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Quality of reduced sodium shrimp paste from shrimp head as alternative source

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Abstract

The significantly decreased availability of the main raw materials for making shrimp paste is a big problem, while most by-products from shrimp processing, especially head, are not yet properly used. Therefore, using shrimp head for shrimp paste production is well motivated. However, traditional shrimp paste is high in salt and can impact health via hypertension, kidney, or cardiovascular disease. This study aimed to produce a reduced sodium shrimp head paste, using 12, 14 or 16-fold amount of shrimp heads relative to salt. The salted shrimp head was fermented for 14-16 hr. Thereafter, it was dried at 60 °C, then ground and fermented further for 30 d. The paste was dried again to 40-45% moisture content and fermented for another 90 d. The total viable count and lactic acid bacteria significantly decreased in all treatments, and no pathogenic microorganisms were detected. After fermentation for 90 d, the a_w had decreased in all treatments. The highest shrimp head proportion (16:1) exhibited the highest total volatile basic nitrogen (TVB-N) and trimethylamine (TMA) contents. In conclusion, shrimp head can serve as an alternative raw material in shrimp paste with comparatively low salt content that may reduce the risks of hypertension, cardiovascular disease, and stroke.

Keywords: shrimp paste; shrimp head; sodium reduction; fermentation.

Practical Application: White shrimp head can be used as an alternative source of shrimp paste.

1 Introduction

Shrimp paste is commonly produced and consumed in Southeast Asian countries, including Burma, Cambodia, Indonesia, Malaysia, Myanmar, Philippines, Vietnam, and Thailand (Hajeb & Jinap, 2012). Although raw materials, salt ratios and fermentation times vary by region, the key production methods involving drying, grinding, and fermentation are quite similar (Hajeb & Jinap, 2012). In Thailand, shrimp paste is commonly used as a condiment and is an important ingredient in some traditional main dishes. It is produced by mixing small shrimp, called krill, of *Acetes* and *Mesopodopsis* species with salt at 3-5:1 ratio of shrimp to salt, then dried, ground and fermented for at least 1 month (Pongsetkul et al., 2014). However, decreasing marine populations of krill have been noted over the recent years (World Wildlife Fund, 2015) including also *Mesopodopsis* raw material of shrimp paste (Pongsetkul et al., 2017). In contrast to the scarcity of krill as raw materials to produce shrimp paste, tremendous amounts of by-products from shrimp processing industry, particularly of the white shrimp (*Litopenaeus vannamei*), are increasingly available. This includes head, shell, and tail, and especially the shrimp head represents up to 45% of the total shrimp weight (Ghorai, 2013). In 2018, Thailand produced around 270,000 tons of shrimp and exported 185,000 tons (Fisheries Economics, 2018) and about 90% of the by-products from shrimp processing are not yet properly utilized. They may be used as animal feed or fertilizer with a low value/price. Recently, shrimp head cost about 0.15 USD/kg, which is much less than the cost of *Acetes vulgaris* as 1.20 USD/kg. Shrimp head is composed of moisture 78.28 ± 0.39%, protein 12.90 ± 0.15%, fat 4.23 ± 0.35% and ash 5.25 ± 0.11% (Reerueangchai, Suwannarat & Hinsua, 2014), while planktonic shrimp (*Acetes vulgaris*) and krill (*Mesopodopsis*) are composed of moisture 86.74%, 86.25%; protein 8.88%, 12.09%; fat 1.25%, 1.26%; ash 2.95%, 2.27%; and salt 1.26%, 1.16%, respectively (Kongpun, & Kongrat, 2013). Therefore, it is hypothesized that shrimp paste might be produced from shrimp head if proper processing is applied.

Generally, a good Kapi (Thai shrimp paste) must have low fishy and ammonia smells, be not too salty, without a bitter taste, and can be of various colors such as pinkish, dark grayish brown and purplish gray. It must contain over 36% table salt (dry basis) (Thailand, 1992) or 14,400 mg sodium in 100 g of shrimp paste. However, one serving size of shrimp paste (15 g) contains 2,160 mg sodium and is therefore considered high sodium food (any food containing more than 20% of daily intake per serving or 460 mg sodium is considered high sodium food) (The United States Food and Drug Administration, 2018). It is well known that the consumption of high sodium food may increase the risk of hypertension, a major cause of death and disability worldwide. Excessive sodium intake not only causes hypertension but also can lead to stroke, calcium loss, osteoporosis, cardiovascular disease, and kidney disease (Malta et al., 2018; Grillo et al., 2019). Therefore, this study aimed to a produce reduced sodium

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shrimp head paste with reduced risk of hypertension from its consumption.

2 Materials and methods

2.1 Shrimp paste production

Shrimp head containing pereiopods along with internal organs was of received from a frozen food manufacturer in Songkhla province, and was used in this experiment. Since, this work aimed to reduce salt content, therefore, ratio of shrimp to salt was further increased. Shrimp head and NaCl were well mixed together at shrimp to salt ratio 12, 14 or 16 as showed in Table 1. These treatments were decided based on preliminary experiments, in which the highest shrimp to salt ratio was 8. The mixture was incubated indoors for 14-16 hr at room temperature (30-33 °C). After that, the mixture was dried in a hot air oven at 60 °C for 6 hours, or until 60% moisture content, and was then ground to make the solids finer in the mixture. After grinding, earthen jar was used to ferment the paste for 30 d on the roof deck at 35-39 °C with humidity 77-81%. The paste was again dried at 60 °C for 5 hours until 40-45% moisture content, following the standard of shrimp paste production (Thailand, 1992). The paste was ground and further fermented for 90 d.

2.2 Physical and chemical quality analyses

Physical quality indicators including color and $a_{w,}$ and chemical quality parameters including proximate composition, pH, TVB-N and TMA, were determined for the shrimp head (raw material) and the finished products.

Proximate analysis (moisture, crude protein, fat, ash, carbohydrate)

-Moisture (Association of Official Analytical Chemists, 2000)

Moisture content was determined by weighing (Sartorius-Sartorius AG, BSA 224 S-CW, Goettingen, Germany) 3 g of sample and drying it in an oven at 105 °C for 3 hours. The sample was reweighed and drying continued until constant weight. After that, the moisture was calculated as Equation 1.

$$\text{Moisture}(\%)=\frac{W_1-W_2}{W_1}\times 100 \quad (1)$$

where W_1 = weight of a sample before drying (g)

W_2 = weight of a sample after drying (g)

- Crude protein (Association of Official Analytical Chemists, 2000)

Table 1. Ratio of shrimp head to salt used in this experiment.

Treatment	Ingredients	
	Shrimp head	Salt (NaCl)
Shrimp head 12: salt 1	92.31%	7.69%
Shrimp head 14: salt 1	93.33%	6.67%
Shrimp head 16: salt 1	94.12%	5.88%

Crude protein content in the sample was determined by adding 0.5-1 g sample to a test tube. Then Kjedahl catalyst was added (mix of K_2SO_4 with $CuSO_4$ in 9:1 ratio) for 5 g along with 200 mL of concentrated H_2SO_4. Blank was prepared by adding Kjedahl catalyst and concentrated H_2SO_4 without a sample to a test tube. Then mixture was boiled until it was clear and then cooled down. After that, 60 ml distilled water was added to the mixture.

The flask was connected with a condenser and NaOH was added, while another flask that contains H_3BO_3 and indicator solution was serving as the receiver. The solution was heated until all NH_3 had been distilled. The distilled solution was removed from receiver and titrated with HCl until it turned colorless. The process was repeated again using the blank. Crude protein content was calculated as Equation 2.

$$\text{Protein}(\%)=(A-B)\times\frac{(A-B)\times N\times 14.007\times 5.6}{W} \quad (2)$$

where A = volume of HCl used to titrate sample (ml)

B = volume of HCl used to titrate blank (ml)

N = Normality of HCl

W = weight of sample (g)

14.007 = atomic weight of nitrogen

5.6 = the protein-nitrogen conversation factor

- Fat content (Association of Official Analytical Chemists, 2000)

Filter paper was used to wrap 3-5 g of sample and weighed. Then the sample was transferred to Soxhlet device. Bottle on the heating mantle was filled with petroleum ether (250 mL) and heated for 14 hours at the rate of 150 drops per minute until there was no more drip of solvent. The bottle was allowed to cool down and was taken to evaporate the solvent until completely dry, and the dried film was weighed. Fat content (%) was calculated as Equation 3.

$$\text{Fat}(\%)=\frac{\text{Weight of fat}}{\text{Weight of sample}}\times 100 \quad (3)$$

- Ash content (Association of Official Analytical Chemists, 2000)

The crucible with 5 g sample was weighed before heating with a Bunsen burner until it no longer produced fumes, and then a furnace was used to heat the sample overnight. After cooling down, ash of the sample was weighed. The ash content was calculated as Equation 4.

$$\text{Ash}(\%)=\frac{\text{Weight of ash}}{\text{Weight of sample}}\times 100 \quad (4)$$

-Carbohydrate content

Total carbohydrate content was calculated by subtracting as Equation 5.

$$\text{Carbohydrate}(\%)=100-(\text{moisture}+\text{crude protein}+\text{total fat}+\text{ash}) \quad (5)$$

Determination of salt content (Association of Official Analytical Chemists, 2000)

The sample and 20 ml 0.1 N $AgNO_3$ were mixed together. After that, 10 ml of concentrated HNO_3 was added. Then the solution was boiled on a hot plate until all solid except for $AgNO_3$ was completely dissolved. Tap water was used to cool down the solution. Ferric alum indicator (ammonium iron sulfate) was added to the solution. After that, the solution was titrated with 0.1 N KSCN until it became light brown. Salt content of the sample was calculated as Equation 6.

$$\text{NaCl}(\%)=\frac{(\text{vol. } AgNO_3\times\text{Conc. of } AgNO_3)-(\text{vol. of KSCN}\times\text{Conc. of KSCN})}{\text{weight of sample (g)}} \quad (6)$$

Water activity (a_w)

Water activity was measured using a water activity analyzer (METER- AQUALAB PRE, Decagon Devices Inc., Washington, USA).

pH determination

The sample was homogenized in distilled water at 1:5 ratio and the pH of the mixture was determined by using a pH meter (Sartorius-Sartorius AG, Docu-pH+ Meter, Goettingen, Germany).

Total volatile basic nitrogen (TVB-N) and trimethylamine-nitrogen (TMA)

Conway's micro-diffusion assay was used to determine TVB-N and TMA according to method of Junsi (2012). 4% trichloroacetic acid (TCA, 2 ml) was used to extract an 8 g sample. Then the extracted mixture was filtered through Whatman No. 41 and 4% TCA was added until the solution reached 10 ml. After that, the filtrate solution was added into the outer ring of a Conway unit, while boric acid with indicator was added into the inner ring. Saturated K_2CO_3 was added in the outer ring opposite to the sample. The Conway unit was rotated to mix K_2CO_3 with the sample solution. The solution was incubated at ambient temperature for 3 hours. After incubation, 0.02 N HCl was used to titrate the indicator in inner ring until it turned to the initial inner ring color. The process was repeated for the blank without a sample, using TCA solution instead. For determination of TMA, 1 ml of 10% formaldehyde solution was added to the sample solution before saturated K_2CO_3. TVB-N and TMA of sample were calculated as Equation 7.

$$\text{TVB-N or TMA}\left(\text{mg.nitrogen per 100g of sample}\right)=\frac{(N)(14)(A-B)(V)(100)}{\text{weight of sample}} \quad (7)$$

where N = normality of HCl

A = ml of HCl used to titrate sample mixture

B = ml of HCl used to titrate blank

V = total volume of sample and TCA in sample preparation

2.3 Microbiological quality analyses

Microbiological quality measures, including TVC, coliforms, *Escherichia coli*, *Clostridium perfringens*, *Salmonella*, *Staphylococcus aureus*, yeast and mold, and lactic acid bacteria (LAB), were determined for the shrimp head (raw material) and the finished products.

Total viable count or total mesophilic count (TVC or TMC)

TVC was analyzed according to the method of The United States Food and Drug Administration (2001a) with some modifications (using 0.85% NaCl solution instead of peptone water). Briefly, sample and 0.85% NaCl in distilled water were mixed together. The mixture was added to blender jar and blended to 10^{-1} dilution. Then appropriate dilutions were made. One ml of each dilution was transferred to a plate. Then 15 ml of plate count agar was added to the plate. The plate was rotated to spread agar, and the agar was allowed to solidify, then it was inverted and incubated at 35 °C for 48 hours. The microbial colonies were counted and recorded in colony forming units per gram (log CFU/g).

Coliform bacteria

Coliform bacteria were analyzed according to the method of The United States Food and Drug Administration (2020) with some modifications (using 0.85% NaCl solution instead of peptone water). The sample was prepared as if it was for TVC. Each dilution was transferred to 3 lactose broth tubes. All tubes were incubated at 35 °C for 24 hours. The tubes containing gas were recorded and further incubated for another 24 hours to record the gas production. One loopful from lactose broth tubes was transferred to brilliant green lactose bile (BGLB) broth tube. The tubes were incubated at 35 °C for 48 hours. All tubes containing active gas were recorded to calculate the most probable number (MPN).

Staphylococcus aureus

Staphylococcus aureus was analyzed according to the method of The United States Food and Drug Administration (2016) with some modifications (using 0.85% NaCl solution instead of peptone water). Sample was prepared similarly as for TVC. One ml of each dilution was divided to 0.1 ml aliquots and transferred to plates with Baird-Parker (BP) agar. The plates were incubated. Plates that contained 20-200 colonies were selected and counted before reporting "detected" or "not detected" per 0.1 g.

Salmonella

Salmonella was analyzed according to the method of The United States Food and Drug Administration (2021) with some modifications (using 0.85% NaCl solution instead of peptone water). Sample and sterile lactose broth were mixed together and added into sterile blending container. The mixture was blended and transferred to container and incubated at room temperature for 1 hour. After incubation, the mixture was transferred to Rappaport-Vassiliadis (RV) medium and Tetrathionate broth (TT). Then RV medium and TT broth were incubated in water

bath at 42 ± 0.2 °C and 43 ± 0.2 °C for 24 ± 2 hours respectively. Thereafter, the mixture was shaken. One loopful of incubated sample in TT broth and RV medium were streaked on bismuth sulfite (BS) agar, xylose lysine desoxycholate (XLD) agar, and Hektoen enteric (HE) agar. Plates containing BS, XLD, and HE agar were incubated at 35 °C for 24 hours. Any plate that had more than one colony of *Salmonella* was selected and sample from the selected plate was streaked on triple sugar iron agar (TSI) and lysine iron agar (LIA) and incubated.

Clostridium perfringens

Clostridium perfringens was analyzed according to the method of The United States Food and Drug Administration (2001b) with some modifications (using 0.85% NaCl solution instead of peptone water). Sample and peptone dilution fluid were added to sterile blender jar. The mixture was homogenized. Dilutions from 10^{-1} to 10^{-6} were made by transferring 10 ml of homogenate to 90 ml of peptone dilution fluid. TSC agar without egg yolk was poured into the plates. Plates were rotated to spread agar. One ml of each dilution was transferred to agar plates and additional TSC agar without egg yolk was poured into plates. Plates were placed in anaerobic jar to ensure anaerobic conditions and incubated. The incubated plates that contained 20-200 black colonies were selected. Number of microbial colonies was counted and recorded as "detected" or "not detected" per 0.01 g.

Yeast and mold

Yeast and mold were analyzed according to the method of The United States Food and Drug Administration (2001c) with some modifications (using 0.85% NaCl solution instead of peptone water). Sample and 0.85% NaCl in distilled water were mixed together to make 10^{-1} dilution. The mixture was homogenized. Appropriate dilutions were made. One ml of each sample dilution was transferred into plates. After that dichloran 18% glyceral (DG18) agar was added. Plates were mixed by swirling clockwise and counterclockwise then incubated in the dark at 25 °C. The incubated plates containing 10-150 colonies were selected for counting. Number of microbial colonies was recorded in log CFU/g.

Lactic acid bacteria (LAB)

LAB was determined according to Pongsetkul et al. (2017) with some modifications (using normal MRS instead of MRS agar plus 10% NaCl) as the sample was prepared similarly as for TVC, and 1 mL of each dilution was transferred to plate. Then De Man, Rogosa, and Sharpe (MRS) agar with 0.0005% bromocresol purple with pH 7.5 was incubated at 30 °C for 3 d.

2.4 Statistical analysis

The experiment was conducted in three replicates and data from experiment were analyzed by one-way analysis of variance and are shown as mean ($\bar{x}$) ± standard deviation (S.D.). Means comparisons were evaluated by Tukey's multiple range test. Completely randomize design (CRD) was used in testing chemical, physical, and microbiological properties.

3 Results and discussion

3.1 Physical and chemical qualities

Proximate composition

At the beginning, moisture, carbohydrate, protein, fat and ash of shrimp head were 75.38 ± 0.51%, 7.95 ± 0.62%, 11.22 ± 0.21%, 0.95 ± 0.07% and 4.51 ± 0.08%, respectively. Proximate composition of the shrimp head used in this experiment was similar to the proximate composition of Brazilian white shrimp (*Litopenaeus vannamei*) head, containing above mentioned components at 75.47 ± 0.43, 4.33, 14.75 ± 0.79, 1.10 ± 0.10 and 4.35 ± 0.28 in the same order (Fernandes et al., 2013), and also close to those of *Acetes vulgaris* and *Mesopodopsis* raw materials used to produce Kapi, which contain moisture at 86.74%, 86.25%; protein 8.88%, 12.09%; fat 1.25%, 1.26% and ash 2.95%, 2.27, respectively (Kongpun & Kongrat, 2013). At the end of experiment (90 d), it was found that moisture content had decreased from 75.38±0.51% down to 42.5%. Similar decreasing trend was found for carbohydrate content, which decreased from 7.95 ± 0.62 to 4.14-5.29%. However, protein content increased from 11.22% to 22.70-26.1% due to both fermentation and drying. Expectedly, the ash increased from 4.51±0.08% to 20.60-24.70% as a result of the salt added. As more salt was added the ash content increased. Comparison of the experimental shrimp head paste with a commercial one indicated a large difference in carbohydrate, maybe due to starch and/or sugar that are usually added to improve texture, flavor and taste attributes, as well as to reduce the price. The proximate composition of commercial Kapi in Thailand had moisture 33.79-52.54%, carbohydrate 2.91-19.41%, protein 17.59-29.77%, fat 0.75-1.76% and ash 17.18-29.68% (Pongsetkul et al., 2014).

Salt content

Salt content of the initial white shrimp head was 1.22 ± 0.08% (4.96 ± 0.32% dry basis), which is similar to the salt contents 1.26% (8.44% dry basis) and 1.16% (9.50% dry basis) reported for *Acetes vulgaris* and *Mesopodopsis*, respectively (Kongpun & Kongrat, 2013). At the end of experiment (90 d), salt contents of shrimp pastes made at ratios 12:1, 14:1 and 16:1 were 13.95 ± 0.62% (24.26 ± 1.04% dry basis), 10.34 ± 1.26% (20.68 ± 2.52% dry basis) and 9.37 ± 0.57% (16.76 ± 1.02% dry basis) which were much lower than the 14.72-22.93% (22.77-35.47% dry basis) of commercial Kapi (Pongsetkul et al., 2014). This indicates that this experimental shrimp pastes made at ratios 14:1 and 16:1 had reduced salt levels (decreased by at least 25%) when compared to a traditional product.

Water activity (a_w)

After salting the a_w in all treatments decreased but still exceeded 0.91 (Figure 1), which normally cannot retard the growth of spoilage microorganisms (Majumdar et al., 2018). However, the a_w decreased further. This may explain the reduction of TVC and LAB in some treatments. Clearly, a_w in all treatments decreased until it reached approximately 0.80. During fermentation, after second drying step, the a_w in all treatments was about 0.78.

The higher the salt ratio (12:1) the lower a_w was obtained, such that supports only halophilic growth (Resnik & Chirife, 1988).

Comparison of a_w with TVC and LAB indicated that a lesser a_w lowered both microbial values. Both salt addition and drying reduced the growth of general microorganisms (Pittia & Paparella, 2016).

pH

After salting the pH in all treatments increased, matching the increased TVB-N and TMA as functions of spoilage microorganisms and endogenous enzymes (Etienne, 2005). Then the pH decreased after drying from evaporation of TVB-N and TMA (O'Neil, 2013). It is interesting that pH of the 16:1 treatment decreased after 15 d, and then increased to equal the other ratios after 30 d. After secondary drying, pH in all treatments decreased again. However, decreasing pH was observed after 45 d except for the ratio 16:1. After that pH in all treatments increased and then stayed constant until 90 d. In addition, there were no differences in pH between the ratios, except for the ratio 16:1 in some process stages. This might be because of the lowest water activities for ratio 16:1.

In general, pH of raw shrimp head was about 8, possibly increased by enzymatic hydrolysis after death of the shrimp (Guo et al., 2019) (Figure 2). The pH results may indicate that the raw material had a high content of alkaline compounds. In addition, pH in all treatments kept increasing after salting. This suggests that there were some chemical reactions taking place. It is known that both TVB-N, TMA and other amine compounds are alkaline. These compounds actually can be derived from endogenous (autolysis) and exogenous enzymes (microorganism enzymes) as time passes (Etienne, 2005). In fact, high activities of digestive enzymes such as protease, lipase and amylase are found in the shrimp head (Kanduri & Eckhardt, 2002). In addition, the digestive tract also carries gut microflora dependent on the food and environment that the shrimp was cultured in (Li et al., 2019). For these reasons, spoilage signs definitely emerge first at the head or a shrimp. It is pointed out that while the shrimp head can be used for a fermented product, some quality parameters such as pH may not be on desired level.

Total volatile basic nitrogen and trimethylamine (TVB-N and TMA)

As shown in Figure 3 and Figure 4, both TVB-N and TMA significantly increased after salting. Particularly the TVB-N increased along the fermentation process. However, every drying step reduced both TVB-N and TMA. This might be because the volatile compounds are sensitive to temperature. However, a different change pattern was found for TMA that seemed to increase with fermentation time. Actually, TMA is used to indicate fishy odor, which also is associated with spoilage or interior quality of a raw seafood product (Herath et al., 2019). However, TMA is also an essential compound for proper smell in some fermented fish products, particularly in fish sauce (Yimdee & Wang, 2016). Generally, changes of both TVB-N and TMA during fermentation were similar to a wave going up and down but not consistently increasing with fermenting time as might be expected. This may be due to some chemical reaction like the browning reaction, which can consume ammonia

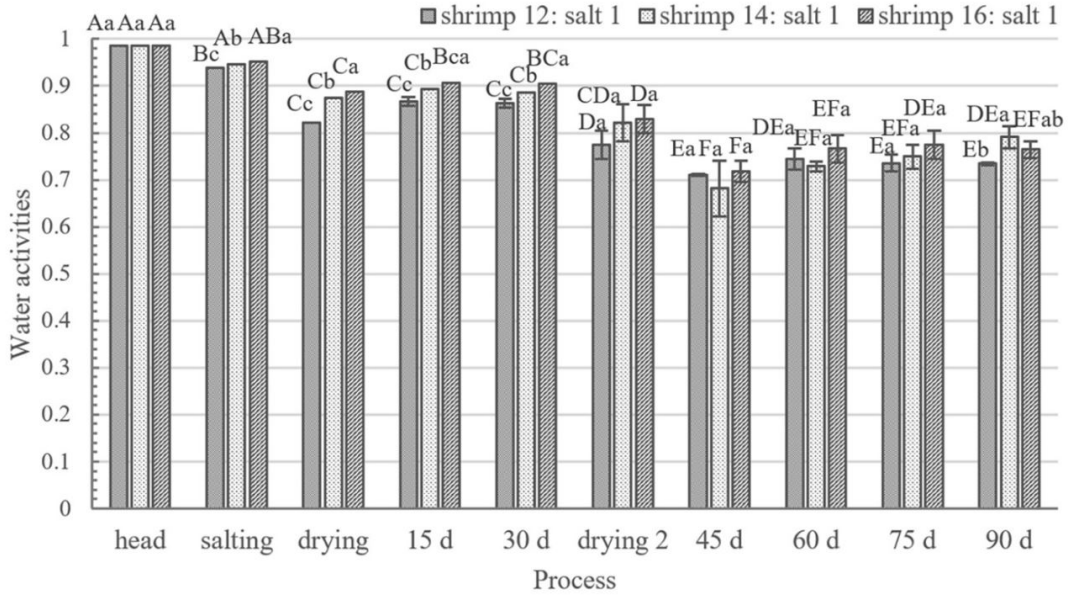

Figure 1. Water activity (a_w) of shrimp head (*Litopenaeus vannamei*) and of shrimp paste with shrimp head to salt ratios 12, 14 or 16. Different uppercase letters indicate significant differences within a salt ratio. Different lowercase letters indicate significant difference within a process ($p < 0.05$). Head means shrimp head; Salting is after salting step; Dry is after primary drying step; 15d is after fermentation for 15 days; 30d is after fermentation for 30 days; Dry2 is after secondary drying; 45d is after fermentation for 45 days; 60d is after fermentation for 60 days; 75d is after fermentation for 75 days; 90d is after fermentation for 90 days (end of fermentation).

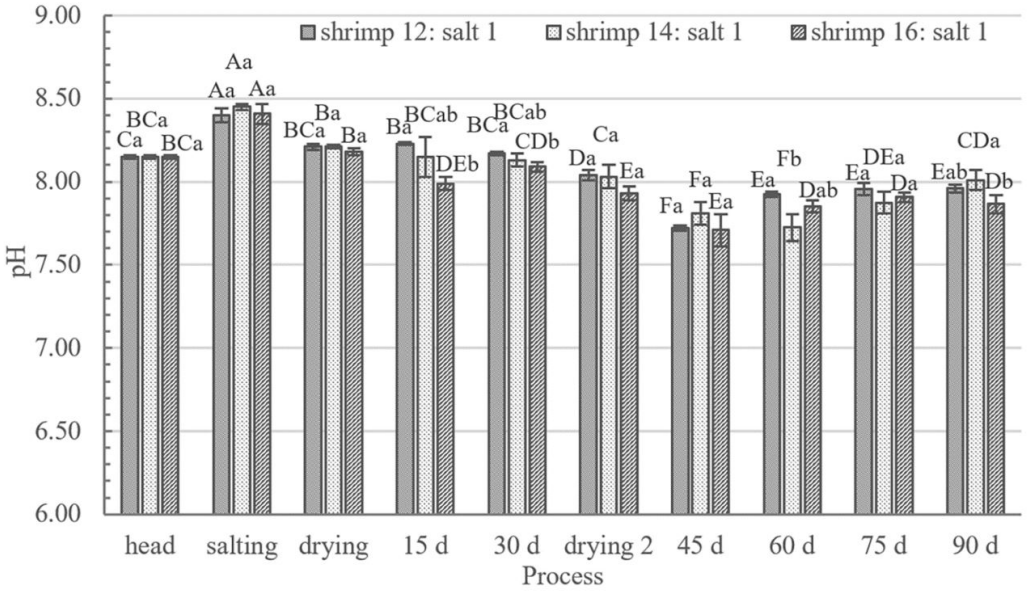

Figure 2. pH of shrimp head (*Litopenaeus vannamei*) and of shrimp paste prepared with shrimp head to salt ratios 12, 14 or 16. Different uppercase letters indicate significant difference within a salt ratio. Different lowercase letters indicate significant difference within a process ($p < 0.05$). Head means shrimp head; Salting is after salting step; Dry is after primary drying step; 15d is after fermentation for 15 days; 30d is after fermentation for 30 days; Dry2 is after secondary drying; 45d is after fermentation for 45 days; 60d is after fermentation for 60 days; 75d is after fermentation for 75 days; 90d is after fermentation for 90 days (end of fermentation).

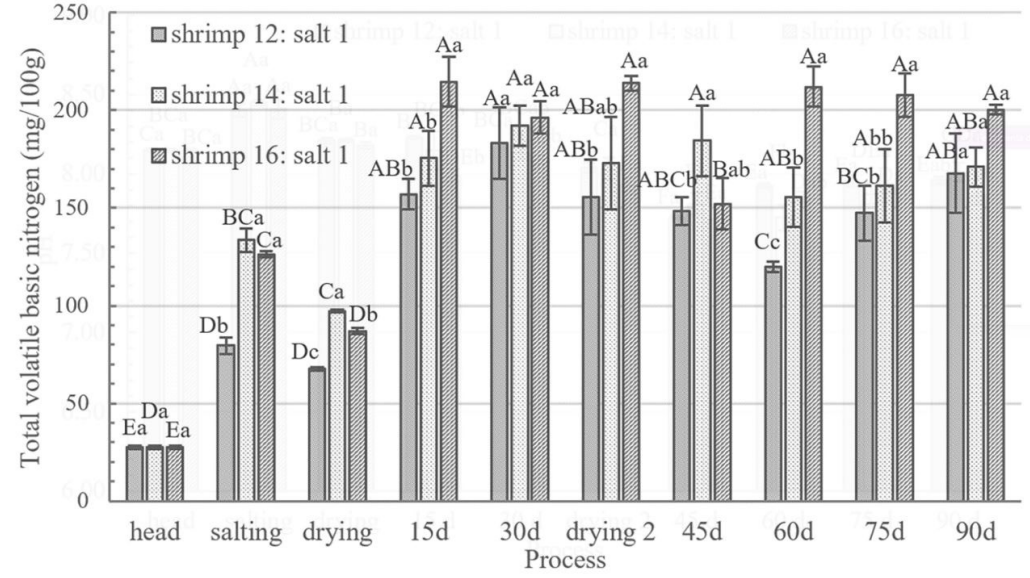

Figure 3. Total volatile basic nitrogen (TVB-N, mg/ 100 g) of shrimp head (*Litopenaeus vannamei*) and shrimp pastes prepared with shrimp head to salt ratio 12, 14 or 16. Different uppercase letters indicate significant difference within a salt ratio. Different lowercase letters indicate significant difference within a process ($p < 0.05$). Head means shrimp head; Salting is after salting step; Dry is after primary drying step; 15d is after fermentation for 15 days; 30d is after fermentation for 30 days; Dry2 is after secondary drying; 45d is after fermentation for 45 days; 60d is after fermentation for 60 days; 75d is after fermentation for 75 days; 90d is after fermentation for 90 days (end of fermentation).

as amine compound to react with carboxylic group of sugar or carbohydrate compound (Izzo & Ho, 1992). The volatile compounds could also change as a result of chemical reactions from both microbial action and its metabolites, as well as from auto-oxidation (Etienne, 2005).

In addition, it was found that at a higher salt content (12:1), TVB and TMA were lower than in other treatments. This may due to the spoilage microorganisms normally not being halophilic bacteria or halo tolerant bacteria (Larsen, 1986). It is known that TVB-N consists of ammonia, trimethylamine and dimethylamine,

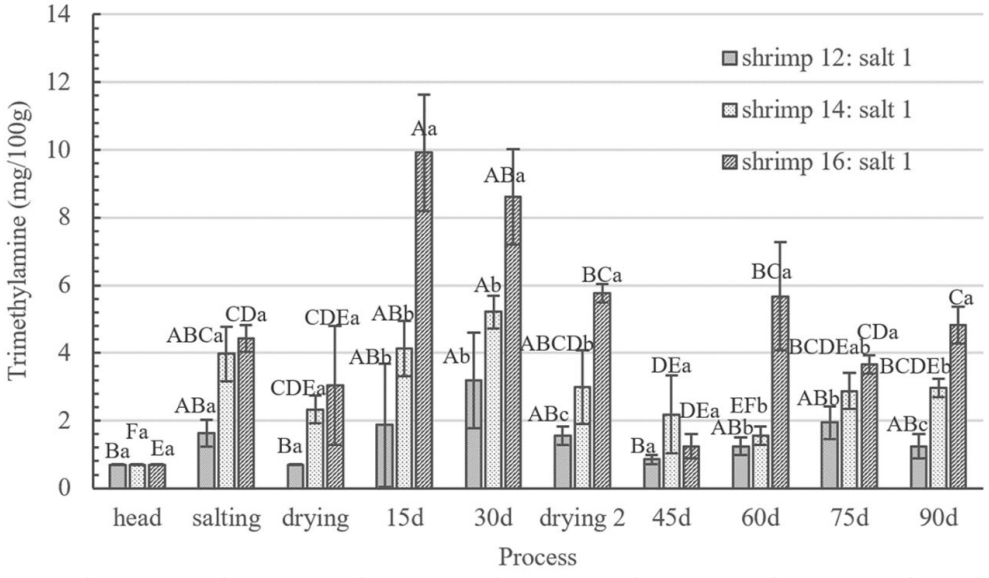

Figure 4. Trimethylamine (TMA, mg/ 100 g) of shrimp head (*Litopenaeus vannamei*) and shrimp paste prepared with shrimp head to salt ratio 12, 14 or 16. Different uppercase letters indicate significant difference within a salt ratio. Different lowercase letters indicate significant difference within a process ($p < 0.05$). Head means shrimp head; Salting is after salting step; Dry is after primary drying step; 15d is after fermentation for 15 days; 30d is after fermentation for 30 days; Dry2 is after secondary drying; 45d is after fermentation for 45 days; 60d is after fermentation for 60 days; 75d is after fermentation; 90d is after fermentation for 90 days (end of fermentation).

which are all volatile alkaline compounds. Therefore, TVB-N should positively correlate with the pH. However, changes in pH of the system actually relate to the amounts of bases, acids and the buffering capacity. An increased TVB-N and TMA may not increase pH, because also acids are generated, or because of the buffering capacity of the system (Pongsetkul et al., 2017).

3.2 Microbiological qualities

Total viable count (TVC)

Interestingly, TVC in all treatments significantly increased after salting and did not decrease after drying as would be expected (Figure 5). This may be due to not having high enough salt content, as indicated by the high a_w (0.910). In addition, temperature of sample during drying was around 41 °C, which is in the optimum temperature range of most mesophilic microorganisms (20-45 °C) (Keenleyside, 2019). A high a_w with not too high temperature (<50 °C) supports microbial growth, particularly microflora in mesophilic and thermophilic groups. Remarkably, the TVC of ratio 12:1 treatment seemed to be higher than for the other treatments, even the highest in some fermentation steps. Based on the percentage of salt there should have been more antimicrobial activity, and TVC of 12:1 treatment should be the lowest among the ratios tested. This may be due to two reasons: (1) microbial profile of the raw material could be halophilic bacteria in brackish water shrimp; and (2) amount of salt used was suitable, supporting bacterial growth as a result of high a_w (Pakdeeto, 2016). Additionally, it was found that TVC in all treatments decreased with fermentation time after the secondary drying step. A decrease of TVC during fermentation may be due to a reduction in a_w mainly from drying. Based on microbiological standards, this indicates that the shrimp paste produced from shrimp head at ratios 12:1, 14:1 and 16:1 to salt was safe to consume. However, it was noted that the smell of treatments with 14:1 and 16:1 ratios was quite foul, similar to a dead rat, after the secondary drying, although that smell was not as strong as before drying. This indicates that a too low salt content may allow growth of some spoilage bacteria. Therefore, TVC may not be an appropriate indicator of quality, instead spoilage bacteria should be determined in a further study.

Lactic acid bacteria (LAB)

Changes in amount of LAB in shrimp paste had trends similar to TVC, with a sharp decrease after the secondary drying (Figure 6). Decrease of LAB after secondary drying may be from decreased water activity (Battcock & Azam-Ali, 2001). An increase in LAB after fermentation for 90 d was noted but it did not exceed 3 log CFU/g. This indicates that LAB population might recover. This was similar to the other fermented products, including fish sauce and soy sauce, because of the natural changes in a live population.

As is known, LAB growth can provide antimicrobial substances such as lactic acid, bacteriocin, hydrogen peroxide and soon (Sudalayandi, 2011), which can reduce or retard the growth of other microorganisms and their metabolites (Saranraj et al., 2013). Therefore, a higher LAB count may inhibit volatile compounds accumulation, which is associated with the pH and TVB-N values.

Pathogenic microorganisms

Staphylococcus aureus, *Clostridium perfringens* and *Salmonella* that are pathogenic bacteria were not found. Coliform and yeast and

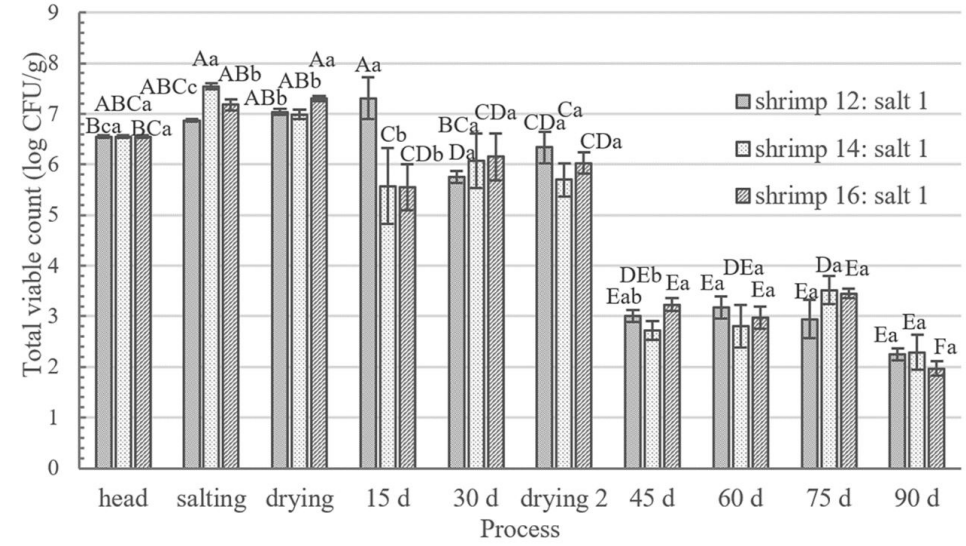

Figure 5. Total viable counts in shrimp head (*Litopenaeus vannamei*) and in shrimp paste prepared with shrimp head to salt ratio 12, 14 or 16. Different uppercase letters indicate significant difference within a salt ratio. Different lowercase letters indicate significant difference within a process ($p < 0.05$). Head means shrimp head; Salting is after salting step; Dry is after primary drying step; 15d is after fermentation for 15 days; 30d is after fermentation for 30 days; Dry2 is after secondary drying; 45d is after fermentation for 45 days; 60d is after fermentation for 60 days; 75d is after fermentation; 90d is after fermentation for 90 days (end of fermentation).

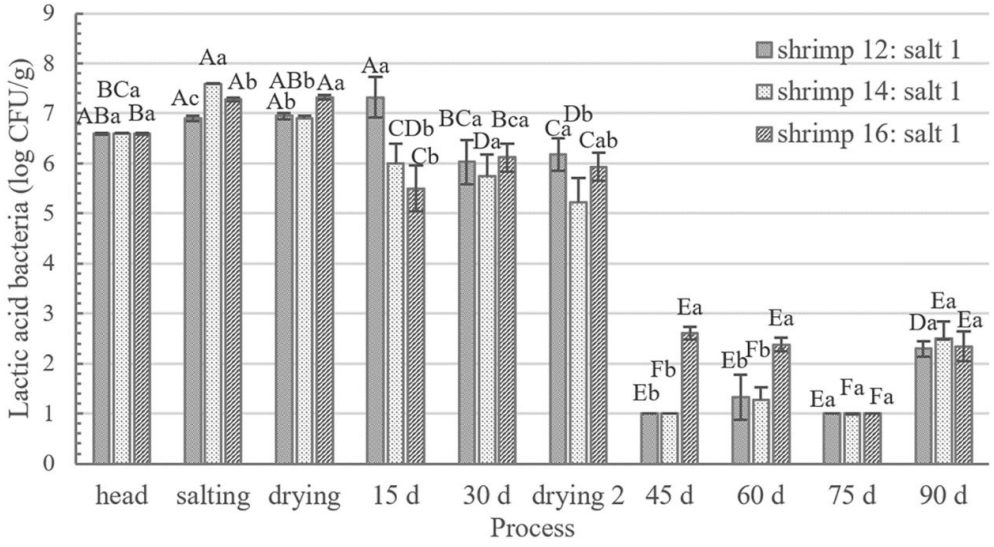

Figure 6. Amount of lactic acid bacteria in shrimp head (*Litopenaeus vannamei*) and shrimp paste prepared with shrimp head to salt ratio 12, 14 or 16. Different uppercase letters indicate significant difference within a salt ratio. Different lowercase letters indicate significant difference within a process ($p < 0.05$). Head means shrimp head; Salting is after salting step; Dry is after primary drying step; 15d is after fermentation for 15 days; 30d is after fermentation for 30 days; Dry2 is after secondary drying; 45d is after fermentation for 45 days; 60d is after fermentation for 60 days; 75d is after fermentation; 90d is after fermentation for 90 days (end of fermentation).

mold were lower than 3 MPN/g and 2.15 log CFU/g, respectively. The results confirmed that initial raw material used in this experiment was of quite high quality in terms of microbiological standards, maybe due to both good agricultural practices (GAP) and good manufacturing practices (GMP). It also proved that the raw material used in this experiment was safe to produce and to later consume. Yeast and mold decreased and disappeared after the first drying step, maybe due to sanitation and fermentation processes.

4 Conclusion

Microbiological quality indicators and pH of shrimp head paste prepared using shrimp head to salt ratios 12:1, 14:1 and

16:1 were quite similar and in the ranges 10-10^2 cfu/g and 7.87-8.01, respectively. However, a higher salt ratio led to reductions in a_w, TVB-N and TMA. The ratio 12:1 exhibited the lowest a_w, TVB-N and TMA at 0.734 ± 0.00, 167.69 ± 20.16 and 1.24 ± 0.36, respectively. In addition, the drying step provided a larger sized effect on a_w than the salt ratios in the range tested. Shrimp head can be a good alternative raw material for making shrimp paste (or actually an equivalent closely similar product). However, sensory evaluations should be further investigated to assess the market potential of such alternative shrimp paste type product.

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