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Quality changes when replacing NaCl with KCl in shrimp head paste

Chanonkarn RUJIRAPONG[1], Sunisa SIRIPONGVUTIKORN[1*], Worapong USAWAKESMANEE[1], Bandhita WANIKORN[2]

Abstract

Traditional shrimp paste was produced by *Litopenaeus vannamei* head as a low-priced by-product instead of the tradition raw material. Partial replacement of sodium chloride with potassium chloride at 0%, 30% and 50% was done to reduce risk of hypertension. The reduction of a_w and increment of pH, total volatile bases nitrogen (TVB-N), trimethylamine (TMA), thiobarbituric acid reactive substances (TBARS) and degree of hydrolysis (DH) were found in all treatments. After fermentation for 90 d, total viable count (TVC) and lactic acid bacteria (LAB) decreased, with no pathogenic microorganisms found. Angiotensin-converting enzyme (ACE) Inhibition (%) of shrimp head was close to captopril at 2.17 ng/mL but lower than shrimp head paste and captopril at 4.35 ng/mL. Sensory score of all treatments were lower than that of commercial shrimp paste. Partial replacement of sodium chloride with potassium chloride at 50% can be done in shrimp head paste but further study required to improve consumer acceptability.

Keywords: shrimp paste; fermentation; reduce sodium; potassium chloride; by-product.

Practical Application: KCl can be used to produce a reduced sodium shrimp paste with comparatively low price and to increase value of by-product from shrimp processing industry. Using the proper process can reduce salt content in the product, which may help reduce the risks of hypertension.

1 Introduction

Various Thai dishes include shrimp paste or Kapi to impart the special typical flavor and umami taste due to high free amino acid contents. Shrimp paste contains many healthy compounds such as high unsaturated fatty acids including docosahexanoic acid (DHA) and eicosapentaenoic acid (EPA) (Pongsetkul et al., 2017a), astaxanthin and antihypertensive peptides (Pongsetkul et al., 2014; Kleekayai et al., 2015b; Prapasuwannakul & Suwannahong, 2015). Raw materials for producing shrimp paste as *Mesopodopsis* and *Acetes vulgaris* have shown declining trends, while large-scale utilization of by-products, especially shrimp head from the shrimp processing industry has not been properly investigated. Recently white shrimp *Litopenaeus vannamei,* is a major by-product in shrimp processing industry in Thailand. Besides being a protein source, shrimp contains various health benefit such as antioxidant, antimutagenic and antiproliferative (Reé-Rodríguez et al., 2022). The contents of shrimp paste are regulated by (1) Thailand Industrial Standards and (2) Thai Community Product Standards as high sodium content with salt not less than 36% (dry basis) and 12% (dry basis), respectively (Ratchakitcha, 1992; Thai Industrial Standard Institute, 2018). Consumption of a high sodium diet is known to cause health problems, particularly hypertension as one of the risk factors of cardiovascular disease, leading to 9.4 million deaths every year (Rajati et al., 2019). However, hypertension is not only caused by high sodium intake but also by low potassium intake (Staruschenko, 2018). Therefore, using KCl as a replacement for high Na content to reduce hypertension is an interesting alternative for use in many food or drink products.

2 Materials and methods

2.1 Replacing NaCl with KCl in shrimp paste production

Shrimp heads containing pereiopods and internal organs were purchased from a frozen food manufacturer in Songkhla Province and used in this experiment. The shrimp heads were divided into three groups based on a_w content 0.916 ± 0.02. The first group was mixed with salt at ratio 12:1 (NaCl 7.69%), while the second and third groups were mixed with salt at ratio 10:1 with replacement KCl 30% (NaCl 6.36%, KCl 2.73%) and 50% (NaCl 4.55%, KCl 4.55%), respectively to achieve similar a_w levels for all treatments. Each mixture was incubated overnight at room temperature and then dried in a hot air oven at 60 °C for 6 h or until reaching 60% moisture content. The sample was then ground to a fine powder. After grinding, an earthen jar was used to ferment the paste for 30 d. The paste was dried again at 60 °C for 5 h until obtaining 40-45% moisture content, following the standard of shrimp paste production (Ratchakitcha, 1992). The paste was then ground and further fermented for 90 d.

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[1] *Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla, Thailand*
[2] *Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand*
*Corresponding author: sunisa.s@psu.ac.th

2.2 Chemical and physical quality analyses

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

Moisture, crude protein, fat and ash were determined according to Association of Official Analytical Chemists (2000), while carbohydrate content was calculated by subtraction as shown in Equation 1.

$$Carbohydrate(\%) = 100 - (moisture + crude\ protein + total\ fat + ash) \quad (1)$$

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

The sample and 20 mL of 0.1 N $AgNO_3$ were mixed and 10 mL of concentrated HNO_3 was added. The solution was then boiled on a hot plate until all solid except for $AgNO_3$ was completely dissolved. Tap water was used to cool down the solution and ferric alum indicator (ammonium iron sulfate) was added. The solution was then titrated with 0.1 N KSCN until it turned light brown. Salt content of the sample was calculated following Equation 2.

$$NaCl(\%) = \frac{(vol.AgNO_3 \times Conc.of\ AgNO_3) - (vol.of\ KSCN \times Conc.of\ KSCN)}{weight\ of\ sample\ (g)} \quad (2)$$

Water activity (a_w)

Water activity was measured using a water activity 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 using a pH meter (Sartorius AG, Docu-pH+ Meter, Goettingen, Germany).

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

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

$$TVB-N\ or\ TMA(mg.nitrogen/100g\ of\ sample) = \frac{(N)(14)(A-B)(V)(100)}{weight\ of\ sample} \quad (3)$$

Where:

N = normality of HCl

A = mL of HCl used to the titrate sample mixture

B = mL of HCl used to titrate the blank

V = total volume of sample and TCA in sample preparation

Determination of thiobarbituric acid reactive substances (TBARS)

The determination of thiobarbituric acid reactive substances followed the method of Subramanyam et al. (2018) with a slight modification using 5,000 x g for 25 min instead of 3,600 x g for 20 min. The sample and thiobarbituric acid (TBA) solution were mixed and heated for 10 min at 95 °C and then centrifuged at 5,000 x g for 25 min. The supernatant was tested for absorbance at 532 nm. Malondialdehyde (MDA) was used as the standard and TBARS was calculated at mg MDA/kg sample.

Degree of hydrolysis (DH)

Degree of hydrolysis was determined according to the method of Baharuddin et al. (2016) with slight modifications. The sample was mixed with 20% TCA solution at 1:1, left for 30 min and then centrifuged at 10,000 x g. The supernatant was then titrated with 0.02 N HCl. DH was calculated following Equation 4.

$$DH\ \% = \frac{(amount\ N\ soluble\ in\ 10\%TCA)(100)}{Total\ N\ in\ sample} \quad (4)$$

2.3 Microbiological quality analyses

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

TVC was analyzed according to the method of Maturin & Peeler (2001) with some modifications (using 0.85% NaCl solution instead of 0.1% peptone water).

Coliform bacteria

Coliform bacteria were analyzed according to the method of Feng et al. (2020) with some modifications (using 0.85% NaCl solution instead of 0.1% peptone water).

Staphylococcus aureus

Staphylococcus aureus was analyzed according to the method of Tallent et al. (2016) with some modifications (using 0.85% NaCl solution instead of 0.1% peptone water).

Salmonella

Salmonella was analyzed according to the method of Andrews et al. (2021) with some modifications (using 0.85% NaCl solution instead of 0.1% peptone water).

Clostridium perfringens

Clostridium perfringens was analyzed at ALS Laboratory Group (Thailand) Co., Ltd. with ISO/IEC 17025:2017 certification.

Yeast and mold

Yeast and mold were analyzed according to the method of Tournas et al. (2001) with some modifications (using 0.85% NaCl solution instead of 0.1% peptone water).

Lactic acid bacteria (LAB)

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

2.4 Angiotensin converting enzyme (ACE) inhibition assay

The sample solution was prepared according to Kleekayai et al. (2015a) with a slight modification using 50 mM tris-HCl buffer (pH 7.0) instead of distilled water. The samples were dried with a hot air oven at 50 °C and then mixed with 50 mM tris-HCl buffer (pH 7.0) at ratio 1:5 (w/v). The mixtures were homogenized and shaken at 150 rpm on an orbital shaker for 1 h at 30 °C before centrifuging at 8,400 × g for 10 min at 4 °C to remove undesired debris. The supernatants were collected and adjusted to pH 7.0 using 1 M HCl.

ACE inhibition assay was determined according to Onuh et al. (2015) with slight modifications. One milliliter of N-[3-(2-Furyl) acryloyl]-L-phenylalanyl-glycyl-glycine (FAPGG) 0.35 mM was dissolved in 50 mM tris-HCl buffer containing 0.3 M NaCl, pH 7.5 and mixed with 20 µl of ACE 20 mU and 200 µl of sample mixture (0.7 mg/mL) dissolved in 50 mM tris-HCl buffer. The absorbance was read by a microplate reader at 340 nm for 30 min at 37 °C. Four controls were prepared by replacing the sample mixture with tris-HCl buffer, tris-HCl buffer with 0.3 M NaCl and tris-HCl buffer containing NaCl and/or KCl at the same concentration of NaCl and/or KCl in the shrimp paste, while positive controls were prepared using captopril (20 nM, 10 nM and 5 nM) instead of the sample solution. ACE inhibition activity was calculated following Equation 5.

$$ACE\ inhibition\ (\%) = \left[\frac{\Delta A\,\min^{-1}(blank) - \Delta A\,\min^{-1}(sample)}{\Delta A\,\min^{-1}\ (blank)}\right] \times 100 \quad (5)$$

Where:

$\Delta A\min^{-1}$ (blank) = ACE activity without sample

$\Delta A\min^{-1}$ (sample) = ACE activity with sample

2.5 Sensory evaluation

After fermentation for 90 d, shrimp head paste was evaluated by a 9-point hedonic scale test with 9 (like extremely) to 1 (dislike extremely). Fifty untrained panelists who were familiar with shrimp paste were used to determine six attributes based on appearance, color, odor, taste, texture and overall acceptability. The evaluation was performed in a laboratory and water, cucumber and raisins were used to refresh the panelists between samples.

2.6 Statistical analysis

The experiment was conducted in three replicates, with data analyzed by one-way analysis of variance as mean ($\bar{x}$) ± standard deviation (SD). Mean comparisons were evaluated by Tukey's multiple range test. Completely randomized design (CRD) was used for testing the chemical, physical and microbiological properties while randomized complete block design (RCBD) was used to determine sensory acceptability.

3 Results and discussion

3.1 Chemical and physical qualities

Proximate compositions and salt content

Proximate composition of the shrimp heads is shown in Table 1. Results were close to shrimp head reported by Rujirapong et al. (2022) with moisture 75.38 ± 0.51%, protein 45.55 ± 0.85% (dry basis), fat 3.86± 0.28%, ash 18.31 ± 0.32% (dry basis) and carbohydrate 32.28 ± 2.52%. However, shrimp head in this experiment contained higher protein and lower carbohydrate compared with *Litopenaeus vannamei* head (Fernandes et al., 2013), with moisture 75.47 ± 0.43% (dry basis), protein 60.13 ± 3.22% (dry basis), fat 4.48 ± 0.40% (dry basis), ash 17.73 ± 1.14% (dry basis) and carbohydrate 4.33% (dry basis). Differences between proximate compositions in shrimp heads of each lot or experiment indicate natural phenomena of raw materials, cultivation and aquaculture condition, particularly when the process steps are not well controlled. The shrimp heads used in this experiment were a by-product of frozen shrimp, leading to faster deterioration confirmed by TVB-N of shrimp head as 16 mg/100 g, compared with TVB-N of 1 mg/100 g of white shrimp head reported by Liu et al. (2021).

After fermentation for 90 d, moisture in all treatments decreased due to salting and drying processes (Table 1). Moisture content of treatment 2 was the lowest while treatment 3 was the highest. This was caused by K^+ inhibiting Na^+ penetration in the muscle by reacting with muscle surface protein, thereby slowing

Table 1. Proximate compositions of shrimp head (*Litopenaeus vannamei*) and shrimp paste added with (1) NaCl 7.69%, (2) NaCl 6.36%, KCl 2.73% and (3) NaCl 4.55%, KCl 4.55% after 90 d of fermentation.

Sample	Proximate compositions (%) (dry basis except moisture)					
	Moisture	Protein	Fat	Ash	Carbohydrate	Salt
Head	76.54 ± 1.40^{a}	54.39 ± 0.38^{a}	6.35 ± 0.05^{a}	18.97 ± 0.78^{d}	20.29 ± 0.70^{b}	14.32 ± 0.55^{c}
1	42.72 ± 0.76^{bc}	33.25 ± 1.12^{b}	5.39 ± 0.08^{b}	36.80 ± 0.28^{c}	24.56 ± 0.77^{a}	27.78 ± 0.24^{ab}
2	40.82 ± 0.93^{c}	34.03 ± 1.51^{b}	3.38 ± 0.26^{c}	39.51 ± 0.76^{b}	23.08 ± 1.63^{ab}	27.03 ± 0.58^{b}
3	43.30 ± 0.25^{b}	32.05 ± 1.02^{b}	3.42 ± 0.07^{c}	41.33 ± 0.35^{a}	23.20 ± 1.32^{ab}	28.28 ± 0.29^{a}

Remarks: Different letters indicate significant differences within value ($p < 0.05$).

the drying rate with higher KCl replacement (Chen et al., 2019). Not surprisingly, ash content of all treatments also increased because of the added salt during shrimp paste production. Treatments 2 and 3 contained higher ash content due to higher salt added compared with treatment 1 (control). Decrease in fat and protein in all treatments after fermentation for 90 d was caused by leaching and melting of internal organs containing a pool of enzymes, protein and fat during salting and fermenting. Penetration of salt during salting caused water loss in the raw material (Pongsetkul et al., 2017b). Moisture, fat and ash contents of all treatments were similar to shrimp head paste reported in Rujirapong et al. (2022) with moisture 42.5%, carbohydrate 9.20% (dry basis), protein 42.78% (dry basis), fat 5.06% (dry basis) and ash 42.96% (dry basis). All treatments in this experiment contained carbohydrate higher than the, while protein was lower due to different raw material, indicating the uncertainty of natural phenomena. However, moisture, carbohydrate, protein and ash contents in this experiment after fermentation for 90 d were similar to proximate compositions of commercial shrimp paste containing moisture 33.79-52.54%, carbohydrate 4.90-32.48% (dry basis), protein 29.44-53.17% (dry basis), fat 1.41-3.67% (dry basis) and ash 33.80-50.50% (dry basis) (Pongsetkul et al., 2014).

Salt content in shrimp heads in this experiment was much higher (14.32 ± 0.55) than in the shrimp head in reported by Rujirapong et al. (2022) with salt content at 4.96 ± 1.09 (dry basis) (Table 1). This was due to the remaining residue of chlorine solution during the washing step because silver nitrate was a main chemical used in the determination of chlorine and salt (The United States Environmental Protection Agency, 1994). Generally, fresh shrimp in frozen plants is washed with different chlorine concentrations based on microbiological quality recorded before the purchasing and harvesting processes. Higher microorganism count would require higher chlorine content application.

Treatment 2 had lower moisture content than the other treatments due to proper replacement of NaCl with KCl to not high until cannot reduce a_w or reabsorbed moisture back during drying and fermentation. NaCl has a higher potency to trap or absorb water in the sample than KCl (Zhang et al., 2020) therefore, it causes more difficult water removal during drying (Fijał-Kirejczyk et al., 2013). After fermentation for 90 d, increase in salt content was found due to drying. Significant differences between treatments 2 and 3 were found but salt content did not exceed 30% as the maximum limit for extreme halophile growth (Pakdeeto, 2016). However, salt contents in all treatments were similar to commercial Kapi, containing salt 22.77-42.48% (dry basis) (Kongpun & Kongrat, 2013; Pongsetkul et al., 2014). Although, initial salt content of shrimp head used in this experiment was high at 14% but the final product had a similar salt content to other experiments or commercial products. This result indicated that salt content in shrimp head was chlorine residue from the washing process as explained earlier. Therefore, using $AgNO_3$ for salt determination may give false positive results. Although, all shrimp head pastes in this experiment contained lower sodium than the production standard of shrimp paste (must higher than 14.4%) but only sample treatments 2 (sodium 7.57% and potassium 4.25%) and 3 (sodium 5.66% and potassium 7.42%) were categorized as reduced sodium shrimp paste (sodium 25% lower than the standard) (Thai Food and Drug Administration, 2018). In addition, sample treatments 2 and 3 were also excellent sources of potassium at more than 30% or 1,050 mg/100 g of the Thai daily potassium intake, recommended at 3,500 mg/d (Thai Food and Drug Administration, 2018).

Water activity (a_w)

After salting, a_w of treatments 2 and 3 reduced from 0.984 to 0.912 and 0.938, respectively (Figure 1). In the initial process, only treatment 1 provided a_w lower than 0.91 that retarded growth of microbials. As known that NaCl can bind to water (water holding capacity) higher than KCl at the same molarity, therefore a_w increased with higher KCl replacement (Xu et al., 2023). However, after drying, a_w of all treatments decreased to lower than 0.91 and remained constant until fermentation for 30 d. An a_w value lower than 0.91 but above 0.7 still allows halophilic bacterial growth but inhibits spoilage microorganism (Majumdar et al., 2018). After secondary drying, a_w of all treatments sharply decreased to lower than 0.75, which can inhibit most microorganism growth including halophilic bacteria (Majumdar et al., 2018). Furthermore, a_w remained constant until the end of fermentation. Most commercial shrimp pastes have a_w 0.669 to 0.774, concurring with Pongsetkul et al. (2014). In addition, a_w of shrimp paste in this experiment and in commercial products was less than 0.85, and below the shrimp paste production standard (Thai Industrial Standard Institute, 2018). However, it was found that replacing NaCl with KCl at 30% (treatment 2) gave the lowest a_w even though salt content was not highest (Table 1). Although, the ionic strength of KCl is lower than NaCl at the same molarity. Higher salt ratio (shrimp head 10: salt 1) in treatment 2 and treatment 3 may help to reduce water activity similarly to treatment 1. Generally,

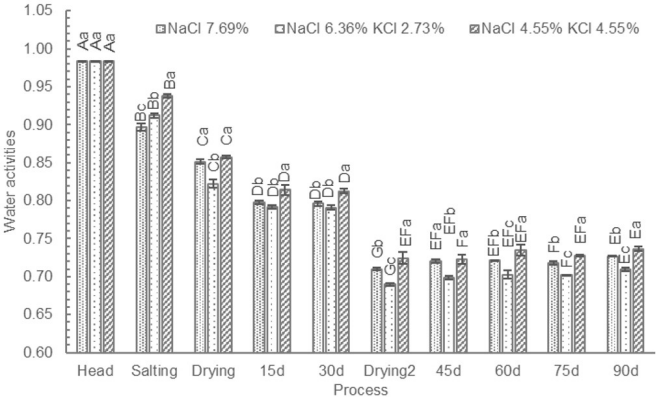

Figure 1. Water activity (a_w) of shrimp head (*Litopenaeus vannamei*) and shrimp paste added with (1) NaCl 7.69%, (2) NaCl 6.36%, KCl 2.73% and (3) NaCl 4.55%, KCl 4.55% after 90 d of fermentation. Different uppercase letters indicate significant differences within a salt ratio ($p < 0.05$). Different lowercase letters indicate significant difference within a process ($p < 0.05$). Head means shrimp head; Salting is after salting step 14-16 h; Drying is after primary drying step; 15 d is after fermentation for 15 d; 30 d is after fermentation for 30 d; Drying2 is after secondary drying; 45 d is after fermentation for 45 d; 60 d is after fermentation for 60 d; 75 d is after fermentation for 75 d; 90 d is after fermentation for 90 d (end of fermentation).

NaCl significantly reduced a_w in raw material, however, NaCl caused slower drying rate due to its high water holding capacity (Chen et al., 2019).

Taken moisture content to compare with a_w each treatment found that there was a good relationship with R^2 between 0.7747 to 0.9774 was found in each treatment. Using 30% KCl to replace NaCl controlled moisture content and a_w better, particularly after the drying and fermenting processes.

pH

After salting, pH increased in all treatments (Figure 2). Increase in pH after salting indicated increased activity of endogenous enzymes and spoilage microorganisms as a result of liberating amine compounds as fermentation of protein and peptide hydrolysis of meat products (Youcai & Tao, 2021). This result was in agreement with increasing of volatile base nitrogen including TVB-N and TMA. However, the pH value in all treatments did not increase with increasing of TVB-N which the highest was found in treatment 3 and the lowest was treatment 1. Changes of pH in all treatments after salting were impacted by acid production, as confirmed by increase in LAB after salting. After drying, pH in all treatments decreased due to heat treatment and remained constant until the end of fermentation. Volatile base compounds are known to be responsible for the increase of pH, however, heat generated during drying also accelerated the evaporation of volatile base nitrogen, causing reduced pH. The final pH of the product was 8.07, similar to commercial shrimp paste at 7.01-8.4 (Pongsetkul et al., 2014). The pH of standard shrimp paste ranges 6.5-7.8 (Ratchakitcha, 1992). However, pH regulation was taken out in the recently shrimp paste production standard (Thai Industrial Standard Institute, 2018). Higher pH of shrimp paste in this experiment was explained by the nature of shrimp head containing internal organs which were easily digested by enzymes and microorganisms as well as not proper management due to low price margin of by-product. pH of shrimp head in this experiment (7.7) seemed to be slightly higher than pH 7.4 when compared with finding of Liu et al. (2021).

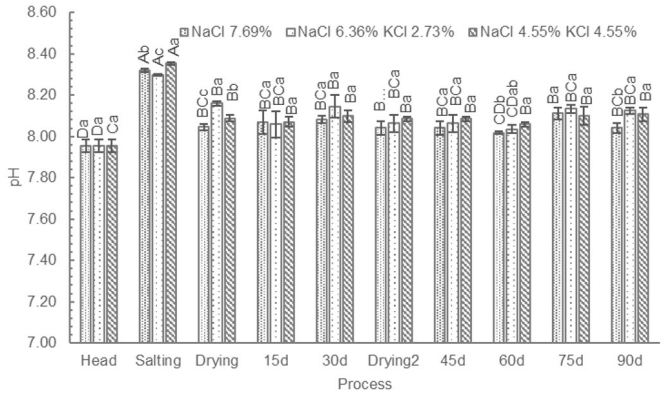

Figure 2. pH of shrimp head (*Litopenaeus vannamei*) and shrimp paste added with (1) NaCl 7.69%, (2) NaCl 6.36%, KCl 2.73% and (3) NaCl 4.55%, KCl 4.55% after 90 d of fermentation. Key: see the caption of Figure 1.

Changes in pH depend on base and acid content as a function of microorganism and enzyme fermentation. The unclearly change of pH after first time drying until end of fermentation may due to stabilization of production of base, acid, and vaporize of volatile acid and base as well as buffering capacity. Although, microorganisms could not grow or even rapidly dead after secondary drying because low a_w, basic and acid compounds derived from extracellular enzyme of the dead microorganisms still provided (Baltar, 2017). In addition, food containing high protein and amino acid such as histidine, cysteine, aspartic acid and glutamic acid were reported as high buffering capacity (Bhagavan & Ha, 2015; Mennah-Govela et al., 2019). Amino acids mentioned above also found in shrimp head especially high glutamic acid (Wu et al., 2021) indicating buffering capacity in shrimp paste leading to unchanged pH.

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

TVB-N and TMA values of all treatments are shown in Figure 3. TVB-N of shrimp head used in this experiment was lower than 25 mg/100 g, and considered not spoilage but also not fresh. In addition, TVB-N of *Acetes vulgaris* (34.08 mg/100 g) used in the production of Indonesian shrimp paste was higher than TVB-N of shrimp head in this experiment (Khairina et al., 2017). However, TVB-N of raw material used in this experiment was lower than Khairina et al. (2017). Differences in TVB-N of raw material were due to differences in the freshness of each lot. TVB-N and TMA of all treatments increased after salting as expected, corresponding with increasing TVC. Salting overnight significantly increased TVB-N and TMA, particularly for the treatment replacing 50% KCl (treatment 3), indicating high protein hydrolysis leading to volatile compounds. However, after drying, TVB-N and TMA of treatment 3 reduced to around 60 mg/100 g. TVB-N of all treatments later significantly increased then seemed to reduce after secondary drying. After that TVB-N of all treatments seemed to increase then kept constant until the end of fermentation. The tendency of TVB-N changes was mainly drying dependent nature. TMA seemed to increase during 30 d of fermentation and then decreased after secondary drying. TMA in all treatments seemed to increase as fermentation time increased but no significant differences were found until the end of fermentation. It was noticed that drying step having both temperature and time can reduce both TVB-N and TMA value due to the nature of volatile phenomenon reduction. As affected of low a_w due to the drying process and salting led to microorganism reduction; however, TVB-N and TMA values remained constant as a result of accumulation during the fermentation process. Throughout this experiment, TVB-N of all treatments seemed to lower than the TVB-N of Indonesian shrimp paste after fermentation for 20 d (150 mg/100 g) (Khairina et al., 2017). The higher TVB-N found in Indonesian shrimp paste with higher salt content (12.5%) and shorter fermentation time may due to the lack of drying process. In addition, Taiwan shrimp paste contained TVB-N and TMA at 73 to 275 mg/100 g (158 ± 45) and 10.3 to 44.9 mg/100 g (26.7 ± 10.2), respectively (Tsai et al., 2006). It pointed out the nature of the fermented protein source yielded a big value of volatile base.

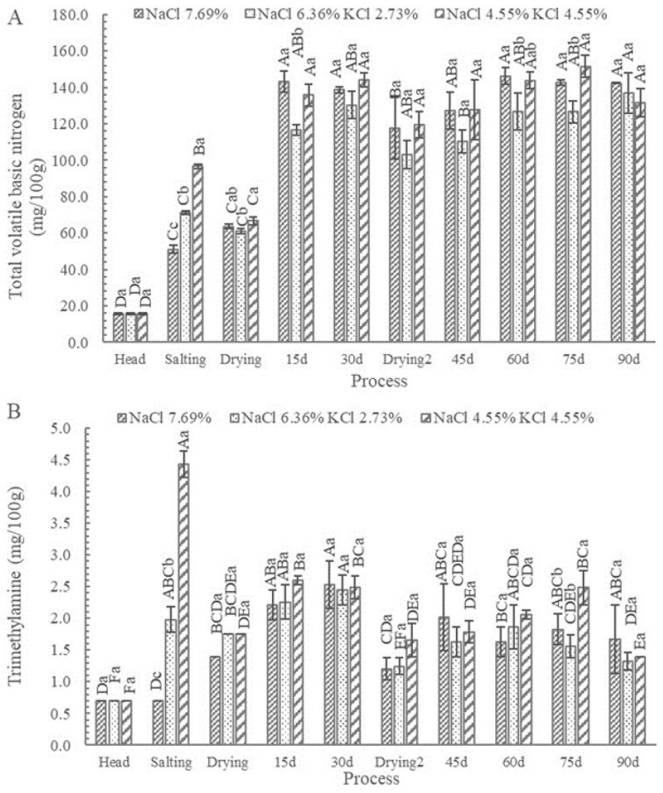

Figure 3. TVB-N (A) and TMA (B) of shrimp head (*Litopenaeus vannamei*) and shrimp paste added with (1) NaCl 7.69%, (2) NaCl 6.36%, KCl 2.73% and (3) NaCl 4.55%, KCl 4.55% after 90 d of fermentation. Key: see the caption of Figure 1.

Volatile alkaline compounds particularly TVB-N consist of ammonia, TMA and dimethylamine. Generally, TVB-N compounds are produced from hydrolysis of protein by spoilage microorganisms and endogenous enzymes. TMA is also responsible for the fishy odor (Herath et al., 2019) and is produced from the degradation of trimethylamine oxide (TMA-O) by both endogenous enzymatic and bacterial action (Summers et al., 2017). TVB-N and TMA are also used to indicate freshness and spoilage of food, especially seafood (Summers et al., 2017). However, in the case of fermented food, increasing of TVB-N from protein degradation turned to indicate a signal of a typical fermentation process. It pointed out that without an increase of TVB-N may indicate unfermented process particularly protein source material. Ammonia in shrimp paste must be lower than 700 mg/100 g and no fishy odor must be detected (Ratchakitcha, 1992). However, no ammonia regulates in recently published shrimp paste production standard (Thai Industrial Standard Institute, 2018). Mitchell & Smith (2016) reported that the odor threshold of TMA less than 100 mg/100 g. Throughout this experiment, TVB-N and TMA were lower than 700 mg/100 g and 100 mg/100 g, respectively which was considered safe for consumption (Ratchakitcha, 1992; Khairina et al., 2017). TVB-N and TMA significantly reduced after drying. Treatment 2 exhibited the lowest TVB-N and TMA values related to lower a_w as explained earlier. Using KCl to replace NaCl may also control the fermentation process as well as improve health benefits.

Determination of thiobarbituric acid reactive substances (TBARS)

TBARS of shrimp head used in this experiment was 4.47 ± 0.28 mg/kg. After fermentation for 90 d, TBARS of all treatments significantly increased to 13.43 ± 0.69 mg/kg 14.01 ± 1.00 mg/kg and 13.08 ± 0.37 mg/kg, respectively but no significant differences. KCl replacement at 30 and 50% did not impact lipid oxidation, in agreement with the finding of Santos et al. (2017). They found that replacement of NaCl with KCl at 50% in dry fermented sausages did not modify lipid oxidation. Lipid oxidation is impacted by the type of lipid, amount of unsaturated fatty acid, light, heat, oxygen, pro-oxidant and antioxidant (Amaral et al., 2018). High lipid oxidation in shrimp paste after fermentation for 90 d was caused by high unsaturated fatty acid at 65.21%, high a_w content in shrimp head, heat applied during drying and salt added which acted as pro-oxidants during fermentation. Vilgis (2015) reported that lipid oxidation still occurred at a_w 0.70. Lipid oxidation in this experiment occurred throughout the fermentation process.

Oxidation of unsaturated fatty acids relates to the rancidity of the product and is normally determined using malondialdehyde (MDA) by the TBARS method (Kumar et al., 2018). Domínguez et al. (2019) stated that TBARS with more than 2.5 mg MDA/kg in meat product indicated rancidity. Therefore, shrimp head used in this experiment exhibited lipid oxidation before the experiment. High unsaturated fatty acid content in shrimp head is susceptible to lipid oxidation from the beginning of post-harvest, particularly when proper attention is not given to the by-product. Unsaturated fatty acid content in this shrimp head (65.21%) was similar to the finding of Takeungwongtrakul (2014). However, lipid oxidation found in whole *Acetes vulgaris* containing high unsaturated fatty acid (56.06%) was only around 0.6 mg MDA/kg (Pongsetkul et al., 2016; Pongsetkul et al., 2017a). Poor management including unchilled, unclean and rough handling of shrimp heads containing high enzymes and microorganism as well as vulnerable structures all supported rapid deterioration and lipid oxidation. It pointed out that quantity of unsaturated fatty acid may and may not well indicate potency of lipid oxidation if post-harvest handling did not specify.

Degree of hydrolysis (DH)

DH of shrimp head in this experiment was around 1.81 ± 0.02% with similar values to DH 2.4% of shrimp head paste reported by Rujirapong et al. (2022). This indicated that the freshness of shrimp head used in these two experiments was close to each other. All shrimp paste samples significantly increased DH from 1.81 ± 0.02% to around 10.74 ± 0.42% to 12.38 ± 0.24% after fermentation for 90 d. These values were similar to commercial shrimp paste (12.68-20.67%) and another experiment (10.72 ± 0.61%) (Pongsetkul et al., 2014).

Comparing the degree of hydrolysis with a_w indicated that the degree of hydrolysis related to a_w with R^2 between 0.7747 to 0.9774. The lower a_w the lesser DH as showed in Figure 1. Treatment 2 with the lowest a_w provided the lowest DH due to enzymatic reaction reduction from both microbial and endogenous enzymes (Gomez et al., 2021). Replacing KCl at 30% led to a_w and degree

of protein hydrolysis reduction during fermentation when the drying step was applied.

3.2 Microbiological qualities

Total viable count (TVC) and lactic acid bacteria (LAB)

TVC and LAB of all treatments increased after salting and fermentation overnight (Figure 4). Increase in TVC and LAB in all treatments was explained by the rule of high a_w and microbial selection (Majumdar et al., 2018). TVC and LAB of all treatments sharply decreased after 45 d of fermentation and then remained constant until the end of fermentation. The reduction of TVC and LAB after secondary drying indicated the low a_w during the fermentation process (drying and salting) and production of secondary metabolites and antimicrobial compounds such as bacteriocins, organic acids as lactic acid, hydrogen peroxide, diacetyl, and carbon dioxide of lactic acid bacteria (Vieco-Saiz et al., 2019; Wu et al., 2023) as well as the natural living cell life cycle (log, stationary and death phase).

Increase in TVC during salting and primary drying as well as further increase during fermentation strongly related to the higher degree of hydrolysis, indicated by the amount of amine and acid content. LAB produce organic acids, especially lactic acid while proteolytic spoilage bacteria are involved with amine production. The more amine production the higher the pH, particularly when acid product is less. Therefore, constant pH may indicate basic and acid production balancing via degree of hydrolysis and LAB growth as well as buffering capacity. TVC and LAB values in this experiment were similar, indicating the predominant microorganism in shrimp head paste as lactic acid bacteria. After fermentation for 90 d, TVC values of all treatments were similar to commercial shrimp paste and varied from 1 to 6 log CFU/g (Kongpun & Kongrat, 2013). The shrimp paste, shrimp paste in this experiment after fermentation for 90 d contained TVC lower than log 5 CFU/g and was safe for consumption (Ratchakitcha, 1992). However, recently published standard of shrimp paste contains no regulation on TVC (Thai Industrial Standard Institute, 2018). Surprisingly, TVC and LAB of all treatments did not decrease after the first and second drying as expected. The drying step did not provide enough heat to suddenly destroy or kill the bacterial cells and spore. However, the drying step injured cells that then died later. Therefore, reduction in TVC and LAB were found after drying. Drying in a hot air oven at 60-65 °C also facilitated growth of mesophiles and thermophiles as well as sporadic bacteria and mold. This changing was under natural rule of number living cell which is dying and growing as growth cycle.

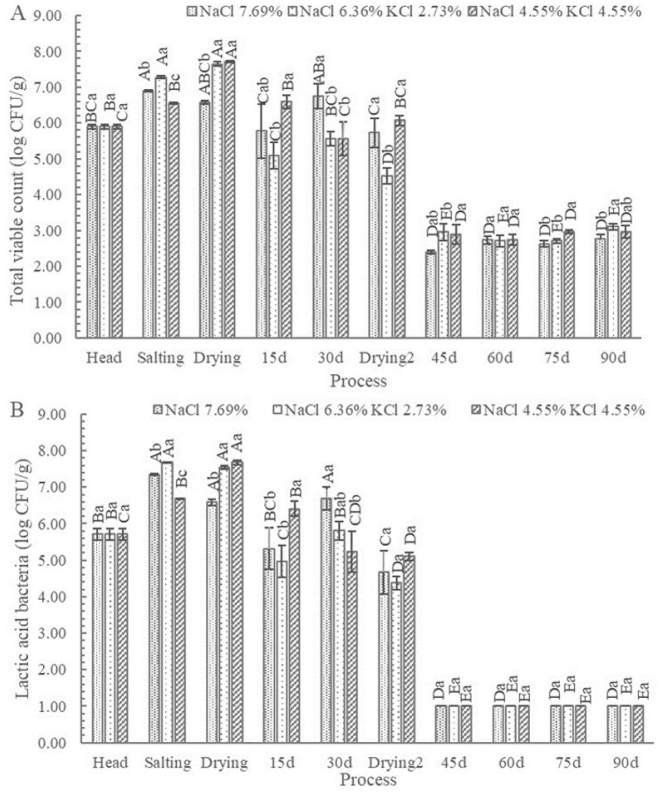

Figure 4. TVC (A) and LAB (B) of shrimp head (*Litopenaeus vannamei*) and shrimp paste added with (1) NaCl 7.69%, (2) NaCl 6.36%, KCl 2.73% and (3) NaCl 4.55%, KCl 4.55% after 90 d of fermentation. Key: see the caption of Figure 1.

Pathogenic microorganisms

Initial pathogenic microorganisms including coliform, *S. aureus*, *C. perfringens Salmonella* and yeast and mold in shrimp head used in this experiment were lower than 3 MPN/g, non-detected in a 0.1 g sample, non-detected in a 0.01 g sample, non-detected in a 25 g sample and 2.97 CFU/g, respectively. Based on safety requirements for agricultural commodities and food such as frozen and chilled shrimp, TVC, *E. coli* and *S. aureus* must contain lower than 6.70 log CFU, 3 MPN/g, 1000 MPN/g and no detected *Salmonella* in a 25 g sample (National Bureau of Agricultural Commodity and Food Standards, 2008). Therefore, the shrimp heads used in this experiment were considered safe. Throughout the experiment *S. aureus*, *C. perfringens Salmonella* were not found in all treatments while coliform bacteria were lower than 3 MPN/g. Yeast and mold of treatment with (1) NaCl 7.69%, (2) NaCl 6.36%, KCl 2.73% and (3) NaCl 4.55%, KCl 4.55% reduced to 1.56, 1.50 and 1.16 log CFU/g after first time drying then gradually reduced to lower than 1 log CFU/g in all treatments after secondary drying due to reduction of a_w and the antimicrobial effect of salt (Cabezas-Pizarro et al., 2018). Generally, all treatments after fermentation for 90 d were safe for consumption based on the industrial standard of shrimp paste which stated that coliform must lower than 3 MPN/g and yeast and mold lower than 1.7 log while *S. aureus*, *C. perfringens* and *Salmonella* must not detect in a sample 0.1 g, 0.01 g and 25 g, respectively CFU/g (Ratchakitcha, 1992). Therefore, replacement of NaCl with KCl at 30% and 50% did not provided any negative impacts on pathogenic microorganism quality of shrimp head paste. Using KCl as a substitute for NaCl did not cause any microbiological quality problem and may help to reduce NaCl consumption which is related to hypertension. It pointed out using KCl substitution for shrimp head paste may be possible to carry on without any negative result.

3.3 ACE inhibition (%)

ACE inhibition values of all treatments are shown in Figure 5. ACE converts angiotensin I to a vasoconstrictor angiotensin II and also promotes degradation of the vasodilator bradykinin leading to increase in blood pressure (Paiva et al., 2017). Therefore, the more ACE inhibition, the less blood pressure detected. No significant difference between ACE inhibition of both the control and blank sample indicated that NaCl and KCl as well as NaCl plus KCl in the fermentation setup did not change the activity of ACE. Increasing ACE inhibition of the positive control (captopril) with increasing captopril concentration was as expected. Generally, captopril is used in medical treatment for hypertension, congestive heart failure and myocardial infarction at minimum dose 25 mg daily to maximum 150 mg (The Electronic Medicines Compendium, 2022). From the result of ACE inhibition, IC_{50} of captopril in this experiment was 4.56 ng/mL (21 nM) which was higher than IC_{50} of captopril 0.39-3.28 ng/mL (1.79-15.1 nM) reported by Henda et al. (2013). Shrimp head used in this experiment contained ACE inhibition activity comparable with captopril at 2.17 ng/mL (10 nM) but significantly lower than captopril at 4.35 ng/mL (20 nM) and shrimp head pastes with or without KCl (S2, S3 and S4). ACE inhibition of shrimp head pastes with or without KCl in this experiment was comparable to captopril at 4.35 ng/mL (20 nM). ACE inhibition of shrimp head paste was higher than fresh shrimp head, indicating that more antihypertensive peptides were generated during fermentation, as also reported by Kleekayai et al. (2015b). Kleekayai et al. (2015a) reported that two antihypertensive dipeptides were found in Kapi with IC_{50} 60.68 ± 1.06 μM (Ser-Val) and 70.03 ± 1.45 μM (Ile-Phe). Paiva et al. (2017) also confirmed that ACE-inhibitory peptides were usually found between peptides with 2 and 30 amino acids. No significant differences between shrimp head pastes (S1, S2 and S3) were found but ACE inhibition reduced as the amount of KCl in treatments increased. However, no significant effect on ACE inhibition was reported by Ayyash et al. (2012) for cheese substitution with KCl up to 75%.

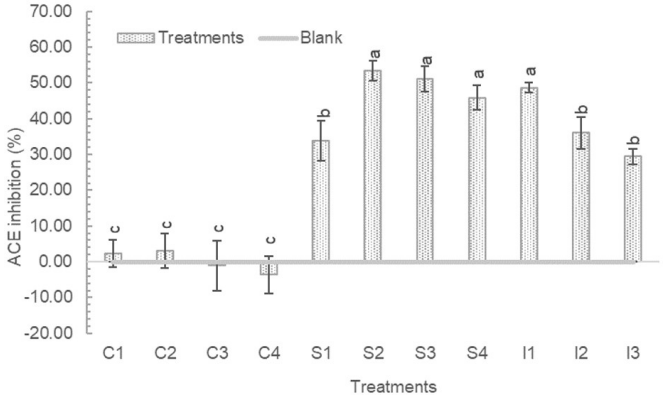

Figure 5. ACE inhibition (%) of shrimp head (*Litopenaeus vannamei*) and shrimp head paste after 90 d of fermentation. Blank tris-HCl; C1 tris-HCl with 1.75% NaCl (0.3 M NaCl); C2 tris-HCl with NaCl 7.69%; C3 tris-HCl with NaCl 6.36%, KCl 2.73%; C4 tris-HCl with NaCl 4.55%, KCl 4.55%; S1 shrimp head; S2 shrimp head paste added with NaCl 7.69%, S3 shrimp head paste added with NaCl 6.36%, KCl 2.73%; S4 shrimp head paste added with NaCl 4.55%, KCl 4.55%; I1 captopril 20 nM; I2 captopril 10 nM; I3 captopril 5 nM. Different letters indicate significant difference within a process ($p < 0.05$).

Shrimp head also contained some natural antihypertensive activity while fermentation did more increasing and replacement of sodium chloride with potassium chloride did not reduce ACE activity. However, blood pressure is not only controlled by ACE, and involves various organs and mechanisms including the renin angiotensin aldosterone system (RAAS) and the kinin-kallikrein system (KKS) (Penton et al., 2015; Bekassy et al., 2022). Determination of antihypertension compounds or compounds with vasodilation properties can be performed by various methods such as nitric oxide (NO) production (induce vasodilation in blood vessels), renin inhibition (inhibits angiotensin I production), gene eNOS (endothelial nitric oxide synthase) expression and increase of nitric oxide synthase (Onuh et al., 2015; Takashima et al., 2017). Penton et al. (2015) also confirmed that the effect of high intake potassium on blood pressure reduction was not by ACE inhibition but stimulated by sodium excretion, while Takashima et al. (2017) reported that L-arginine of garlic induced NO production from nitric oxide synthase (NOS). Penton et al. (2015) reported that high potassium intake altered plasma K^+ levels and led to reduced stiffness of endothelial cells and vasodilation by increasing nitric oxide (NO), while K^+ reduced excessive water by increasing sodium excretion, reducing sympathetic nervous system activity and hyperpolarization of endothelial and vascular smooth muscle cells leading to vasodilation, an altered response of arterial baroreceptors and reduced renal renin. Vio et al. (2020) detected decreased renal renin, angiotensin I converting enzyme (ACE) and angiotensin converting enzyme II in rats that received high potassium diet (3%) compared to rats that received normal potassium (0.9%). Increasing potassium in blood was detected by inwardly rectifying potassium channels 4.1 and 5.1 (Kir 4.1 and Kir 5.1) in the basolateral membrane, leading to inhibition of the chlorine channel. Accumulation of intracellular chlorine then led to inhibition of apical sodium chloride cotransporter (NCC) and decreasing sodium reabsorption (Vio et al., 2020).

3.4 Sensory evaluation

The sensory scores of shrimp head paste and commercial shrimp paste were significantly different (Table 2). Differences in sensory acceptability between shrimp head paste and commercial shrimp paste reflected many causative factors including freshness of raw material, different types of used raw material (whole krill for commercial one), and process improvement of sensory attributes (sugar, monosodium glutamate and other sweet potatoes) (marketing survey and personal data during shrimp paste plant visiting). However, inferior qualities in appearance and textural attributes of shrimp head paste compared to the commercial variety were the coarse appearance and rough texture with 30% of total panelists. This problem was generated by the thickness and toughness of shrimp head due to high calcium content (3,394 mg/100 g) which was double the value in commercial shrimp paste (1,565 mg/100 g) (Benjakul et al., 2011). The odor score of shrimp head paste was also inferior compared with the commercial variety may due to the high

Table 2. Sensory scores of shrimp head (*Litopenaeus vannamei*) paste added with (1) NaCl 7.69%, (2) NaCl 6.36%, KCl 2.73% and (3) NaCl 4.55%, KCl 4.55% after 90 d of fermentation and a commercial variety.

Sample	Treatment					
	Appearance	Color	Texture	Odor	Taste	Overall
1	6.02 ± 1.02[b]	6.13 ± 1.18[b]	5.87 ± 1.12[b]	6.25 ± 1.13[b]	6.48 ± 1.12[b]	6.18 ± 1.05[b]
2	6.07 ± 0.95[b]	6.18 ± 1.01[b]	5.71 ± 1.14[b]	5.83 ± 1.11[b]	5.85 ± 1.18[b]	5.79 ± 1.15[b]
3	5.86 ± 1.28[b]	6.13 ± 1.14[b]	5.76 ± 1.22[b]	5.81 ± 1.28[b]	6.21 ± 1.32[b]	6.08 ± 1.31[b]
Commercial shrimp paste	7.60 ± 0.96[a]	7.67 ± 0.88[a]	7.50 ± 0.86[a]	7.58 ± 1.11[a]	7.55 ± 1.12[a]	7.64 ± 1.09[a]

Remark: Different letters indicate significant differences ($p < 0.05$).

deterioration rate of internal organs therefore more 32% of total panelists noted the pungent smell in all treatments. Based on sensory score indicated that appearance, texture and odor played an important role for fermented shrimp head paste. However, this problem could be resolved by increasing the salt ratio to control both autolysis enzymes and microbial growth and increase primary drying time. Although, sensory scores of shrimp head paste in this experiment were lower than for commercial shrimp paste, substitution of KCl at 30 and 50% did not show any significant taste difference. Using KCl to replace NaCl at 50% did not cause a bitter taste or any negative effect on sensory evaluation. At least 70% of panelists accepted this product with higher score 5/9.

4 Conclusion

No differences were found in physical, chemical and microbiological quality in any treatment substituted with KCl up to 50%. Reduction of moisture, a_w and degree of hydrolysis were noticed with increase of KCl. Inhibition of ACE was found in shrimp head even lower than shrimp head paste, with no difference in shrimp head paste among treatments using 0% KCl and substituted with KCl up to 50%. Bitter taste was not detected in any treatment with added KCl, however, further improvements in sensory qualities are required to solve the poor texture and odor problems.

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