

Preliminary Study of Some Aroma Compounds of Salted Shrimp Paste (Kapi): Using Chemometrics to Categorize Characteristics of Different Manufacturers

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ABSTRACT

Shrimp paste (kapi) is a fermented condiment made from planktonic shrimp widely used in Southeast Asian cooking. Its flavor is formed from a complex combination of aroma compounds (ACs). The present study examined kapi that was produced by six manufacturers that used different shrimp species and varying ratios of shrimp and salt. A preliminary study of AC was conducted using GC-MS during six months of fermentation. Correlations were found among production factors and significant ACs determined by chemometric methods. Thirty-six peaks were detected in kapi samples, with only 11 ACs playing an important role in odor. S-containing compounds (dimethyl disulfide and dimethyl trisulfide) and aldehydes (2-methyl-propanal, 3-methyl-butanal, and 2-methyl-butanal) were the key ACs in all types of kapi. Eleven ACs were discriminated effectively by principal component analysis (82.02 % cumulative variance), hierarchical cluster analysis, and linear discriminant analysis (99.66 % cumulative variance). Kapi samples produced from the same species (*Acetes* sp.) and salt quantity showed the best AC correlation. The formula with added sugar showed the highest content of N-containing compounds (N, N-dimethyl methylamine, 3-ethyl-2,5-dimethyl pyrazine) and S-containing compounds, whereas 2,3-butanedione was only found in kapi produced from *Mesopodopsis* sp., which was also sun-dried for a longer period. Thus, the developed aroma of kapi was significantly affected by the species of planktonic shrimp, the formulation, and the fermentation period. It should be noted that this study used SPME GC-MS technique with DB-1ms capillary column and thus may have captured only a small fraction of the ACs present in kapi.

Keywords: HS-SPME/GC-MS, Multivariate statistical analysis, Planktonic shrimp, Volatile compound

INTRODUCTION

Salted shrimp paste (kapi) is a traditional fermented condiment of Southeast and East Asia. In Thailand, kapi processing plants are located along the coasts of the lower central, eastern and southern regions, since the main raw material, planktonic shrimp, namely *Acetes* sp. and *Mesopodopsis* sp., is captured from estuaries. For kapi manufacturing,

planktonic shrimp is mixed with salt at a ratio of 5-12 to 1 (shrimp to salt, w/w). The mixture is then drained overnight and sun-dried for 1 to 3 days. After that, the moist salted shrimp is thoroughly crushed in a grinding machine, mortar, or by kneading into a paste before being packed into containers. The salted shrimp is fermented at ambient temperature for 3 to 6 months until the characteristic aroma is developed. Although the

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processes are quite similar in all regions, the suite of flavors detected by human senses are different, which may be due to variations in shrimp species, formula, and geographic area (Lopetcharat *et al.*, 2001; Pongsetkul *et al.*, 2015a; 2019).

In Thailand, *Acetes* sp. and *Mesopodopsis* sp. are the planktonic shrimp species most commonly used for making kapi. *Acetes* sp. is in the family Sergestidae with a larger size (10.0-39.9 mm) than that of *Mesopodopsis* sp. (4.0-9.9 mm) (Chaitiamvong, 1980), which is in the family Mysidae. The habitat of *Acetes* sp. is commonly distributed along the coastal areas of the Thai gulf and Andaman Sea. Conversely, *Mesopodopsis* sp. is found only in mangrove areas of Samuthsongkram, Petchaburi and Surat Thani provinces (Chaitiamvong and Youdee, 1979).

Kapi can release flavor compounds similar to fish sauce and soy sauce, and the mechanisms involved in generating these compounds are complex. The products from protein, carbohydrate, and lipid catabolism are reactive, and some are influenced by the Maillard reaction. Previous studies (Cha and Cadwallader, 1995; Pongsetkul *et al.*, 2015a; Kleekayai *et al.*, 2016) reported the composition of shrimp paste's volatile compounds, which include aldehydes, ketones, alcohols, N-containing compounds, S-containing compounds and others. Moreover, N-containing compounds such as pyrazines are predominant among all of the flavor compounds in shrimp paste. According to Rowe (2011), pyrazines are formed from amino acids and sugars in the Maillard reaction, of which the common product is 2,3,5-trimethylpyrazine. The Maillard reaction is dependent on temperature and water content, since it will be inactive if the temperature is low or the water content is high. In addition, the Maillard reaction can be enhanced by adding sugar and amino acids (Winkel, 2005).

For statistical data analysis, chemometrics uses multivariate statistics to extract relevant information from large amounts of data and indicates correlation between variables by various methods. In studies of flavor, principal component analysis (PCA), hierarchical clustering analysis (HCA) and discriminant analysis (DA) have commonly

been used (Aishima and Nakai, 1991; Zervos and Albert, 1992). PCA and HCA are exploratory methods to identify similarities and hidden variables among samples by showing relationships between data and their grouping. It is a very powerful way to create subgroups within a data set and allows very similar observational values to be included in the same cluster. PCA is used to identify the largest total variance among groups and minimize the variation within groups. HCA is used to group similar properties into clusters, and then place distinct clusters into a hierarchy. LDA is a classification method that decreases data dimensionality (Granato *et al.*, 2018) and identifies separate groups by maximizing variance between groups.

Many studies have been conducted with completely ripened kapi, but research has rarely examined aroma compounds either during fermentation or from different species and manufacturing processes, which might affect the various aromas of kapi. Since consumers normally experience kapi odor at room temperature, the evaluation of low molecular weight compounds may be appropriate for comparing samples. Therefore, to understand the phenomenon of aroma compound (AC) production, a preliminary study was conducted. The objective of the study was to characterize by GC-MS some of the ACs of kapi made by six producers in three geographic regions. The main differences among the manufacturers were the planktonic shrimp species used and the ratio of shrimp and salt. The volatile compounds were collected at months 1, 3, and 6 of fermentation, and all data were categorized to explore correlations between ACs and manufacturing variables by the chemometric method.

MATERIALS AND METHODS

Materials

Fresh planktonic shrimp, harvested from estuary or mangrove areas of Thailand in November, 2017 were randomly sampled from manufacturers located in lower central, eastern and southern regions for chemical determinations. The samples were randomly taken from two lots of shrimp from each

manufacturer and the samples were pooled before analyses. The salted planktonic shrimp were collected from six commercial processors, among which slight variations in production formula and process exist. After mixing the shrimp with sea salt, draining for one night, sun drying and mincing, the samples were transported to the processing plant within one day. The samples were coded as follows: C1 (Samutsakhon Province) and C2 (Phetchaburi Province) for the lower central region, E1 (Trat Province) and E2 (Rayong Province) for the eastern region, and S1 (Satun Province) and S2 (Songkhla Province) for the southern region. Most manufacturers used *Acetes* sp. except C2, where *Mesopodopsis* sp. was used. The size of *Acetes* sp. was about 25 to 35 mm, while that of *Mesopodopsis* sp. was 6 to 12 mm. The shrimp were mixed with sea salt at various ratios of shrimp to salt (w/w): 12:1 (C1), 10:1 (E1, E2, S2) and 5:1 (C2); the formula of S1 also included sugar at the ratio of shrimp: salt: sugar of 13.8: 1: 0.38 (w/w). The sun-drying duration was 1-2 days, except for C2, which was 15-20 days. Thirty-kg samples were packed tightly in pottery jars and covered with plastic sheets. Each treatment was duplicated and stored at ambient temperature (about 33 °C). During fermentation, one kilogram of each treatment was randomly sampled at months 1, 3 and 6 for chemical composition and volatile compound determination.

Chemicals

Standards (a series of n-Alkanes; C5 to C30) and internal standard (2,4,6-trimethyl pyridine) were obtained from Sigma (Sigma Aldrich Pte. Ltd., Germany and Switzerland, respectively). The other chemicals used for proximate composition analysis were AR grade from Sigma Aldrich Pte. Ltd., USA.

Chemical composition analysis and pH determination of raw planktonic shrimp and fermented shrimp

Moisture, protein (N×6.25) and ash contents of raw and fermented planktonic shrimp were determined by standard methods AOAC 950.46, 928.08 and 938.08, respectively (AOAC, 1995). The pH was measured from a 10 % sample

solution in water by pH meter (Sartorius, Germany). Fat and salt content were analyzed by the method of Bligh and Dyer (1959) and volumetric method by titration with silver nitrate (FAO, 1981), respectively. Carbohydrate content was calculated by subtracting the percentages of the other chemical contents (moisture, protein, fat and ash) from 100 %.

Extraction of volatile compounds

A fermented shrimp sample of 6.00 g was put into a 22-mL vial, and spiked with 10 µL of 1000 µg·mL⁻¹ of 2,4,6-trimethylpyridine solubilized in methanol as an internal standard and immediately sealed. Then, solid phase micro extraction (SPME) fiber (50/30 µm DVB/CarboxenTM/PDMS StableFlexTM; Supelco, Tokyo, Japan) was inserted into the headspace of the sealed vial, which was kept at 40 °C for 69 min in a water bath. Thereafter, the SPME fiber was introduced into the GC injector port to desorb the volatiles at 270 °C for 15 min before injection into the GC/MS system.

GC-MS analysis

Desorbed volatiles were injected into the GC/MS-6890N/5975MSD (Agilent G&W, USA) which was an Agilent 6890 GC connected to a 5975 MSD (mass-spectrometer detector) with a DB-1ms capillary column (30 m × 0.25 mm I.D.; 0.25 µm film thickness). Splitless injection was conducted with an injector temperature of 270 °C. The column temperature gradient was initially kept at 35 °C (held for 5 min), then increased from 35 °C to 185 °C at 3 °C·min⁻¹, and increased to 235 °C at 10 °C·min⁻¹ and held for 10 min. The carrier gas was helium (99.999 % purity) at a constant flow rate of 1 mL·min⁻¹. The apparent peaks were scanned m/z at 25-500 at 3.25 scans·s⁻¹. To identify the compounds, n-Alkanes (C5 to C30) standard mixture was injected under the same GC conditions as the samples and the retention index (RI) of the peaks of interest in the chromatogram was calculated using the following formula (Van den Dool and Kratz, 1963):

$$RI = 100 (n) + 100 \{t_r(\text{unknown}) - t_r(n) / t_r(n) - t_r(n)\}$$

Where n is the number of carbon atoms in the smaller alkane, N is the number of carbon atoms in the larger alkane, and t_r is the retention time of the compound. Compounds were identified by comparing retention index and mass spectra of samples with those in the mass spectral library (Wiley Libraries version 7.0).

Quantification of volatile compounds of kapi

The peak area (from GS-MS analysis) of each volatile compound was determined and the concentration of volatile compound (M_v) was normalized by 2,4,6-trimethyl pyridine equivalents (assuming all response factors were 1) and evaluated by the equation below (unit modification from Marco *et al.*, 2007).

$$M_v (\mu\text{g}\cdot\text{kg}^{-1}) = \frac{A_v \times M_{is}}{A_{is}} \times 1000$$

Where A_v is the peak area of volatile compound, M_{is} is the concentration of the internal standard (2,4,6-trimethyl pyridine) and A_{is} is the peak area of the internal standard.

Odor activity values calculation

The relationship between volatile concentration and sensory aspect was evaluated as odor activity value (OAV). It was calculated by dividing the concentration of a volatile compound (M_v) by its odor threshold as published by Van Gemert (2011). Compounds with an OAV greater than 1 were selected as compounds which played an important role in odor.

Statistical analysis

The data describing chemical composition and pH of raw shrimp among different areas, and of fermented shrimp among different areas and fermentation times, were analyzed by one-way ANOVA. Differences between means were tested by Duncan's new multiple range test (Steel and Torrie, 1980). The critical probability was set at 0.05.

The concentrations of 11 significant ACs were processed by chemometrics. Principal component analysis (PCA), hierarchical cluster analysis (HCA), and discriminant analysis (DA) were performed to discriminate samples. PCA and HCA were used to visualize chromatographic data by score and load plots to find the variance and similarity among samples, which were labeled by manufacturer (6 formulas) and fermentation time (1, 3, or 6 months). DA was applied for better sorting and discrimination among samples, which were labeled only by manufacturer. Data analyses were facilitated by the computer package XLSTAT, version 2019.1.1.

RESULTS AND DISCUSSION

Chemical composition and pH of raw planktonic shrimp

The chemical compositions of planktonic shrimp are shown in Table 1. The moisture content of the shrimp varied by species and geographic area, with a range from 77.30 % to 86.24 %. *Mesopodopsis* sp. from the lower central region had 83.95 % moisture content and 75.31 % (dry basis) protein content. *Acetes* sp. from the same area had 86.24 % moisture content and the lowest protein content of 64.53 % ($p < 0.05$), but it was rich in fat (9.08 % dry basis), carbohydrate (4.95 %), and ash (21.44 %). In contrast, *Acetes* sp. from the southern region had the highest protein content (77.56 %) but the lowest fat (6.40 %) and ash content (12.58 %), with a lower carbohydrate content (3.47 %) and the highest pH (8.40). The pH of *Acetes* sp. (8.30-8.40) was higher than that of *Mesopodopsis* sp. (7.60).

In commercial practice, the production capacity of manufacturers who produce kapi from *Acetes* sp. is quite high, and processors usually keep an abundance of raw material ready to enter the process. Therefore, the decomposition of some planktonic shrimp might occur, resulting in increasing pH during the later post-mortem changes due to the formation of basic compounds. In contrast,

Table 1. Chemical composition based on wet weight and dry weight (in parentheses) and pH (mean±SD) of raw planktonic shrimp used for kapi production in this study.

| Parameters | Raw planktonic shrimp | | | |
|------------------|---|--|---|---|
| | Lower central region | | Eastern region | Southern region |
| | <i>Mesopodopsis</i> sp. | <i>Acetes</i> sp. | <i>Acetes</i> sp. | <i>Acetes</i> sp. |
| Moisture (%) | 83.95±0.78 ^b | 86.24±0.37 ^a | 77.30±0.38 ^c | 77.50±0.28 ^c |
| Protein (%) | 12.09±0.68 ^c (75.31±0.58 ^b) | 8.88±0.33 ^d (64.53±0.64 ^d) | 16.27±0.17 ^b (71.68±0.46 ^c) | 17.45±0.23 ^a (77.56±0.03 ^a) |
| Fat (%) | 1.26±0.06 ^c (7.85±0.03 ^b) | 1.25±0.03 ^c (9.08±0.04 ^a) | 2.09±0.01 ^a (9.21±0.09 ^a) | 1.44±0.01 ^b (6.40±0.02 ^c) |
| Carbohydrate (%) | 0.43±0.04 ^b (2.69±0.39 ^b) | 0.68±0.08 ^a (4.95±0.75 ^a) | 0.83±0.06 ^a (3.65±0.19 ^b) | 0.78±0.03 ^a (3.47±0.17 ^b) |
| Ash (%) | 2.27±0.08 ^c (14.15±0.16 ^c) | 2.95±0.10 ^b (21.44±0.15 ^a) | 3.51±0.14 ^a (15.46±0.36 ^b) | 2.83±0.07 ^b (12.58±0.16 ^d) |
| Salt (%) | 0.32±0.01 ^{bc} (1.16±0.00 ^a) | 0.40±0.13 ^{ab} (1.26±0.13 ^a) | 0.64±0.12 ^a (1.41±0.11 ^a) | 0.08±0.01 ^c (0.83±0.11 ^b) |
| pH | 7.60±0.00 ^c | 8.30±0.00 ^b | 8.30±0.00 ^b | 8.40±0.00 ^a |

Note: Different superscripts in the same row indicate significant difference ($p < 0.05$).

those who produce kapi from *Mesopodopsis* sp. are small-scale processors, and the planktonic shrimp incurs less spoilage before salting. Some researchers reported that fresh *Acetes vulgaris*, stored at room temperature (28-30 °C) for up to 15 h, increased in TVB-N from 10.15 mg N·100 g⁻¹ (at 0 h) to 74.31 mg N·100 g⁻¹ (at 15 h), and pH changed from 7.37 to 7.45 (Pongsetkul *et al.*, 2015b). In contrast, *Acetes* sp. stored in ice for up to 11 days showed increases of TVB-N from 5.13 mg N·100 g⁻¹ (zero days) to 21.07 mg N·100 g⁻¹ (at 11 days), and changes in pH from 6.56 to 8.09 (Keer *et al.*, 2018).

Salt content among all samples ranged from 0.83-1.41 % (dry basis). It was noteworthy that *Acetes* sp. from the southern region had lower salt content (0.83 %) than all but one other sample (*Mesopodopsis* sp. from lower central region) and the highest pH (8.40) ($p < 0.05$). The reason for the variation of chemical contents of planktonic shrimp can be attributed to the differences in species and geographic area. The physical characteristics of the two shrimp species are different. *Acetes* sp. is larger with a thick shell, while *Mesopodopsis* sp. has a smaller size but higher yield due to its thinner shell.

Chemical composition and pH of fermented shrimp

The kapi manufacturing from the six sources differed, especially by species used and ratio of shrimp to salt (w/w); the formulations could be divided into four groups: (1) C1 (*Acetes* sp., 12:1) (2) C2 (*Mesopodopsis* sp., 5:1) (3) a grouping of E1, E2, and S2 (*Acetes* sp., 10:1) and (4) S1 (*Acetes* sp., 13.8:1 plus sugar at 0.38). However, the sun-drying duration of C2 was 15-20 days, much longer than the other groups, and only the formula of S1 had sugar added, along with the lowest proportion of salt.

The moisture content showed significant variation among manufacturers, with values ranging from 36.86 % to 53.88 % (Table 2). The highest moisture content was found in C2 (53.41-53.88 %) because of the greater addition of salt (a ratio of shrimp to salt of 5:1); it retained water even though it was sun-dried for many days. E1 and S1 (in the sixth month) contained the lowest moisture content (36.86-39.32 %). Apart from moisture, the major components of kapi based on dry weight were protein (40.42-53.92 %), ash (36.28-48.70 %) and salt (29.28-42.11 %), while the minor components

Table 2. Chemical composition based on wet weight and dry weight (in parentheses) and pH (mean±SD) of fermented shrimp from different manufacturers and fermentation times.

| Parameters | Lower central region | | | | | | Eastern region | | | | | | Southern region | | | | | |
|--------------|-------------------------|-------------------------|-------------------------|-------------------------|--------------------------|-------------------------|-------------------------|-------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| | C1 | | C2 | | E1 | | E2 | | S1 | | S2 | | S1 | | S2 | | S1 | |
| | 1 month | 6 month | 1 month | 6 month | 1 month | 6 month | 1 month | 6 month | 1 month | 6 month | 1 month | 6 month | 1 month | 6 month | 1 month | 6 month | 1 month | 6 month |
| Moisture (%) | 46.26±0.14 ^b | 46.16±0.15 ^b | 53.41±0.50 ^a | 53.88±0.63 ^a | 38.10±1.11 ^{de} | 36.86±0.64 ^e | 45.05±0.00 ^b | 43.31±0.02 ^c | 38.64±2.03 ^{de} | 39.32±0.83 ^d | 46.89±0.47 ^b | 46.71±0.13 ^b | 46.89±0.47 ^b | 46.71±0.13 ^b | 46.89±0.47 ^b | 46.71±0.13 ^b | 46.89±0.47 ^b | 46.71±0.13 ^b |
| Protein (%) | 27.97±0.23 ^b | 29.04±0.13 ^a | 22.22±0.40 ^d | 22.15±0.86 ^d | 29.44±0.44 ^a | 29.13±0.71 ^a | 24.00±0.00 ^f | 24.63±0.00 ^e | 29.91±0.22 ^a | 29.88±0.53 ^a | 21.47±0.23 ^d | 22.53±0.75 ^d | 21.47±0.23 ^d | 22.53±0.75 ^d | 21.47±0.23 ^d | 22.53±0.75 ^d | 21.47±0.23 ^d | 22.53±0.75 ^d |
| Fat (%) | 3.24±0.14 ^{ab} | 3.36±0.21 ^{ab} | 2.45±0.06 ^{cd} | 2.34±0.43 ^d | 3.10±0.83 ^{abc} | 3.42±0.05 ^{ab} | 3.55±0.04 ^a | 3.49±0.08 ^a | 3.31±0.42 ^{ab} | 3.10±0.14 ^{abc} | 2.69±0.12 ^{bcd} | 2.68±0.02 ^{bcd} | 2.69±0.12 ^{bcd} | 2.68±0.02 ^{bcd} | 2.69±0.12 ^{bcd} | 2.68±0.02 ^{bcd} | 2.69±0.12 ^{bcd} | 2.68±0.02 ^{bcd} |
| Carb (%) | 3.00±0.27 ^a | 1.92±0.40 ^b | 1.11±0.11 ^d | 0.78±0.17 ^d | 3.22±0.48 ^a | 3.47±0.35 ^a | 0.96±0.04 ^d | 6.15±0.12 ^{ab} | 5.39±0.51 ^{ab} | 5.11±0.16 ^b | 3.10±0.39 ^a | 2.16±0.23 ^b | 3.10±0.39 ^a | 2.16±0.23 ^b | 3.10±0.39 ^a | 2.16±0.23 ^b | 3.10±0.39 ^a | 2.16±0.23 ^b |
| Ash (%) | 19.53±0.24 ^b | 19.54±0.18 ^b | 20.82±0.07 ^b | 20.87±0.49 ^b | 26.15±1.03 ^a | 27.14±0.36 ^a | 26.45±0.00 ^a | 27.33±0.05 ^a | 26.32±1.41 ^a | 26.63±1.24 ^a | 25.87±0.02 ^a | 25.93±0.37 ^a | 25.87±0.02 ^a | 25.93±0.37 ^a | 25.87±0.02 ^a | 25.93±0.37 ^a | 25.87±0.02 ^a | 25.93±0.37 ^a |
| Salt (%) | 20.02±1.07 ^b | 20.48±1.02 ^b | 28.91±0.31 ^a | 29.84±1.56 ^a | 29.24±2.42 ^a | 29.45±2.15 ^a | 29.92±0.18 ^a | 29.53±0.87 ^a | 33.72±3.77 ^a | 29.22±4.62 ^a | 29.91±0.59 ^a | 31.09±0.70 ^a | 29.91±0.59 ^a | 31.09±0.70 ^a | 29.91±0.59 ^a | 31.09±0.70 ^a | 29.91±0.59 ^a | 31.09±0.70 ^a |
| pH | 7.55±0.07 ^a | 7.45±0.07 ^a | 6.85±0.07 ^b | 6.85±0.07 ^b | 7.55±0.07 ^a | 7.60±0.28 ^a | 7.40±0.00 ^a | 7.60±0.00 ^a | 7.35±0.07 ^a | 7.35±0.07 ^a | 7.60±0.00 ^a | 7.50±0.00 ^a | 7.60±0.00 ^a | 7.50±0.00 ^a | 7.60±0.00 ^a | 7.50±0.00 ^a | 7.60±0.00 ^a | 7.50±0.00 ^a |

Note: C1, C2, E1, E2, S1 and S2 are fermented shrimps collected from Samutsakhon, Phetchaburi, Trat, Rayong, Satun and Songkhla provinces, respectively. Different superscripts in the same row indicate significant difference ($p<0.05$).

were fat (5.02-6.45 %) and carbohydrate (1.69-5.82 %). Protein content is calculated from total nitrogen, which is composed of amino acid nitrogen (protein nitrogen) and volatile-based nitrogen. Interestingly, protein content of kapi varied considerably ($p < 0.05$) among the manufacturers. The kapi from C1 had the highest protein, whereas S2 had the lowest protein content, although they were both produced from the same *Acetes* sp. The reason might be due to natural variation of shrimp collected from different areas. High ash content was attributed to high salt content and inorganic substances in the shrimp shell. During fermentation, chemical composition from each processor showed no significant difference ($p > 0.05$) between months 1 and 6 except for moisture content of E2, carbohydrate content of C1, S1, and S2, and salt content of S1. The trend of pH for all kapi samples was consistent with values for the raw planktonic shrimp. Kapi produced from *Acetes* sp. had higher pH (7.35-7.60) than *Mesopodopsis* sp. (6.85) from C2 ($p < 0.05$). The pH of kapi was lower than for raw shrimp, which might be due to the elimination of basic compounds in the draining process as well as the evaporation of volatile basic components during sun drying.

Faithong *et al.* (2010) found that shrimp paste from the southern part of Thailand, produced from *A. vulgaris*, contained 36.78-49.93 % moisture with 31.85-43.12 % protein, 41.04-50.93 % ash, 2.42-4.10 % fat, 4.58-24.70 % carbohydrates (dry basis), 19.29-24.73 % salt (wet basis), and had pH of 7.44-7.66. Similarly, Prapasuwannakul and Suwannahong (2015) found that kapi from the same area as C2 contained 37.36-46.85 % moisture with 33.79-44.45 % protein, 35.80-46.24 % ash, 1.25-3.55 % fat, 7.63-28.79 % carbohydrates, 34.42-41.97 % salt (dry basis), with pH 7.01-7.71. The slightly different results could be attributed to the geographic area, manufacture process and formula, as well as the ripening period, which might also affect AC production.

Identification of some aroma compounds using GC-MS

Thirty-six volatile components were detected in kapi samples from six manufacturers at

fermentation times of 1, 3, and 6 months (Table 3). These components were classified into eight classes of compounds: N-containing compounds, alcohols, ketones, aldehydes, acids, S-containing compounds, esters and alkanes.

N-containing compounds are predominant flavor compounds of salted shrimp paste, which was consistent with previous studies (Cha and Cadwallader, 1995; Pongsetkul *et al.*, 2015a; Kleekayai *et al.*, 2016). The N-containing compounds consisted of two groups: N, N-dimethyl methylamine (or trimethylamine, TMA) and pyrazines. Even though protein content did not decrease during fermentation (Table 2), N-containing compounds either decreased or increased depending on manufacturer. Since the total nitrogen is the sum of protein nitrogen and volatile-based nitrogen, the chemical reactions that occurred during ripening could change nitrogen compounds to a mixture of various new substances. TMA alone exhibits an ammonia-like, fishy off-odor, which is produced through the reduction of trimethylamine oxide by microbial or endogenous enzyme degradation (Lindsay, 1994; Durnford and Shahidi, 1998). The concentrations of TMA had a tendency to decrease dramatically upon fermentation, except for those of C2, S1 and S2, which showed a slight decrease. The TMA content of C2 was low throughout the fermentation period. This was probably due to the sun-drying step, which took a longer time (15-20 days) before further fermentation, resulting in evaporation of some volatile TMA or TMAO. It is believed that kapi that is fermented for at least six months has a good odor, which might be partly due to TMA reduction.

Pyrazines contribute attractive flavor compounds after high temperatures; the Maillard and pyrolysis reactions can both generate pyrazines through Strecker degradation in thermal processes (Whitfield, 1992; Cha *et al.*, 1997). Six alkyl pyrazines were detected in kapi, namely methyl pyrazine, 2,5-dimethyl pyrazine, 2,3,5-trimethyl pyrazine, 3-ethyl-2,5-dimethyl pyrazine, tetramethyl pyrazine, and 3,5-diethyl-2-methyl pyrazine. The Strecker degradation is an important reaction of sugar degradation products with amino acids. S1 had a large amount of 2,5-dimethyl pyrazine, 2,3,5-trimethyl pyrazine and 3-ethyl-2,5-dimethyl

| Peak No. | Compounds | RI DB-1ms | Compound concentration (µg·kg ⁻¹ sample) | | | | | | | | | | | | | | | | | |
|------------------------|-------------------------------|-----------|---|--------|--------|---------|---------|---------|--------|--------|--------|--------|--------|--------|---------|--------|---------|--------|--------|--------|
| | | | C1 | | | C2 | | | E1 | | | E2 | | | S1 | | | S2 | | |
| | | | 1 m | 3 m | 6 m | 1 m | 3 m | 6 m | 1 m | 3 m | 6 m | 1 m | 3 m | 6 m | 1 m | 3 m | 6 m | 1 m | 3 m | 6 m |
| N-containing compounds | | | | | | | | | | | | | | | | | | | | |
| 1 | N,N-dimethyl Methylamine | <600 | 563.65 | 430.51 | 222.28 | 212.44 | 190.95 | 219.64 | 561.06 | 227.20 | 236.38 | 601.95 | 281.48 | 345.74 | 757.66 | 730.13 | 675.86 | 548.15 | 483.09 | 414.73 |
| 18 | Methyl pyrazine | 886 | 38.44 | 33.37 | nd | nd | nd | nd | nd | 48.20 | 22.14 | 22.96 | 18.21 | nd | 20.73 | nd | 34.48 | nd | nd | 31.53 |
| 23 | 2,5-dimethyl pyrazine | 1098 | 519.78 | 197.58 | 34.93 | 17.23 | 51.46 | 169.84 | 100.17 | 152.70 | 329.64 | 119.71 | 86.42 | 85.53 | 1008.16 | 889.57 | 1289.66 | 124.43 | 218.01 | 167.28 |
| 31 | 2,3,5-trimethyl pyrazine | 1181 | 142.48 | 22.14 | 41.28 | 583.09 | 382.22 | 549.81 | 51.79 | 53.18 | 77.14 | 418.73 | 203.09 | 49.87 | 268 | 316.39 | 494.83 | 153.72 | 233.09 | 249.05 |
| 33 | 3-ethyl-2,5-dimethyl pyrazine | 1269 | nd | nd | nd | 81.38 | 63.39 | 99.81 | 15.82 | nd | nd | 107.49 | 62.04 | 60.75 | 670.80 | 744.54 | 1005.17 | nd | nd | nd |
| 34 | Tetramethyl pyrazine | 1272 | nd | nd | nd | 1156.60 | 1017.99 | 1727.33 | nd | nd | nd | nd | nd | nd | nd | nd | 44.83 | nd | nd | nd |
| 35 | 3,5-diethyl-2-methyl pyrazine | 1346 | 73.12 | 70.70 | nd | nd | nd | nd | nd | nd | nd | 26.87 | 13.27 | 12.39 | 34.18 | 73.51 | 98.28 | 24.16 | 31.62 | 26.19 |
| Alcohols | | | | | | | | | | | | | | | | | | | | |
| 2 | Ethanol | <600 | 132.03 | 70.37 | 89.82 | 286.28 | 91.00 | 149.71 | 213.37 | nd | nd | 127.52 | 113.27 | 129.05 | nd | nd | 61.21 | nd | nd | nd |
| 8 | 2-methyl-1-propanol | 606 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | 40.35 | nd | 30.17 | nd | nd | nd |
| 11 | 1-Penten-3-ol | 640.5 | 46.80 | 134.14 | nd | nd | 435.56 | 176.85 | 33.80 | 41.36 | 48.21 | 62.54 | 30.25 | 36.87 | 67.25 | nd | 80.17 | 22.95 | nd | 18.71 |
| 14 | 2-methyl-1-butanol | 689 | nd | nd | 9.98 | nd | nd | nd | nd | nd | nd | nd | nd | nd | 95.27 | nd | 75.86 | 15.10 | 26.84 | 27.79 |
| 15 | 2,3-Butanediol | 833 | nd | nd | nd | 289.15 | 251.05 | 91.05 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 16 | 2-Propanol | 850 | nd | nd | nd | 179.04 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 27 | 2-propyl-1-pentanol | 1167 | 94.43 | 202.53 | nd | 171.38 | 166.32 | 505.16 | 71.33 | 80.86 | 99.29 | 100.65 | 78.70 | 86.43 | 75.09 | 69.54 | 116.38 | 20.23 | nd | nd |
| 30 | 1-Octen-3-ol | 1174 | nd | nd | nd | 79.47 | 89.12 | 199.61 | 15.82 | 24.26 | 36.79 | 63.03 | 49.07 | 44.43 | 51.56 | nd | 114.66 | nd | nd | nd |
| Ketones | | | | | | | | | | | | | | | | | | | | |
| 3 | 2-Propanone | <600 | nd | nd | nd | nd | nd | nd | nd | 81.17 | 120.36 | 52.77 | nd | nd | nd | nd | nd | 63.12 | 156.99 | 300.89 |
| 5 | 2,3-Butanedione | <600 | nd | nd | nd | 59.36 | 20.08 | 56.03 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 6 | 2-Butanone | <600 | 68.52 | 19.16 | 47.63 | nd | 59.00 | 39.40 | 62.03 | 25.50 | 54.29 | 111.89 | 31.79 | 41.71 | 49.88 | nd | 104.31 | 59.80 | 87.13 | 189.73 |
| 21 | 2-Heptanone | 1081 | 44.29 | 21.15 | 97.53 | 232.66 | 115.48 | nd | 104.82 | 142.74 | 176.79 | 126.06 | 164.20 | 82.51 | 49.88 | 52.65 | 91.38 | 32.32 | 133.82 | 40.62 |
| 25 | 6-methyl-2-heptanone | 1146 | nd | nd | nd | nd | nd | nd | 31.32 | 38.87 | 31.43 | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 28 | 1-Hepten-6-one | 1170 | nd | nd | nd | nd | nd | nd | 18.61 | 17.42 | 24.64 | 23.45 | 13.89 | 15.4 | | | | | | |

Table 3. Cont.

| Peak No. | Compounds | RI DB-1ms | Compound concentration (µg·kg ⁻¹ sample) | | | | | | | | | | | | | | | | | |
|------------------------|-------------------------------|-----------|---|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|-------|--------|--------|--------|--------|--------|--------|
| | | | C1 | | | C2 | | | E1 | | | E2 | | | S1 | | | S2 | | |
| | | | 1 m | 3 m | 6 m | 1 m | 3 m | 6 m | 1 m | 3 m | 6 m | 1 m | 3 m | 6 m | 1 m | 3 m | 6 m | 1 m | 3 m | 6 m |
| Aldehydes | | | | | | | | | | | | | | | | | | | | |
| 4 | 2-methyl-propanal | <600 | 60.17 | 38.66 | 18.15 | nd | nd | 47.28 | 56.13 | 25.19 | 70.36 | 32.74 | 32.72 | 31.13 | 26.34 | nd | 55.17 | 47.72 | 113.24 | 172.09 |
| 9 | 3-methyl-butanal | 617 | 36.77 | 32.71 | 36.29 | nd | 21.34 | 77.04 | 49.93 | 51.62 | 46.43 | 37.62 | 18.83 | 20.25 | 31.38 | nd | 25.00 | 136.21 | 151.84 | 468.17 |
| 10 | 2-methyl-butanal | 624 | 65.18 | 58.48 | 24.50 | nd | 37.66 | 84.92 | 81.25 | 95.16 | 94.29 | 59.61 | 30.25 | 36.87 | 38.11 | nd | 37.93 | 121.11 | 170.22 | 466.57 |
| 24 | Benzaldehyde | 1136 | 105.71 | 122.25 | nd | nd | nd | 119.94 | 22.64 | 34.52 | 31.07 | 44.95 | 35.49 | 25.69 | 30.82 | 74.50 | 142.24 | nd | nd | 26.72 |
| Acids | | | | | | | | | | | | | | | | | | | | |
| 7 | Acetic acid | 605 | nd | nd | nd | 350.43 | nd | 247.76 | 181.74 | 154.87 | 147.50 | 85.99 | 105.56 | 65.28 | 79.58 | nd | 86.21 | 46.81 | 100.74 | 277.91 |
| 12 | Propionic acid | 674 | nd | nd | nd | nd | nd | nd | 64.82 | 77.75 | 53.57 | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 19 | 2-oxo-butanoic acid | 1044 | nd | nd | nd | 121.60 | 49.58 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 20 | 3-methyl-ethyl- butanoic acid | 1076 | nd | nd | nd | nd | 64.02 | nd | nd | nd | nd | 114.33 | nd | nd | nd | nd | nd | nd | nd | nd |
| 22 | 2-methyl-butanoic acid | 1087 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | 25.31 | 36.87 | 17.93 | nd | nd | nd | nd | nd |
| S-containing compounds | | | | | | | | | | | | | | | | | | | | |
| 13 | Dimethyl Disulfide | 684 | 90.25 | 30.40 | 249.04 | 201.06 | 109.83 | 102.43 | 40.32 | 22.08 | 24.64 | 50.33 | 25.62 | 36.87 | 430.95 | 238.91 | 817.24 | 98.76 | 166.54 | 122.39 |
| 26 | Dimethyl Trisulfide | 1148 | 191.78 | 237.22 | 225.45 | 267.13 | 252.93 | 139.20 | nd | nd | 17.86 | 80.13 | 63.58 | 84.32 | nd | 113.25 | 377.59 | nd | nd | 39.01 |
| 36 | Dimethyl tetrasulfide | 1377 | 45.54 | 78.96 | nd | nd | nd | 106.81 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Ester | | | | | | | | | | | | | | | | | | | | |
| 17 | Ethyl buterate | 876 | nd | nd | 67.14 | 217.34 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Alkane | | | | | | | | | | | | | | | | | | | | |
| 32 | n-Decane | 1234 | nd | nd | nd | nd | nd | nd | 22.95 | 33.90 | 42.14 | 16.61 | nd | 12.09 | nd | nd | nd | nd | nd | nd |

Note: *C1, C2, E1, E2, S1 and S2 are fermented shrimps collected from Samutsakhon, Phetchaburi, Trat, Rayong, Satun and Songkhla provinces, respectively.

nd = Not detected peak of volatile compound

pyrazine, which is probably due to added sugar in the formula participating in the Maillard reaction (Milić and Piletić, 1984; Rowe, 2011). The concentrations of these three ACs increased from 1 month to 6 months of the fermentation period.

Alcohol is the product of carbohydrate catabolism by microbes, decomposition of secondary hydroperoxides of fatty acids, lipoxygenase on fatty acids, oxidative decomposition of fat, or reduction of a carbonyl (Spurvey *et al.*, 1998). Eight alcohols were detected in kapi, namely ethanol, 2-methyl-1-propanol, 1-penten-3-ol, 2-methyl-1-butanol, 2,3-butanediol, 2-propanol, 2-propyl-1-pentanol, and 1-octen-3-ol. These alcohols were found in all of the samples except 2-methyl-1-propanol, 2-methyl-1-butanol, 2,3-butanediol, and 2-propanol. C2 contained high levels of ethanol, 2,3-butanediol, 2-propyl-1-pentanol, and 1-octen-3-ol, whereas S1 notably contained 2-methyl-1-butanol, 1-penten-3-ol, and 1-octen-3-ol, which might be formed through lipid oxidation (Spurvey *et al.*, 1998; Pongsetkul *et al.*, 2015a), while butanol derivatives might be formed by bacterial fermentation of lactic acid (Pongsetkul *et al.*, 2015a).

Ketones may be produced by thermal oxidation/degradation of fatty acids, amino acid degradation or microbial oxidation (Spurvey *et al.*, 1998). Seven ketones were detected in kapi, namely 2-propanone, 2,3 butanedione, 2-butanone, 2-heptanone, 6-methyl-2-heptanone, 1-hepten-6-one, and 6-methyl-5-hepten-one. The first four substances were found in high amounts in all kapi samples. E1 and E2 contained almost all components except 2,3 butanedione. It was noted that 2,3 butanediol and 2,3 butanedione were only found in C2, which might be the result of the long sun-drying time and the shrimp species used. 2,3 butanedione can be generated from the Maillard reaction and is a characteristic volatile compound in cooked food (Spurvey *et al.*, 1998); however, it was not detected in S1. During the long sun drying period (15-20 days) of C2, 2,3 butanedione generation might have been enhanced.

Alkanals are degradation products of lipid oxidation, resulting in oxidized flavors.

The formation of hexanal, heptanal, and octanal can be attributed to the decomposition of lipid hydroperoxides and peroxy radicals (Sink, 1973). In some cases, branched aldehydes are generated by catabolism of the branched amino acids (Ordóñez *et al.*, 1999). In addition, aldehydes can be reduced to the corresponding alcohols or oxidized to the corresponding acids. All samples were found to contain three branched aldehydes (2-methyl-propanal, 3-methyl-butanal and 2-methyl-butanal) and one aromatic aldehyde (benzaldehyde). The amounts of the first three substances were highest in S2 at the sixth month. Since *Acetes* sp. from the southern region had high protein content, those branched aldehydes may have been produced from amino acid catabolism. However, S1 showed lower amounts of the branched aldehydes, higher benzaldehyde, as well as higher alcohol content (only in the sixth month), which might be due to aldehyde reduction forming alcohol. It was reported that benzaldehyde is probably derived from the Strecker degradation of amino acids (Mason *et al.*, 1967).

Volatile fatty acids are formed by fat oxidation or aldehyde oxidation. Acetic acid was the major acid among five acids generated in kapi, followed by lower amounts of propionic acid, 2-oxo-butanoic acid, 3-methyl-ethyl- butanoic acid and 2-methyl-butanoic acid. The acetic acid content was highest in C2 (months 1 and 6) and S2 (month 6).

Only straight-chain sulfur-containing compounds were found in kapi. The major components were dimethyl disulfide and dimethyl trisulfide, whereas a minor component was dimethyl tetrasulfide. Varlet and Fernandez (2010) found that the main pathways for generation of aliphatic sulfur-containing compounds such as thiols and polysulfides in seafood are microbial-mediated enzymatic reactions. In contrast, Maillard reactions are involved in the generation of dimethyl disulfide and dimethyl trisulfide in most thermally processed meats (Wang *et al.*, 2012). Both compounds usually affect the overall food aroma due to their low threshold values (Spurvey *et al.*, 1998). Dimethyl disulfide imparts an onion- or cabbage-like aroma, similar to spoiled eggs (Spurvey *et al.*, 1998).

Comparing all samples, S1 had the highest dimethyl disulfide and dimethyl trisulfide content, especially in the sixth month. Sugar added in the S1 formula probably enhanced the Maillard reaction during ripening at the high temperature in summer, while these substances in C1, E2 and S2 tended to increase with ripening time, which might be considered as the result of microbial activity. On the contrary, these contents in C2 decreased with ripening time, suggesting that high salt addition might have retarded enzyme activity of microbes. Giri *et al.* (2010a) reported that dimethyl disulfide and dimethyl trisulfide have a significant impact in fish miso, while Jeleń *et al.* (2013) and Feng *et al.* (2015) found only dimethyl trisulfide as the key aroma for tempeh and soy sauce, respectively.

Identification the aroma compounds by odor activity values (OAVs)

The main aroma components in kapi were identified by comparing their concentrations to threshold values, which are represented by odor activity values (OAV). An OAV greater than 1 indicates a significant aroma compound (Gao *et al.*, 2014). Only 11 compounds were selected as compounds that play important role in the odor of kapi. (Table 4). S-containing compounds (dimethyl disulfide, dimethyl trisulfide) and aldehydes (2-methyl- propanal, 3-methyl-butanal, 2-methyl-butanal) were considered the key aroma compounds of kapi, while N-containing compounds (N, N-dimethyl methylamine, 3-ethyl-2,5-dimethyl pyrazine), alcohols (2-methyl-1-butanol, 1-octen-3-ol), ketone (2,3-butanedione) and acetic acid were also detected, although they were not found in all treatments and also had low OAV. Dimethyl trisulfide mostly had higher OAV in a comparison of the key ACs among treatments, which may be due to its low threshold values. Interestingly, 2,3-butanedione was only found in kapi of C2. Since *Mesopodopsis* sp. was used for manufacturing C2, and it was the only treatment with a longer sun drying time, 2,3-butanedione might be the characteristic aroma of kapi produced from this species using the special manufacturing technique. However, a study of the exact mechanism of 2,3-butanedione formation

should be done in the future. The strongest aldehyde aroma was from 3-methyl-butanal. The key compounds of kapi were also found in fish miso (Giri *et al.*, 2010a) and fish sauce (Giri *et al.*, 2010b). The key compounds of the fish miso, considering the lower threshold perception and higher OAVs, were 2-methyl-butanal, 3-methyl-butanal, methional, isoamyl acetate, dimethyl disulfide, dimethyl trisulfide, 2,3-butanedione, 3-methylethyl butanoate, 3-methyl-1-butanol, ethyl hexanoate, 1-octen-3-ol, heptanol, heptanal and 2-undecanone (Giri *et al.*, 2010a).

Categorization of aroma compounds by chemometrics

The GC-MS data of all samples were analyzed by chemometrics using PCA, HCA and DA.

Principal component analysis (PCA)

PCA was performed on 11 variables to extract the first three principal components (PCs), which are responsible for 82.02 % of the information. PC1, PC2 and PC3 explained 33.47 %, 27.85 %, and 20.70 % of the variance, respectively. The three related PCs had eigenvalues >1 (3.681, 3.064, and 2.277, respectively), indicating that these PCs are appropriate (Granato *et al.*, 2018). A factor loading score higher than 0.59 indicates that a compound has an impact on the aroma of kapi. PC1 separated the kapi samples depending on three aldehydes (2-methyl- propanal, 3-methyl-butanal, 2-methyl-butanal), which were negatively correlated with two S-containing compounds (dimethyl disulfide, dimethyl trisulfide) (Figure 1). Based on PC2, two N-containing compounds (N, N-dimethyl methylamine, 3-ethyl-2,5-dimethyl pyrazine) and 2-methyl-1-butanol were shown to be important for the aroma. PC3 was primarily associated with 1-octen-3-ol, 2,3-butanedione and acetic acid. The score plots of only PC1 and PC2 (explaining 61.32 % of the total variance) and loading plots are shown in Figure 1. It shows good clustering for S1 and C2, and overlaps for C1, E1, E2, and S2 between early-fermented and mature salted shrimp paste.

Table 4. Odor activity values (OAV) of some aroma compounds of fermented shrimp from different manufactures and fermentation times.

| Compounds | Threshold µg.kg ⁻¹ | odor activity values (OAV) | | | | | | | | | | | | | | | | | |
|-------------------------------|----------------------------------|----------------------------|-------|-------|---------|--------|--------|--------|--------|--------|--------|--------|--------|--------|-------|--------|--------|--------|--------|
| | | C1 | | | C2 | | | E1 | | | E2 | | | S1 | | | S2 | | |
| | | 1 m | 3 m | 6 m | 1 m | 3 m | 6 m | 1 m | 3 m | 6 m | 1 m | 3 m | 6 m | 1 m | 3 m | 6 m | 1 m | 3 m | 6 m |
| N-containing compounds | | | | | | | | | | | | | | | | | | | |
| N,N-dimethyl Methylamine | 500 ^a | 1.13 | 0.86 | 0.44 | 0.42 | 0.38 | 0.44 | 1.12 | 0.45 | 0.47 | 1.20 | 0.56 | 0.69 | 1.52 | 1.46 | 1.35 | 1.10 | 0.97 | 0.83 |
| 3-ethyl-2,5-dimethyl pyrazine | 8.6 ^a | | | | 9.46 | 7.37 | 11.61 | 1.84 | | | 12.50 | 7.21 | 7.06 | 78.00 | 86.57 | 116.88 | | | |
| Alcohols | | | | | | | | | | | | | | | | | | | |
| 2-methyl-1-butanol | 15.9 ^a | | | 0.63 | | | | | | | | | | | | | | | |
| 1-Octen-3-ol | 1.5 ^a | | | | 52.98 | 59.41 | 133.07 | 10.54 | 16.17 | 24.52 | 42.02 | 32.72 | 29.62 | 34.37 | 76.44 | | | | |
| Ketones | | | | | | | | | | | | | | | | | | | |
| 2,3-Butanedione | 0.059 ^a | | | | 1006.13 | 340.40 | 949.68 | | | | | | | | | | | | |
| Aldehydes | | | | | | | | | | | | | | | | | | | |
| 2-methyl-propanal | 1.5 ^a | 40.11 | 25.77 | 12.10 | | | 31.52 | 37.42 | 16.79 | 46.90 | 21.82 | 21.81 | 20.75 | 17.56 | | 36.78 | 31.81 | 75.49 | 114.73 |
| 3-methyl-butanal | 1.1 ^a | 33.43 | 29.74 | 32.99 | | | 19.40 | 70.04 | 45.39 | 46.93 | 42.21 | 34.20 | 17.12 | 18.41 | 28.53 | 22.73 | 123.83 | 138.03 | 425.61 |
| 2-methyl-butanal | 50 ^a | 1.30 | 1.17 | 0.49 | | | 0.75 | 1.70 | 1.63 | 1.90 | 1.19 | 0.60 | 0.74 | 0.76 | | 0.76 | 2.42 | 3.40 | 9.33 |
| Acids | | | | | | | | | | | | | | | | | | | |
| Acetic acid | 0.41 ^a | | | | 854.70 | | 604.30 | 443.26 | 377.74 | 359.76 | 209.74 | 257.45 | 159.22 | 194.09 | | 210.26 | 114.18 | 245.70 | 677.83 |
| S-containing compounds | | | | | | | | | | | | | | | | | | | |
| Dimethyl Disulfide | 23 ^b | 3.92 | 1.32 | 10.83 | 8.74 | 4.78 | 4.45 | 1.75 | 0.96 | 1.07 | 2.19 | 1.11 | 1.60 | 18.74 | 10.39 | 35.53 | 4.29 | 7.24 | 5.32 |
| Dimethyl Trisulfide | 8 ^a | 23.97 | 29.65 | 28.18 | 33.39 | 31.62 | 17.40 | | | 2.23 | 10.02 | 7.95 | 10.54 | | 14.16 | 47.20 | | | 4.88 |

Note: *C1, C2, E1, E2, S1 and S2 are fermented shrimps collected from Samutsakhon, Phetchaburi, Trat, Rayong, Satun and Songkhla provinces, respectively.

^{a,b} Threshold values in water^(a) and air^(b) sources were published by Van Gemert (2011).

Blank cell means the OAV was not calculated for the sample.

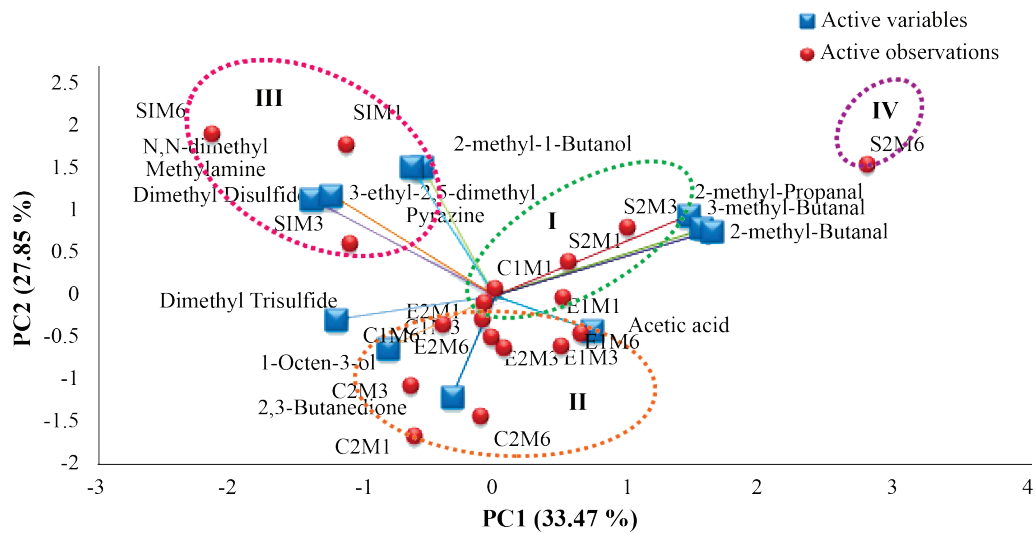


Figure 1. Principal component analysis score and loading plots of aroma compounds and fermentation time of fermented planktonic shrimp and the ellipses of active observations of clusters obtained from hierarchical cluster analysis.

Hierarchical clustering analysis (HCA)

To visualize the relationships among samples, HCA was applied, and the resulting dendrogram shows that the 18 samples from six manufactures ripened for 1, 3, and 6 months could be grouped into four clusters (Figure 2). Samples of C1, C2, E1, E2 and S2 were mixed to form larger clusters (Cluster I and II), which indicates the similarity of AC profiles in these samples. The four clusters (shown by ellipses of dotted lines) were applied to the PCA plot for clear observation (Figure 1). Cluster I (C1M1, C1M3, E1M1, E2M1, S2M1 and S2M3) indicated the relation of AC profiles during early fermentation, whereas cluster II (C1M6, C2M3, C2M1, C2M6, E1M3, E1M6, E2M3 and E2M6) indicated the relation of most compounds around later fermentation. Both clusters contained the same moderate amounts of N-containing compounds and S-containing compounds, while cluster II also had a high 1-octen-3-ol, 2,3-butanedione and acetic acid content. These three compounds were found in large quantities in C2. Therefore, these special compounds might be related to the *Mesopodopsis* sp. used for making kapi of C2, as well as the distinctive manufacturing process.

Cluster III (S1M1, S1M3 and S1M6) was obviously separated from the others, which was a result the highest content of N- and S-containing compounds in S1, due to the addition of sugar in the formula, which participated in the Maillard reaction. Cluster IV (S2M6) was also clearly separated, which was associated with the variables loaded on PC1 in the positive direction, primarily the high aldehyde content (2-methyl-propanal, 3-methyl-butanal and 2-methyl-butanal). Though the manufacturing processes of E1, E2, and S2 used the same shrimp species and ratios of shrimp and salt, the resulting aroma compounds of S2 was different in the sixth month of ripening.

Linear discriminant analysis (DA)

In order to reduce the number of ACs needed to separate the six manufacturing processes, discriminant analysis was used. The six processes could be separated into four groups by a combination of 11 ACs and by fermentation time. These factors explained 99.66 % of the total variance. Function 1 explained 98.24 % of the variance, and function 2 accounted for 1.42 %. A plot of F1 vs. F2 is presented in Figure 3, which shows the four clearly separated groups: 1) C2, 2) C1, 3) a grouping of

E1, E2, and S2, and 4) S1. It is notable that the resulting groups were the same as the four different manufacturers, as mentioned previously, with differences in species and the ratio of shrimp and salt. Therefore, the variation of chemical composition of fermented planktonic shrimp could be attributed to the differences in species (C2 with

Mesopodopsis sp.), a special ingredient (S1 with added sugar), and ratio of shrimp and salt (C1 with lower salt), whereas the geographic area (E1, E2, and S2 with same *Acetes* sp. and salt content) had minimal effect on the resulting composition even though the protein content of the raw *Acetes* sp. used was different.

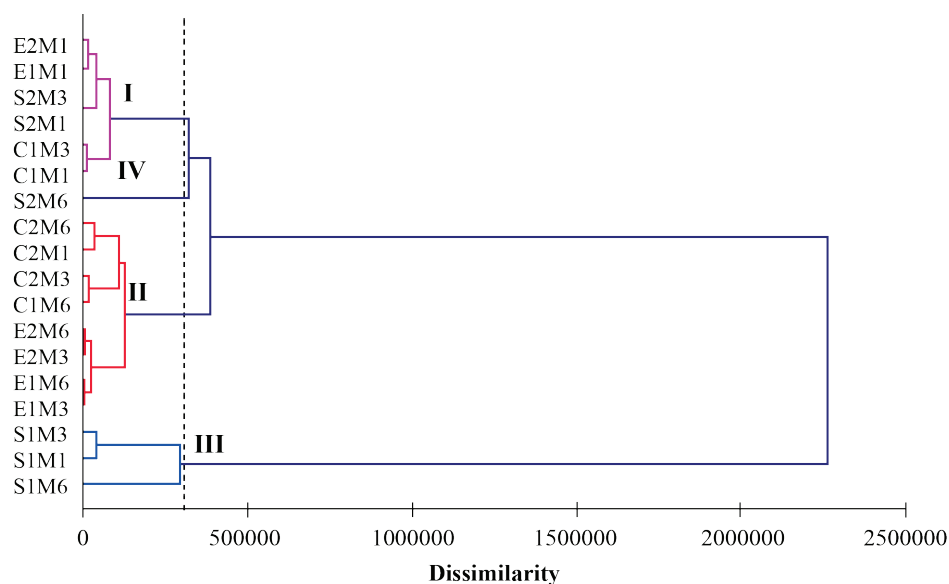


Figure 2. Hierarchical cluster analysis dendrogram of the aroma compounds of fermented planktonic shrimp obtained from six manufacturers at three fermentation times.

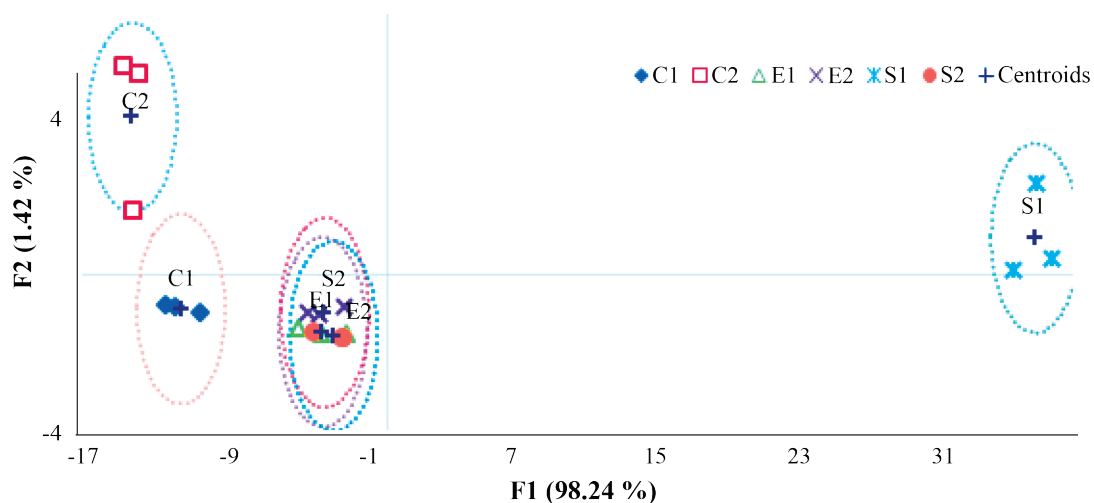


Figure 3. Discriminant analysis results for aroma compounds of fermented planktonic shrimp from six manufacturers.

CONCLUSION

Chemical composition of planktonic shrimp varied by species and geographic area. Salted shrimp paste (kapi) is produced by various manufacturing processes involving different shrimp species, sun-drying techniques, and salt content. However, the compounds which played an important role in odor were identified as S-containing compounds (dimethyl disulfide, dimethyl trisulfide), aldehydes (2-methyl- propanal, 3-methyl-butanal, 2-methyl-butanal), N-containing compounds (N, N-dimethyl methylamine, 3-ethyl-2,5-dimethyl pyrazine), alcohols (2-methyl-1-butanol, 1-octen-3-ol), a ketone (2,3-butanedione) and acetic acid. Of these compounds, only the S-containing compounds and aldehydes were found in all kapi samples. Chemometrics can differentiate the similar aroma compounds into groups. Kapi with sugar inclusion in the formula (S1, cluster III) was obviously unique, owing to the highest amounts of N-containing compounds (N, N-dimethyl methylamine (TMA), 3-ethyl-2,5-dimethyl pyrazine) and S-containing compounds, which is caused by enhancing the Maillard reaction. Moreover, 2,3-butanedione was only found in kapi produced from *Mesopodopsis* sp. with a special manufacturing technique (a long sun-drying period and high added salt) (C2, PC3). Further study should be done to clarify whether the 2,3-butanedione formation is influenced by drying time or the use of *Mesopodopsis* sp. The aroma compounds of kapi that are produced from the same species (*Acetes* sp.) and salt content were quite similar. Therefore, the variation of chemical components and aroma compounds of fermented planktonic shrimp were based on the species, special manufacturing techniques, formula, and the fermentation period, whereas geographic area was less relevant. In this study, only SPME GC-MS with one column type was used to identify low-molecular weight volatile compounds in kapi. Therefore, further study using different column types to collect other volatile fractions as well as GC-O analyses are suggested, as they will increase the understanding of the key aroma compounds of kapi.

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