

Microbiological and chemical changes of shrimp *Acetes vulgaris* during *Kapi* production

Jaksuma Pongsetkul¹ · Soottawat Benjakul¹ · Kitiya Vongkamjan¹ · Punnanee Sumpavapol¹ · Kazufumi Osako²

Revised: 17 July 2017 / Accepted: 10 August 2017 / Published online: 4 September 2017
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Abstract Microbiological and chemical changes in shrimp *Acetes vulgaris* during production of *Kapi* (salted shrimp paste of Thailand) including salting, drying and fermentation were monitored. Moisture content of samples decreased rapidly after salting and drying steps. The lower water activity was found in the final product (0.694). The pH decreased within the first 10 days of fermentation and continuously increased as fermentation progressed. Protein underwent degradation throughout *Kapi* production as indicated by increasing TCA-soluble peptides and degree of hydrolysis. The increases in peroxide value as well as thiobarbituric acid reactive substances value revealed that lipid oxidation occurred throughout all processes. Total viable count, halophilic, proteolytic and lipolytic bacteria counts increased continuously during *Kapi* production, while lactic acid bacteria count slightly decreased at the final stage of fermentation. Thus, proteolysis and lipolysis took place throughout *Kapi* production, and contributed to the characteristics of finished product. These changes were governed by both endogenous and microbial enzymes.

Keywords Salted shrimp paste · *Kapi* · Fermentation · *Acetes vulgaris* · Microbial population

Introduction

Kapi, a purplish gray or dark grayish brown salted shrimp paste of Thailand, has been widely used as a condiment in several Thai foods (Pongsetkul et al. 2014). *Kapi* is known with different names in each region, e.g. *Terasi Udang* in Indonesia, *Belacan* in Malaysia, *Bagoong-alamang* in the Philippines, *Mam ruoc* in Vietnam, etc. (Hajeb and Jinap 2015). Although those products were different in term of raw material, the ratio of salt used, fermentation process and time, they can provide a salty and umami taste to cuisines. Different shrimp are used for *Kapi* production in various regions. Stocks of krill (*Mesopodopsis orientalis*), a traditional raw material for *Kapi* production, have decreased by 3% per year since 1990 (Meland and Willassen 2007). Other raw materials for *Kapi* production have been researched. *Acetes vulgaris* is one of small shrimp found in the southern part of Thailand throughout the years (Pongsetkul et al. 2015). Our previous study revealed that *Kapi* prepared from *A. vulgaris* had comparable sensory properties to commercial products (Pongsetkul et al. 2015).

For *Kapi* production, there are 2 major ingredients including shrimp and salt. The ratio between shrimp and salt varies from 1:6 to 1:2 (w/w) (Hajeb and Jinap 2015). After salting overnight, salted shrimp is dried with sunlight, ground into a fine paste and fermented under anaerobic condition at ambient temperature (28–30 °C) up to 30 days or until the typical aroma is formed (Faithong and Benjakul 2012). Salting and drying are two ancient practices used for food preservation and yield a particular flavor (Rodrigo et al. 1998). During fermentation, microbiological and biochemical change occur. Proteolysis is an essential biochemical reaction occurring during the fermentation of *Kapi*. It is induced by endogenous proteases in shrimp as well as those produced by halophilic bacteria

✉ Soottawat Benjakul
soottawat.b@psu.ac.th

¹ Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand

² Department of Food Science and Technology, Tokyo University of Marine Science and Technology, 5-7 Konan 4, Minato-ku, Tokyo 108-8477, Japan

surviving under high salt condition (Peralta et al. 2008). Proteolysis affects both texture and flavor of fermented products by inducing the formation of low molecular weight compounds, e.g. peptides, amino acids, aldehydes, organic acids and amines (Mizutani et al. 1987). Lipolysis during fermentation is also important since free fatty acids (FFA) released undergo oxidation, responsible for aroma development (Lizaso et al. 1999). Oxidation products include aldehydes and ketones (Takeungwongtrakul and Benjakul 2013) and more likely contribute to the development of typical taste and flavor of fermented product (Lizaso et al. 1999). Furthermore, Amano (1962) revealed that growth of anaerobes in high salt environment contributes to the typical flavor of fermented products. Initial microflora, in both the raw materials and salt, have the influence on the numbers of microorganisms in final product, especially during the early stages of fermentation (Rodrigo et al. 1998). Microaerophilic or anaerobic conditions with high salt during *Kapi* fermentation could favor the proliferation of some bacteria, leading to different flavor or odor in the finished products. However, a little information concerning chemical and microbiological changes during *Kapi* processing/fermentation exists. Therefore, this study aimed to monitor the changes in chemical compositions and microbial populations of shrimp *A. vulgaris* during *Kapi* production.

Materials and methods

Sample collection and preservation

Shrimp (*A. vulgaris*) were caught off and collected from the Ko-yo area, Songkhla province, Thailand. After capture, shrimp were delivered to the Department of Food Technology, Prince of Songkla University, Hat Yai, Thailand, in ice using a shrimp/ice ratio of 1:2 (w/w) in a polystyrene container, within approximately 2 h.

Preparation of *Kapi*

Upon arrival, shrimp [R] were mixed with solar salt at the ratio of 5:1 (w/w). The mixture was placed in the basket, covered with a cheese cloth and allowed to salting overnight at room temperature (28–32 °C) [S]. Subsequently, salted shrimp were drained, mashed, followed by spreading out on fiberglass mats to dry with sunlight until the moisture content reached 35–40% [D]. After drying, samples were compacted into earthen jars and covered with plastic bag tightly (in the close system). The samples were allowed to ferment at room temperature (28–32 °C) for 1 month. During fermentation, the samples were taken at day 10 [F1], 20 [F2] and 30 [F3] of fermentation. All

samples were subjected to determination of chemical composition and microbial load.

Changes in chemical compositions

Moisture content, water activity (A_w) and pH

Moisture content of all samples was measured as per AOAC method (2000) with the analytical No. of 35.1.13. A_w was determined by a water activity analyzer (Thermoconstanter, Novasina, Switzerland). The pH of samples was analyzed by a pH meter (Sartorius, Gottingen, Germany) following the method of Nirmal and Benjakul (2009).

Free amino acid composition

Free amino acid composition of samples was determined according to the method of Minh-Thuy et al. (2014). Free amino acids were firstly extracted using 6% (v/v) perchloric acid. The extracts were subsequently neutralized and filtrated as per the method of Minh-Thuy et al. (2014). The filtrate was used for amino acid analysis using an amino acid analysis system (Prominence; Shimadzu, Kyoto, Japan) equipped with a column (Shim-pack Amino-Li, 100 mm × 6.0 mm i.d.; column temperature, 39.0 °C; Shimadzu) and pre-column (Shim-pack ISC-30/S0504 Li, 150 mm × 4.0 mm i.d.; Shimadzu). Amino acids were detected using a fluorescence detector (RF-10AXL; Shimadzu). The content was reported in term of mg/g dry weight sample.

TCA-soluble peptide content

TCA-soluble peptide content of samples was determined according to the method of Pongsetkul et al. (2015). Ground sample (3 g) was homogenized with 27 mL of cold 5% TCA using a homogenizer at a speed of 11,000 rpm for 1 min. The homogenate was stored in ice for 30 min, followed by centrifugation at 5000 g for 20 min at 4 °C using a refrigerated centrifuge (Model RC-B Plus centrifuge Newtown, CT). Soluble oligopeptide content in the supernatant was measured according to the Lowry method (Lowry et al. 1951) and expressed as mmol tyrosine equivalent/g dry weight sample.

Degree of hydrolysis (DH)

DH of all samples was determined following the method of Benjakul and Morrissey (1997). DH was calculated and expressed as the percentage as follows:

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

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

Peroxide value (PV)

PV was determined as described by Sae-leaw et al. (2013). Cumene hydroperoxide at a concentration range of 0.5–2 ppm was used for standard preparation. PV was calculated and expressed as mg hydroperoxide/kg dry sample.

Thiobarbituric acid reactive substances (TBARS)

TBARS value was examined following the method of Nirmal and Benjakul (2009). A standard curve was prepared using malondialdehyde bis (dimethyl acetal) (0–2 ppm). TBARS value was calculated and expressed as mg malondialdehyde (MDA)/kg dry sample.

Changes in microbial load

Total viable count

Total viable count was determined using a standard plate count agar containing 10% NaCl (pH 7.5) and the incubation was performed for 3–5 days at 30 °C (BAM 2001). Samples (25 g) were mixed with 225 mL of peptone water containing 10% (w/v) NaCl in a Stomacher 400 Lab Blender (Seward Ltd., Worthing, UK) at high speed for 3 min. Peptone water containing 10% (w/v) NaCl was used for sample dilution. The sample appropriately diluted in serial tenfold steps was used for analysis by the spread plate technique. Microbial load was reported as colony forming units/g dry sample (CFU/g dry sample).

Halophilic bacteria count

Halophilic bacteria content was measured using JCM media (Namwong et al. 2009). Diluted sample (0.1 mL) was applied on the surface of media, spread and incubated for 5–7 days at 30 °C.

Proteolytic bacteria count

Proteolytic bacteria count was determined using a standard plate count agar having 10% NaCl and 1% (w/v) sodium caseinate (pH 7.5) with the incubation for 3–5 days at 30 °C (Tanasupawat et al. 2011). Proteolytic bacteria, which showed clear zone around colonies on plate, were counted.

Lipolytic bacteria count

Lipolytic bacteria count was determined using a standard plate count agar including 10% NaCl and 1% (w/v) tributyrin (pH 7.5). The incubation for 3–5 days at 30 °C was

performed (Chappe et al. 1994). Lipolytic bacteria showing clear zone around colonies on plate were counted.

Lactic acid bacteria (LAB) count

LAB count was determined using De Man, Rogosa, and Sharpe (MRS) agar containing 10% NaCl following the method of Tanasupawat et al. (2011). Aliquot of 0.1 ml of appropriately decimally diluted samples was introduced on MRS agar containing CaCO₃ (1%) and 10% NaCl (pH 7.5) and incubated for 3–5 days at 30 °C.

Statistical analysis

All experiments were conducted in triplicate using three lots of samples. Statistical analysis was done by using one-way analysis of variance (ANOVA). Mean comparison was conducted using the Duncan's multiple range test (Steel et al. 1980). Analysis was performed using the SPSS package (Version 10.0) (SPSS for windows, SPSS Inc., Chicago, IL, USA).

Result and discussion

Changes in chemical compositions of shrimp during *Kapi* production

Changes in moisture content, water activity (A_w) and pH

Moisture content of shrimp during *Kapi* production was monitored (Fig. 1a). Fresh shrimp had the moisture content of 83.53%. Moisture content of shrimp after being salted overnight was rapidly lowered to 66.03% ($P < 0.05$). During salting, salt was able to penetrate into the shrimp meat, whereas water was removed from shrimp via the osmotic pressure. This led to the decrease in moisture content (Chaijan 2011). During the first 10 h of fish flesh salting, water content decreased more rapidly during dry salting and the amount of exudate was related with lowered moisture content (Bellagha et al. 2007). Moisture content of salted shrimp sharply decreased after drying with the sunlight and low moisture content (32.43%) was obtained after this process. Moisture content of samples was slightly increased after the first 10 days of fermentation ($P < 0.05$). Partially dried sample more likely absorbed the water vapor from the environment to some degree. However, no difference was observed among samples as fermentation time was extended up to 30 days ($P > 0.05$). Moisture content directly affected the consistency of *Kapi*. In general, consistency of commercial *Kapi* was varied from soft and pasty to dry and hard, depending upon the processes used (Faithong et al. 2010). Moisture content was within the moisture range of commercial *Kapi* produced in

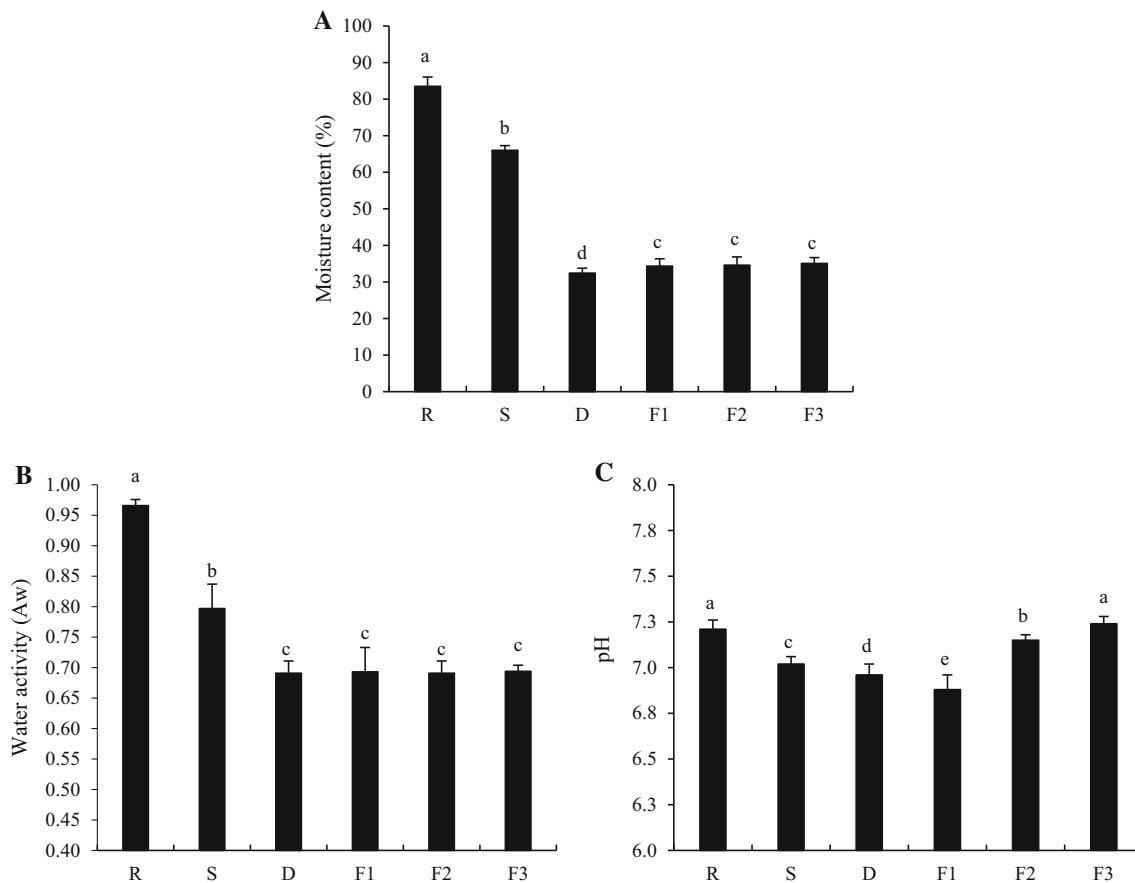


Fig. 1 Changes in moisture content (a), water activity (A_w) (b) and pH (c) of shrimp during *Kapi* production. Bars represent the standard deviation ($n = 3$). R raw material, S after salting, D after drying, F1,

F2 and F3 after fermentation for 10, 20 and 30 days. Different letters on the bars indicate the significant differences ($P < 0.05$)

Thailand (33.79–52.54%) (Pongsetkul et al. 2014). After 30 days of fermentation, our product had the moisture content of 35.07%. This was associated with texture, because it became slightly dry and hard. Moreover, the result also suggested that salting and drying step for *Kapi* production were the main steps to reduce the moisture content.

Changes in water activity (A_w) of all samples exhibited the similar trends with those of moisture content throughout *Kapi* production processes as depicted in Fig. 1b. Decreases in A_w of shrimp during salting and drying were observed ($P < 0.05$) and no changes in A_w were obtained during 30 days of fermentation ($P > 0.05$). A_w of final product (after 30 days of fermentation) was 0.694, corresponding to low moisture content. Food having A_w range of 0.6–0.7 could be classified as an intermediate moisture food (Fennema 1996). Since *Kapi* samples had A_w in this range, the growth of food pathogens and spoilage microorganisms could be retarded.

Changes in the pH of shrimp throughout all processes were found. Fresh shrimp had the pH of 7.21. pH of sample was slightly dropped during salting (7.02) and drying (6.96) ($P < 0.05$) as shown in Fig. 1c. Continuous decrease in pH

within the first 10 days of fermentation was observed ($P < 0.05$). Some microorganisms, particularly LAB, were able to produce some acids, which could result in pH lowering. These LAB produced lactic acid, leading to the decrease in pH of samples. However, pH turned to increase when fermentation time was more than 20 days ($P < 0.05$). This was related with the decrease in LAB. Furthermore, the increase of pH during fermentation was plausibly associated with the formation of degradation products or volatile base compounds such as ammonia produced by endogenous or microbial enzymes (Pongsetkul et al. 2015). After 30 days of fermentation, the pH of final product was 7.24.

Changes in free amino acid composition

Free amino acid composition of shrimp throughout all processes is given in Table 1. Total free amino acid (TFAA) of fresh shrimp was 44.75 mg/g dry sample. There was no change in TFAA when fresh shrimp were subjected to salting and drying ($P > 0.05$). However, TFAA was continuously increased during fermentation and reached 64.66 mg/g dry sample after the fermentation was

performed for 30 days ($P < 0.05$). Change in total essential amino acid (TEAA) was fluctuated during *Kapi* production, but there were no differences between fresh shrimp and final product ($P > 0.05$). On the other hand, total non-essential amino acid (TNEAA) contents were different among samples ($P < 0.05$). The increases in TNEAA during all processes were in agreement with the increases in TFAA. TNEAA reached 49.17 mg/g dry sample after 30 days of fermentation.

Fresh shrimp *A. vulgaris* contained Glu/Gln, Arg, Asp/Asn, Ala, Pro and Gly as the major free amino acids and their total amount was more than 50% of total free amino acids. The result was in agreement with the other shrimp species including *Acetes chinensis* (Chung and Lee, 1976),

Penaeus notialis (Akintola, 2015), *Penaeus kerathurus* (Zlatanov et al. 2009), etc. It was noted that changes in amount of each amino acid varied during different processes. As processing proceeded, some amino acids, especially Glu/Gln, Asp/Asn increased, while other amino acids, particularly Ile, Arg and Ser decreased. Some amino acids, which were not found in fresh sample, were generated during *Kapi* processing. Those included Trp, Ans, Car, Cit, Cys, etc. These changes in free amino acid during processing might be influenced by endogenous proteases in raw material and microbial proteases proliferated during processes. Our previous study revealed that proteolytic activity of fresh shrimp was decreased after being salted and dried, but continuously increased during fermentation

Table 1 Changes in free amino acid composition of shrimp during *Kapi* production

Free amino acid composition (mg/g dry weight sample)	Sample					
	R	S	D	F1	F2	F3
Essential amino acid						
Histidine (His) ^a	0.89 ± 0.01ab ^{*,**}	0.93 ± 0.02a	0.85 ± 0.01b	0.54 ± 0.01c	0.55 ± 0.07c	0.59 ± 0.01c
Isoleucine (Ile)	1.56 ± 0.03a	1.30 ± 0.01b	1.32 ± 0.01b	1.21 ± 0.02c	1.18 ± 0.01c	1.18 ± 0.01c
Leucine (Leu)	2.71 ± 0.01c	2.81 ± 0.01c	3.25 ± 0.01b	3.99 ± 0.01a	3.99 ± 0.03a	4.05 ± 0.01a
Lysine (Lys)	2.88 ± 0.03 cd	3.01 ± 0.01c	3.03 ± 0.01c	3.95 ± 0.05b	4.26 ± 0.02a	4.38 ± 0.03a
Methionine (Met)	0.72 ± 0.01b	0.75 ± 0.02b	0.72 ± 0.00b	1.12 ± 0.01a	1.09 ± 0.01a	1.16 ± 0.01a
Phenylalanine (Phe)	2.10 ± 0.06a	2.09 ± 0.04a	2.24 ± 0.01a	2.05 ± 0.01a	1.66 ± 0.01b	1.43 ± 0.01b
Threonine (Thr)	1.98 ± 0.02a	1.72 ± 0.04a	1.33 ± 0.01a	0.05 ± 0.00b	0.08 ± 0.01b	0.03 ± 0.00b
Tryptophan (Trp)	ND	ND	ND	ND	0.03 ± 0.00a	0.03 ± 0.01a
Valine (Val)	2.34 ± 0.01b	2.32 ± 0.01b	2.36 ± 0.04b	2.55 ± 0.03a	2.39 ± 0.02ab	2.64 ± 0.03a
Total EAA	15.18 ± 0.06a	14.93 ± 0.04b	15.10 ± 0.03b	15.46 ± 0.05a	15.23 ± 0.06a	15.49 ± 0.03a
Non-essential amino acid						
Alanine (Ala)	3.57 ± 0.02c	3.58 ± 0.04c	4.1 ± 0.04b	5.23 ± 0.06a	5.62 ± 0.04a	5.66 ± 0.02a
Anserine (Ans)	ND	ND	ND	ND	0.03 ± 0.00b	0.06 ± 0.00a
Arginine (Arg)	5.16 ± 0.06a	5.02 ± 0.03a	4.55 ± 0.01b	4.05 ± 0.01c	4.05 ± 0.04c	3.14 ± 0.01d
Aspartic acid/Asparagine (Asp/Asn)	4.13 ± 0.04c	4.55 ± 0.01c	4.53 ± 0.04c	6.69 ± 0.08b	8.04 ± 0.09a	8.51 ± 0.04a
Carnosine (Car)	ND	ND	ND	ND	ND	0.03 ± 0.00
Citrulline (Cit)	ND	ND	ND	ND	0.07 ± 0.00a	0.09 ± 0.01a
Cysteine (Cys)	ND	ND	0.04 ± 0.01c	0.06 ± 0.01b	0.06 ± 0.00b	0.12 ± 0.01a
Glycine (Gly)	3.31 ± 0.02d	3.54 ± 0.01c	3.91 ± 0.05a	3.55 ± 0.01c	3.75 ± 0.03b	3.66 ± 0.02bc
Glutamic acid/Glutamine (Glu/Gln)	5.42 ± 0.10e	5.35 ± 0.04e	6.02 ± 0.04d	9.11 ± 0.03c	14.15 ± 0.07b	18.18 ± 0.05a
Hydroxylysine (Hyl)	ND	ND	ND	ND	ND	0.02 ± 0.00
Hydroxyproline (Hyp)	ND	ND	ND	ND	ND	ND
Ornithine	ND	ND	ND	1.04 ± 0.01b	1.15 ± 0.01b	2.02 ± 0.02a
Proline (Pro)	3.48 ± 0.02c	3.55 ± 0.01c	3.28 ± 0.02c	4.22 ± 0.01b	4.59 ± 0.02ab	5.01 ± 0.01a
Serine (Ser)	2.94 ± 0.05a	2.05 ± 0.02b	2.15 ± 0.01b	1.64 ± 0.02c	0.92 ± 0.00d	0.93 ± 0.01d
Taurine (Tau)	0.02 ± 0.00c	0.02 ± 0.00c	0.02 ± 0.00c	0.07 ± 0.01a	0.05 ± 0.00b	0.05 ± 0.00b
Tyrosine (Tyr)	1.54 ± 0.01d	1.88 ± 0.01b	1.95 ± 0.01ab	2.11 ± 0.01a	1.82 ± 0.01b	1.69 ± 0.03c
Total NEAA	29.57 ± 0.08d	29.54 ± 0.03d	30.58 ± 0.04d	37.77 ± 0.09c	44.3 ± 0.09b	49.17 ± 0.04a
Total free amino acid (TAA)	44.75 ± 0.08d	44.47 ± 0.06d	45.68 ± 0.05d	53.23 ± 0.09c	59.53 ± 0.10b	64.66 ± 0.05a

^aEssential amino acid in children

R raw material, S after salting, D after drying, F1, F2 and F3 after fermentation for 10, 20 and 30 days

*Mean ± SD (n=3)

**Different letters in the same row indicate the significant difference ($P < 0.05$)

up to 30 days (Pongsetkul et al. 2016). Those proteases more likely played a profound role in protein degradation, thus contributing to the liberation of free amino acids throughout *Kapi* production. The decrease or fluctuation of amino acids might be from the utilization of those amino acids by microorganisms for their growth.

Kapi fermented for 30 days contained Glu/Gln and Asp/Asn as the dominant free amino acids. The sharp increases in Glu/Gln and Asp/Asn were observed during fermentation and reached 18.18 and 8.51 mg/g dry sample, respectively ($P < 0.05$) at day 30 of fermentation. This result was in accordance with the high amount of Glu/Gln and Asp/Asn in *belacan* (Hajeb and Jinap 2015), *terasi* (Mizutani et al. 1987), *seinsanga-pi* (Tyn 1983) and *bagoong-alamang* (Peralta et al. 2008), shrimp pastes of Malaysia, Indonesia, Myanmar and the Philippines, respectively. In general, the taste and odor of salted shrimp paste were governed by amino acids (Hajeb and Jinap 2015). Some amino acids known as sweet compounds for fermented foods included Lys, Pro, Ala (Chung and Lee, 1976), Asp (Kim et al. 2005), Gly, Ser and Thr (Liu, 1989). Glu/Gln were associated with meaty or umami taste as reported by Chung and Lee (1976).

Based on the result, free amino acid compositions of final *Kapi* product was similar to those found in raw material used, inferring that different raw material with different amino acids more likely yielded *Kapi* with various tastes or flavors. However, those amino acids could be altered during processing, especially during fermentation.

Changes in TCA-soluble peptide contents and degree of hydrolysis

As shown in Fig. 2a, fresh shrimp contained TCA-soluble peptides of 80.62 mmol/g dry weight sample. Pongsetkul et al. (2015) reported that fresh *A. vulgaris* contain some TCA-soluble peptides, which could be associated with rapid degradation after death, particularly during transportation. The initial level of TCA-soluble peptides might represented endogenous oligopeptides in sample and the degradation products generated during post-harvest handling (Benjakul and Morrissey 1997). The increase in the contents of TCA-soluble peptides during *Kapi* production suggested that the degradation mediated by either endogenous or microbial proteases in the presence of high salt still took place. Slight increase in TCA-soluble peptide contents was found after drying process, while the higher rate of increase was noticeable when dried shrimp were fermented at room temperature ($P < 0.05$). After 30 days of fermentation, TCA-soluble peptide content was 288.96 mmol/g dry weight sample, which was 3.58-fold higher than that obtained in raw material. This suggested the continuous proteolysis during fermentation. Increasing TCA-soluble peptides throughout *Kapi* production process was

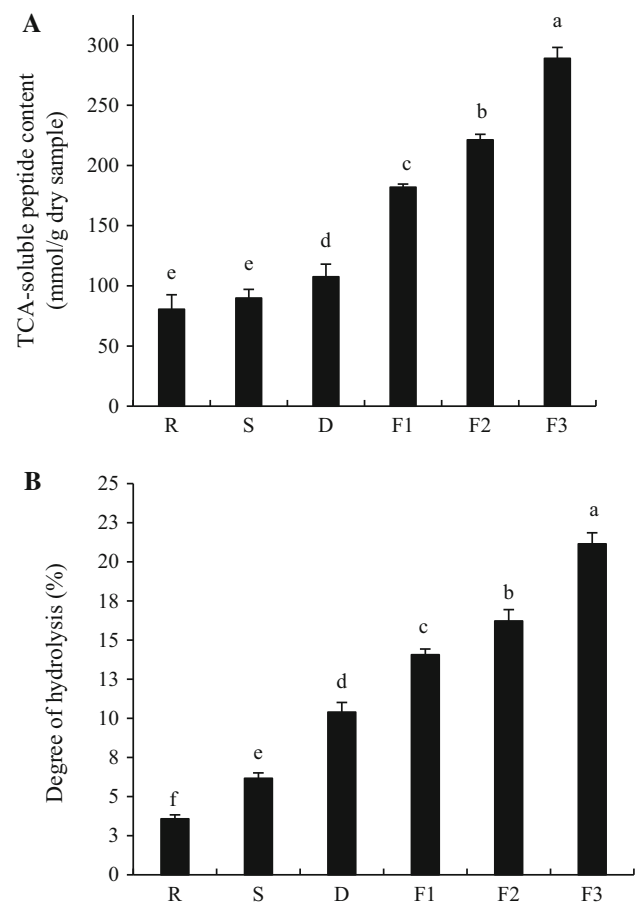


Fig. 2 Changes in TCA-soluble peptide content (a) and degree of hydrolysis (b) of shrimp during *Kapi* production. Bars represent the standard deviation ($n = 3$). R raw material, S after salting, D after drying, F1, F2 and F3 after fermentation for 10, 20 and 30 days. Different letters on the bars indicate the significant differences ($P < 0.05$)

coincidental with the increase in degree of hydrolysis (DH). DH of *Kapi* samples increased from 3.81% in raw material to 21.12% after 30 days of fermentation as shown in Fig. 2b. Rapid increase in DH was also found during fermentation process. With increasing fermentation time, short chain peptides were more produced, affecting the characteristics, bioactivities as well as acceptability of *Kapi*.

Changes in lipid oxidation products

Lipid oxidation of shrimp during *Kapi* production expressed as PV and TBARS values was monitored (Fig. 3a, b). PV of fresh shrimp was 0.08 mg hydroperoxide/kg dry sample, suggesting that lipid oxidation occurred after harvest or during transportation in ice. There were no differences in PV between fresh sample and that after salting ($P > 0.05$). However, PV increased after drying with sunlight and continuously increased during fermentation up to 20 days. Subsequently, a decrease in PV was noticeable

at day 30 of fermentation ($P < 0.05$). The increase in PV during processing and the first period of fermentation more likely resulted from the formation of hydroperoxide, a primary lipid oxidation products. Lipid hydroperoxides are formed by various pathways. Those include the reaction of singlet oxygen with unsaturated fatty acids or the lipoxygenase-catalyzed oxidation of PUFA (Sae-leaw et al. 2013). The decrease in PV after 30 days of fermentation was probably due to the decomposition of hydroperoxide to the secondary oxidation products. Shrimp oil was rich in polyunsaturated fatty acids (Takeungwongtrakul and Benjakul 2013). The result indicated that lipid oxidation occurred during *Kapi* production, probably owing to the high content of polyunsaturated fatty acids. Lipid oxidation products could affect odor and flavor characteristics of *Kapi*.

TBARS values of shrimp during *Kapi* production is shown in Fig. 3b. TBARS value of 0.23 mg MDA/kg dry sample was obtained in fresh shrimp before salting process, suggesting the

presence of lipid oxidation products. No changes in TBARS value were noticeable after being salted ($P > 0.05$). Thereafter, a continuous increase in TBARS value during drying and fermentation was found ($P < 0.05$). TBARS value is the good index of concentration of relatively polar secondary reaction products, especially aldehydes, which are considered to be responsible for the off-flavors in meat (Cagdas and Kumcuoglu 2015). As fermentation time increased, lipid oxidation proceeded in sample as evidenced by the increases in TBARS value. At the end of process (30 days of fermentation), TBARS value was increased to 0.94 mg MDA/kg dry sample (fourfold higher than that of fresh sample). As fermentation time increased, shrimp matrix was loosened, caused by autolysis or microbial action. Thus, lipids bound with matrix were more released and exposed to oxidation, as indicated by the increases in both PV and TBARS values.

Changes in microbial populations of shrimp during *Kapi* production

Initial total viable count (TVC) of shrimp used as raw material was 3.50 log CFU/g dry sample and slightly decreased after salting and drying process ($P < 0.05$) (Fig. 4a). Nevertheless, TVC continuously increased during fermentation at room temperature ($P < 0.05$) and reached 5.84 log CFU/g dry sample after 30 days of fermentation. Microorganisms found in fresh shrimp could be contaminated during handling or washing using seawater. In addition, sorting to remove foreign materials is commonly carried out, which is typically done by hand. This might introduce microorganisms to shrimp. The presence of microorganisms during fermentation contributes to the degradation of proteins and development of flavor and aroma (Majumdar et al. 2015). For the production of *Kapi*, salt plays a key role in preservation by inhibiting the growth of spoilage and pathogenic microorganisms. Phithakpol (1993) reported that putrefactive microorganisms are inhibited by salt at level higher than 6–8%. Salting and drying processes led to the lower moisture content and water activity (A_w) of shrimp as shown in Fig. 1a, b, respectively. These processing conditions could be both eradicate and select microorganisms. It was found that slight reduction of TVC after drying process was observed ($P < 0.05$). In contrast, TVC rapidly increased as fermentation increased ($P < 0.05$), especially within the first 10 days of fermentation. The selected strains might grow well under fermentation condition. The increase in microbial population was correlated well with the rapid increase in TCA-soluble peptides and DH (Fig. 2a, b) during fermentation. Latorre-Moratalla et al. (2011) revealed that free amino acids and other soluble non-nitrogenous substances derived from proteolysis could be considered as available nutrients for microorganisms. In general, the

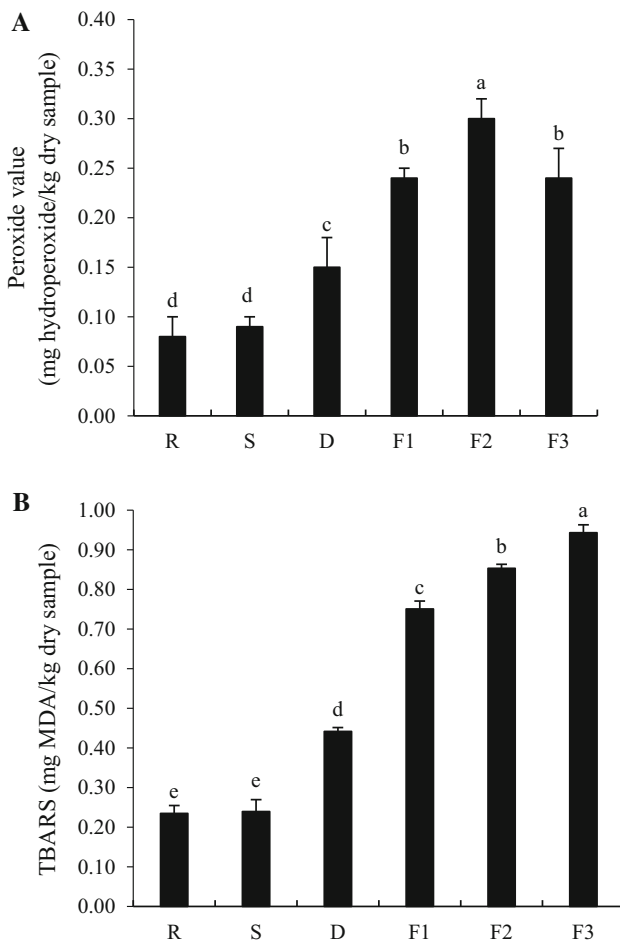


Fig. 3 Changes in peroxide values (a) and TBARS values (b) of shrimp during *Kapi* production. Bars represent the standard deviation ($n = 3$). R raw material, S after salting, D after drying, F1, F2 and F3 after fermentation for 10, 20 and 30 days. Different letters on the bars indicate the significant differences ($P < 0.05$)

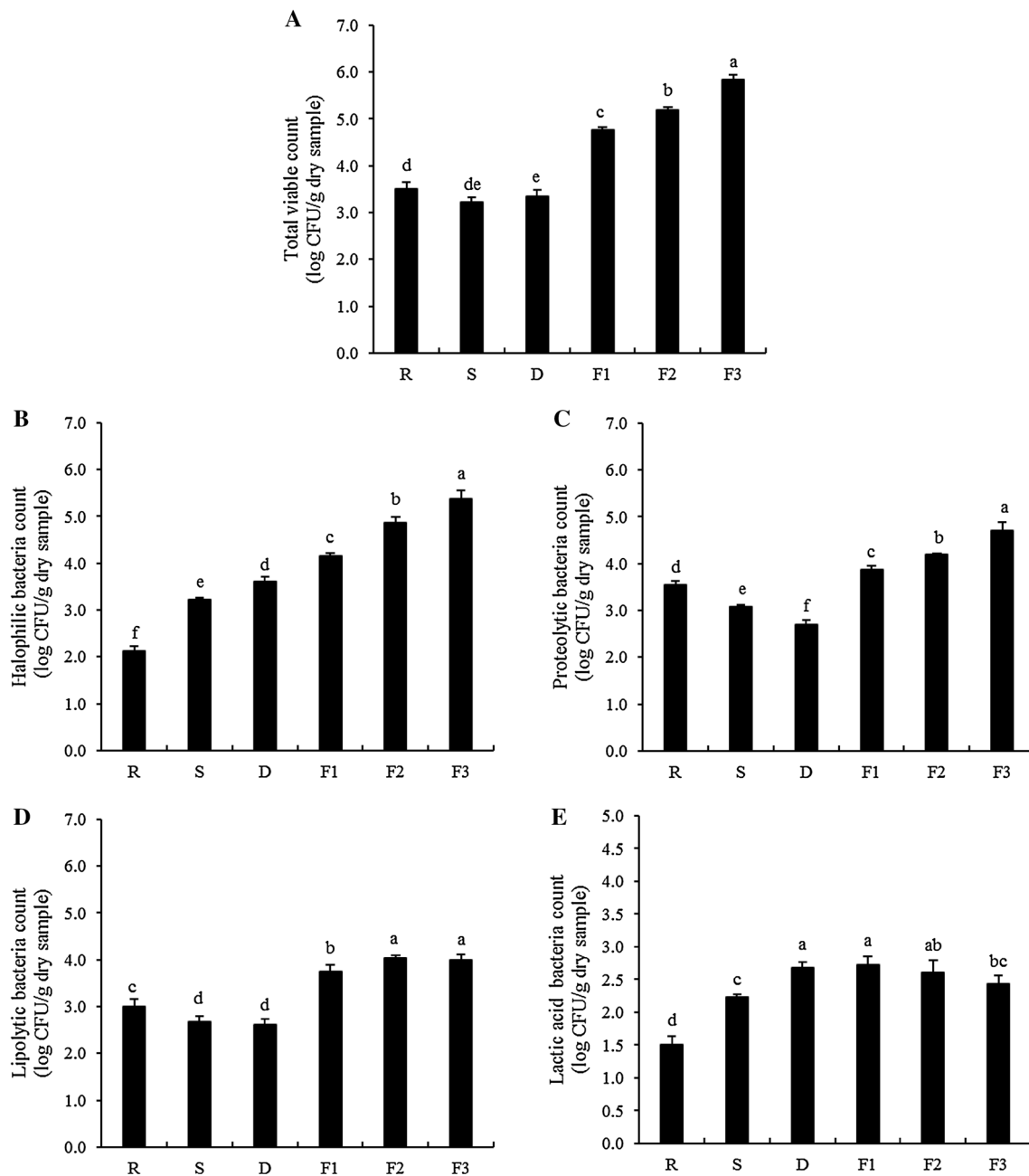


Fig. 4 Changes in total viable count (a), halophilic bacteria (b), proteolytic bacteria (c), lipolytic bacteria (d) and lactic acid bacteria (e) of shrimp during *Kapi* production. Bars represent the standard

deviation ($n = 3$). *R* raw material, *S* after salting, *D* after drying, *F1*, *F2* and *F3* after fermentation for 10, 20 and 30 days. Different letters on the bars indicate the significant differences ($P < 0.05$)

increase in TVC was in accordance with increasing free amino acids throughout the process (Table 1). Moreover, decomposition and oxidation of lipids during *Kapi* fermentation, as indicated by PV and TBARS (Fig. 3a, b), also occurred as induced by the increase in these microorganisms.

An increase in the number of halophilic or halo-tolerant bacteria was observed after salting and drying and also continuously increased during fermentation up to 30 days as shown in Fig. 4b. The high salt environment and

microaerophilic or anaerobic conditions during *Kapi* processing and fermentation more likely resulted in the proliferation of halophilic bacteria. The increased halophilic bacteria count during *Kapi* production coincided well with the increasing TVC. This result suggested that dominant microorganisms in *Kapi* were halophilic bacteria. Chuon et al. (2014) revealed that halophilic and halotolerant bacteria genera, *Staphylococcus* and *Tetragenococcus*, were predominant microorganisms found in *Kapi* from

Cambodia. However, Kobayashi et al. (2003) reported that *Bacillus licheniformis* and *Bacillus sphaericus* were found in Indonesian fermented shrimp (*Terasi*). Moreover, halophilic bacteria found in *Kapi* showing proteolytic activity included *Oceanobacillus kapialis* (Namwong et al. 2009), *Lentibacillus kapialis* (Pakdeeto et al. 2007) and *Virgibacillus halodenitrificans* (Tanasupawat et al. 2011), etc.

The increase in proteolytic bacteria count during fermentation was also observed in the present study (Fig. 4c). Furthermore, as shown in Fig. 4d, lipolytic bacteria count also increased during fermentation of *Kapi*. Halophilic bacteria might contain lipolytic enzymes. This resulted in the release of free fatty acids, which were susceptible to oxidation as indicated by the increases in PV and TBARS values (Fig. 3a, b). The initial proteolytic and lipolytic bacteria counts in fresh shrimp were 3.55 and 3.00 log CFU/g dry sample, respectively. Both types of bacteria showed similar changes throughout *Kapi* production. Both proteolytic and lipolytic bacteria counts of fresh shrimp were slightly decreased after drying process ($P < 0.05$). The presence of high salt as well as the lowered water activity after drying in this product could retard the growth of proteolytic or lipolytic microorganisms. However, both proteolytic and lipolytic bacteria counts increased during fermentation and reached the highest level at day 30 of fermentation ($P < 0.05$).

Figure 4e shows the changes in lactic acid bacteria (LAB) count of shrimp during *Kapi* production. The initial LAB count was quite low (about 1.50 log CFU/g dry sample). LAB count increased continuously until drying process was complete ($P < 0.05$). No further change in LAB count occurred during fermentation up to 20 days ($P < 0.05$). Conversely, slight decrease was found as fermentation was performed for 30 days ($P < 0.05$). In general, LAB species, especially belonging to *Tetragenococcus*, are known to be dominant microorganisms in many high-salt-containing fermented foods (Chuon et al. 2014). LAB plays a profound role in flavor of those products due to their fermentation of carbohydrates. The limited increase in LAB count after salting, drying as well as the first 10 days of fermentation was observed with the slight decrease in pH (Fig. 1c). No further growth of LAB was noticeable after 20 days of fermentation, while pH was slightly increased. It was suggested that LAB were not predominant or played an important role in *Kapi* fermentation with the extended fermentation time. Also, proteins in *Kapi* plausibly exhibited buffering capacity, thereby preventing the pH lowering. Furthermore, *Kapi* contained low amount of carbohydrate as reported by Pongsetkul et al. (2014) and Faithong et al. (2010). This could result in the reduction of LAB during fermentation because of insufficient substrates for their growth.

Overall, halophilic bacteria were the dominant microorganisms, which could grow and produce some

proteolytic or lipolytic enzymes. These microbial enzymes, as well as endogenous enzymes play a profound role in degradation/decomposition of proteins and lipids in shrimp throughout the process. Those degradation products might contribute to the typical characteristics of final products, especially flavor or taste. As reported by Phithakpol (1993), the stronger aroma was obtained in *Kapi* with increasing fermentation time. Therefore, the development of flavor or taste of *Kapi* could be related with the increasing microbial load during *Kapi* fermentation.

Conclusion

Chemical compositions and microbial populations of shrimp underwent changes throughout the processes used for *Kapi* production. Salting and drying were mainly implemented to lower moisture content and A_w , in order to prolong the shelf-life of this product. Protein degradation and lipid oxidation occurred throughout *Kapi* production. For microorganisms, TVC increased continuously and halophilic bacteria were prevalent. Proteolytic and lipolytic bacteria were also increased throughout all processes. Therefore, proteolysis and lipolysis, mediated by both endogenous and microbial enzymes, were involved throughout the process and contributed to the characteristics of finished product. Isolation and identification of these bacteria isolated from *Kapi* production will be further studied. The use of the selected isolates as starter culture for production of *Kapi* with desired quality needs to be investigated.

Acknowledgements Grant-in-Aid for dissertation from Graduate School, Prince of Songkla University, Thailand was acknowledged. Authors are grateful for the TRF Distinguished Research Professor Grant.

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