ABTS radical scavenging assay has been used to determine both hydrophilic and lipophilic antioxidants. FRAP is generally used to measure the capacity of a substance in reducing TPTZ-Fe(III) complex to TPTZ-Fe(II) complex (Sun and Tanumihardjo, 2007).

Overall, DPPH, ABTS radical scavenging activity and FRAP of water extracts of *Kapi* increased when the post-mortem time of shrimp used as raw material for *Kapi* production increased ($P<0.05$). Water extract of *Kapi* produced from shrimp with 18 h of post-mortem time showed the highest DPPH, ABTS radical scavenging activity and FRAP. On the other hand, the extract of *Kapi* produced by fresh shrimp (0 h) had the lowest activities.

During the extended post-mortem storage prior to salting, low molecular weight peptides and amino acids could be generated to a higher

extent. Protein hydrolysis or degradation during fermentation step also led to the formation of substantial active peptides which could interact with free radical and terminate the chain reaction of auto-oxidation.

This correlated with pronounced degradation of proteins in shrimp stored for a longer time before salting (Fig.2). The extracts from salted shrimp paste have been reported to possess antioxidant activities (Pongsetkul et al., 2015). Furthermore, Maillard reaction products might be partially involved in antioxidant activity. Maillard reaction products were reported to have antioxidative activity (Benjakul et al., 2005). Maillard reaction products were also increased in *Kapi* prepared from unfresh (Table1). Therefore, antioxidative activity of *Kapi* was governed by post-mortem time of shrimp prior to salting.

3.2.6. Volatile compounds

Volatile compounds in *Kapi* produced from shrimp with different post-mortem times analysed by SPME GC-MS are shown in Table2. Thirty-six volatile compounds were isolated and identified. Those compounds could be classified as aldehydes (5), ketones (6), alcohols (9), nitrogen-containing compounds (7), hydrocarbons (4) and others (5). Lipid-derived components such as aldehydes, alcohols as well as nitrogen-containing compounds were the major volatile compounds in *Kapi.*

3-methyl-butanal, pentanal, 4-heptanal, hexanal and benzaldehyde were the prevalent aldehydes found in all *Kapi* samples. *Kapi* with varying abundance of aldehydes might influence the flavour acceptability differently. In general, the presence of these compounds is related with lipid oxidation, which was more likely generated during storage or fermentation (Dissaraphong et al., 2006). Shrimp contained high amounts of ω-3 fatty acids, which were highly susceptible to lipid oxidation (Takeungwongtrakul et al., 2012). *Kapi* produced from shrimp stored for 18 h before salting showed higher abundance in all aldehydes, except 4-hexanal.

This was coincidental with the highest TBARS value of shrimp used as raw material stored for 18 h (Fig.1D). Furthermore, Steinhaus and Schieberle (2007) reported that branched short chain aldehydes or aromatic aldehydes plausibly resulted from deamination of amino acids. This was also in agreement with the highest amino nitrogen content found in this sample (Fig.3). Ketones were also found in *Kapi* including 2-heptanone, 1-(2-pyridinyl)-ethanone, 3,5-Octadien-2-one, etc. The higher abundance of most ketone compounds was obtained in *Kapi* produced from shrimp with 18 h of post-mortem time. This confirmed that delayed post-mortem salting allowed more lipid oxidation in raw material to take place. Those products were still presented in resulting *Kapi.* However, such compounds with low concentrations and high odour threshold values might not significantly contribute to flavour of salted shrimp paste (Cha and Cadwallader, 1995). Normal and branched alcohols, which were quite low in abundance, also detected in *Kapi*. 3-methyl, 1-butanol was found at higher abundance than others. This alcohol was increased in *Kapi* prepared from shrimp with increasing post-mortem times. However, Cha and Cadwallader (1995) reported that alcohols might not have a paramount impact on *Kapi* flavour because of their high flavour thresholds. All resulting *Kapi* consisted of 7 nitrogen-containing compounds, which were all pyrazine derivatives. 2-ethyl-5-methyl-pyrazine was dominant in all samples, followed by 2,5-dimethyl-pyrazine and 3-ethyl-2,5-dimethyl-pyrazine. Jaffres et al. (2011) reported that pyrazines derivatives associated with meaty flavour of shrimp sauce. Abundance of most pyrazine derivatives of resulting *Kapi* increased when the post-mortem time of shrimp used as raw material increased (P<0.05). Pyrazines were reported to be formed by Maillard reaction through strecker degradations from various nitrogen sources such as amino acids (Cha and Cadwallader, 1995).

Table 2. Volatile compounds of *Kapi* produced from shrimp with different post-mortem times.

Volatile compounds	Peak area (Abundance) × 10^6			
	0 h	6 h	12 h	18 h
Aldehydes				
3-methyl-butanal	215.22	206.11	411.12	732.51
Pentanal	294.58	199.76	408.81	466.25
4-heptanal	117.22	132.99	130.19	98.66
Hexanal	54.51	501.11	511.19	526.63
Benzaldehyde	162.8	203.42	452.61	688.22
Ketones				
1-phenyl-ethanone	128.71	134.34	205.55	205.13
1-(2-pyridinyl)-ethanone	237.93	304.49	314.51	505.82
1-(2-aminophenyl)-ethanone	150.54	68.88	102.25	111.33
2-Heptanone	597.82	413.22	499.65	404.52
2-Octanone	265.60	244.12	300.64	305.77
3,5-Octadien-2-one	117.14	332.26	304.55	502.65
Alcohols				
Benzenemethanol	42.95	35.55	69.32	167.83
2-Butoxy-ethanol	56.14	181.22	555.92	608.73
3-methyl, 1-butanol	299.19	625.55	632.72	820.24
1-Pentanol	225.61	203.44	144.41	28.32
1-Penten-3-ol	188.13	55.67	59.11	9.88
1-Hexanol	404.33	133.92	129.29	266.51
2-ethyl-hexanol	122.97	166.92	208.82	277.01
1-Octen-3-ol	171.89	204.45	229.33	301.99
Octa-1,5-dien-3-ol	57.04		106.66	169.72
Nitrogen-containing compounds				
Methyl-pyrazine	214.34	506.66	511.23	495.95
Trimethyl-pyrazine	252.17	345.52	304.41	406.77
2,5-dimethyl-pyrazine	898.28	933.49	925.67	1055.61
2,6-dimethyl-pyrazine	90.42	215.62	307.66	313.49
2-ethyl-5-methyl-pyrazine	1524.77	1866.17	1908.22	1855.52
2-ethyl-2,5-dimethyl-pyrazine	155.54	260.08	477.71	503.31
3-ethyl-2,5-dimethyl-pyrazine	644.91	601.98	802.22	899.13
Hydrocarbon				
2,6,10,14-tetramethyl-pentadecane	21.53		19.22	111.19
2-Undecyne		13.31	455.63	109.92
Hexadecane			61.63	13.34
Cyclododecane	23.98	50.66	45.61	77.84
Others				
2-methyl-propanoic acid	68.42	56.33		23.18
Octanoic acid	126.10	15.03		102.31
Methyl-ester-octadecanoic acid		19.44	18.49	
Phenol	261.08	505.55	635.48	904.41
1H-Indole	711.41	805.92	8908.23	9181.46

ND: non-detectable * Value in the parenthesis represent the abundance of compound in each sample.

This was in accordance with increasing browning intensity when post-mortem time of shrimp was extended. Thus, different freshness of raw material more likely had the influence on meaty flavour, as well as browning colour to some degrees.

Four hydrocarbon compounds were found in *Kapi*. Only cyclododecane was detected in all *Kapi. Kapi* produced from shrimp with 18 h of post-mortem time showed the higher abundance than others. Alkanes and alkenes are mainly formed from lipid autooxidation of fatty acids released from triglycerides (Latorre-Moratalla et al., 2011).

Additionally, all samples contained phenol and indole and *Kapi* produced from shrimp with 18 h of post-mortem times had the highest abundance than other samples. Cha and Cadwallader (1995) reported that phenol give an undesirable aroma in seafoods, whilst Lakshmanan et al. (2002) reported that indole is the degradation product from tryptophan and has been used as the index for shrimp spoilage. The higher abundance of indole in *Kapi* correlated well with the increased spoilage of shrimp as indicated by increased TVB, TMA as well as TVC.

Therefore, several reactions might be involved in formation of volatiles. Lipid hydrolysis and autooxidation, proteolysis and transformation of amino acids to several compounds could be generated during *Kapi* production. *Kapi* produced from shrimp with different post-mortem times showed varying volatile compounds. Thus, post-mortem times of shrimp used as raw material might have a great influence on the volatile composition which impact on the final flavour and odour of resulting *Kapi*.

Table 3. Likeness score of *Kapi* produced from shrimp with different post-mortem times.

Attributes	Post-mortem time			
	0 h	6 h	12 h	18 h
Appearance	6.86±0.77^{a}	6.81±0.93^{a}	6.92±0.87^{a}	6.95±0.74^{a}
Colour	6.90±0.83^{a}	7.05±0.92^{a}	7.24±0.83^{a}	7.14±0.91^{a}
Odour	6.90±0.97^{ab}	7.38±1.02^{a}	6.52±0.93^{b}	5.81±1.08^{c}
Texture	6.95±0.80^{a}	7.00±0.95^{a}	7.05±0.97^{a}	6.57±0.68^{a}
Flavour	6.67±1.08^{ab}	7.10±0.70^{a}	6.38±0.74^{b}	5.48±1.02^{c}
Overall	6.57±0.68^{b}	7.29±0.96^{a}	6.19±1.27^{bc}	5.86±1.02^{c}

Values are given as mean ± SD (n = 3).
Score are based on a 9-point hedonic scale (1: Dislike extremely, 5: Neither like nor dislike, 9: Like extremely).
Different lowercase superscripts within the same row indicate the significant differences (P<0.05).

3.2.7. Sensory properties

Likeness scores of *Kapi* produced from shrimp with different post-mortem times are shown in Table3. Amongst all samples, appearance, colour and texture likeness scores were not different (P>0.05). However, *Kapi* produced from shrimp with 18 h of post-mortem time showed the lowest odour and flavour likeness score (P<0.05). The result suggested that this sample might have undesirable volatiles, which were associated with deterioration products, particularly formed during the extended post-mortem storage prior to salting. This was in agreement with the highest abundance of aldehydes, ketones, phenol along with indole found in this sample. Furthermore, *Kapi* produced from shrimp stored for 18 h prior to salting also had the lowest overall likeness score (P<0.05). Different tastes or flavors were possibly caused by differences in volatile compounds (Table2), governed by freshness of raw material used. It was noted that *Kapi* prepared from shrimp stored for 6 h prior to salting showed the

highest overall likeness score (P<0.05). This might be caused by partial decomposition of nucleotides, in which some derivatives, especially inosine monophosphate (IMP) was formed. IMP has been known to have the umami taste (Hajeb and jinap, 2015). Non-significantly higher score for flavour likeness was also found in this sample. Based on overall likeness score, shrimp should not be stored more than 12 h before salting.

4. Conclusions

Small shrimp (*M. lanchesteri*) could be used as an alternative raw material for *Kapi* production. Protein degradation as well as lipid oxidation proceeded during the extended storage. Freshness of shrimp affected *Kapi* characteristics, in which *Kapi* became darker but had higher antioxidative activity as the raw material was unfresh. The delay in salting of shrimp should not exceed 12 h, when the resulting *Kapi* still had the sensorial property equivalent to that prepared from fresh shrimp.

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