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EFFECTS OF LOW SODIUM CHLORIDE SUBSTITUTES ON PHYSICO-CHEMICAL AND SENSORY PROPERTIES OF KAPI, A FERMENTED SHRIMP PASTE, DURING FERMENTATION

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ABSTRACT

The effects of low sodium chloride substitutes on physico-chemical and sensory properties of Kapi, a fermented shrimp paste during fermentation period were investigated. Changes in sodium chloride content, thiobarbituric acid reactive substances (TBARS), antioxidant activities as determined by DPPH (1, 1-diphenyl-2-picryl hydrazine) and ABTS (2,2-axino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical, water activity (Aw), color values, weight loss content and sensory evaluation were monitored. During fermentation, the Aw was decreased in all samples ($P \leq 0.05$). The samples using sodium chloride substitute contented lower sodium chloride than control (100% NaCl) ($P \leq 0.05$). We found that a replacement by KCl and $CaCl_2$ decreased intensity of reactions to lipid oxidation, while 100% NaCl had a significantly higher TBARS value than other samples ($P \leq 0.05$). The percentage of inhibition DPPH and ABTS radical scavenging activity significant increased ($P \leq 0.05$) with increasing fermentation periods, but there was no significant difference between treatments. The result of sensory evaluation revealed that fermented shrimp paste with 25 and 50% KCl had the highest overall acceptance scores with the $CaCl_2$ replacement ($P \leq 0.05$), there was no significant difference from 100% NaCl.

Keywords: Kapi, sodium chloride substitutes, physico-chemical and sensory properties

INTRODUCTION

Salt is an inorganic compound. It's made when Na (sodium) and Cl (chloride) come together to form white, crystalline cubes. Salt imparts a number of functional properties in meat products, contributing to the water-holding capacity, color, fat binding properties and flavor.
Excessive salt consumption kills an estimated 2.5 million people worldwide. WHO recommends consumption of less than 5 grams of salt per day in order to improve population health.**Thais** consume an average of 10.8 grams of salt per day (over 4,000 milligrams of sodium). This rate is more than double the recommended daily amount of salt. The main sources of salt derive from salt added during cooking, packaged food, and street food. High dietary sodium consumption is associated with high blood pressure **(WHO, 2007; He and MacGregor, 2008)** which is a major risk factor for cardiovascular disease **(WHO, 2012)**.
Fermentation is a common practice in food preservation and it plays an important role in improvement of nutritional and functional properties of foods **(Steinkraus, 2002)**. Fermented food products are good source of peptides and amino acids **(Rajapakse *et al.*, 2005; Sathivel *et al.*, 2003)**. During fermentation process, a specific amino acid is synthesized in large quantities. The selected microorganism is cultured with nitrogen and carbohydrate sources and during the process of fermentation L-form of the amino acid is produced. Fermented shrimp products are mainly categorized into sauces, pastes, and lacto-fermented products. Those products are known with diverse names by local population of each nation. While, most of the people in the countries in Southeast Asia region regularly use shrimp sauce and paste in their daily cooking, fermented shrimp products are only produced and consumed by the people of Malaysia, Thailand and the Philippines. Depending on the species of shrimp, the quantity of salt used, and the treatment of raw materials prior to fermentation, different types of products are produced **(Mizutani *et al.*, 1992)**. In order to produce fermented shrimp products, salt is mixed with cleaned fresh or dried shrimp and allowed to be fermented for several months in order to enable the indigenous enzymes to auto-digest the meat and create products with high amino acids content. The enzymatic fermentation of shrimp mediated by indigenous proteases yields short chain peptides and free amino acids which render the typical flavour and taste. Salt is added to prevent deterioration and food poisoning as well as to yield meaty-savoury flavour **(Steinkraus, 2002)**.
Thai traditional fermented shrimp paste is commonly known as Kapi. It is widely used as a condiment in many Thai cuisines, in particular chili pastes, curry pastes and sauces. Kapi is traditionally prepared from shrimp or mysid shrimp mixed with salt at the ratio of 3–5:1 and then sun-dried to decrease the moisture content, and finally it is homogenized to obtain a homogenous product (paste). During ripening period, it is replaced and allowed to ferment for 2–6 months to develop desirable and unique flavors and aromas. The mixture is sun-dried and thoroughly ground before being compacted in a container usually earthen jar. Fermentation of Kapi is generally taken place until the typical aroma is developed and the fermentation time varies with each manufacturers **(Phithakpol, 1993)**.
In Thailand, Kapi can be classified into two distinct types: Kapi Ta Dam (black paste) and Kapi Ta Deang (red paste) obtained from mangrove canals and seagrass beds, respectively. Only one species of mysid shrimp, *Mesopodopsis orientalis*, found in mangrove canals of the Andaman Sea is used solely for production of Kapi Ta Dam. In contrast, three different species of shrimps from the genus Acetes; *Acetes indicus*, *Acetes japonicas*, and *Acetes crythraeus*, found in seagrass beds in the Andaman Sea are used for production of Kapi Ta Deang **(Pengchumrus and Upanoi 2005)**.
Due to the harmful effects and increasing consumption of sodium, health authorities are increasingly interested in reducing salt intake. Reducing salt levels in processed food has been one of the goals of the food industry **(World Health Organization, 2011)**. Another way to reduce sodium is partially replaced by other salt. The most commonly used is potassium chloride (KCl), which has similar properties to NaCl and is recognized as safe, can be used without loss of functionality **(Thaisa *et al.*, 2017)**. Potassium chloride provides one of the most direct substitutions due to the similarity in molecular composition, but its use can be limited due to negative sensory attributes **(Askar *et al.*, 1994)**. However, with respect to fermentation shrimp paste (Kapi), there has been very little research on the use of these salts on physico-chemical and sensory properties. Therefore, the objective of this research is aimed to elucidate the effects of low sodium chloride substitutes on physico-chemical and sensory properties of Kapi, a Thai fermented shrimp paste, during fermentation.

MATERIALS AND METHODS

Preparation of fermented shrimp paste (Kapi)

Shrimp (*Acetes vulgaris*) were obtained from Samuth sakorn province, the Gulf of Thailand in June 2017 and placed in ice with an ice-to-shrimp ratio of 2:1 (w/w). Upon arrival, shrimp were divided into five groups. Each group was mixed with ground solar salt, using the following formulations: Formulation I: 100% NaCl salt, formulation II: NaCl and KCl at 50% each, formulation III 75% NaCl and 25% KCl, formulation IV NaCl and $CaCl_2$ at 50% each and formulation V 75% NaCl and 25% $CaCl_2$. The mixture was kept overnight in plastic woven basket. The extrudes were drained continuously through the basket in order to lower moisture content. The mixture was sun-dried for 6 hr. and then blended thoroughly with hammer mill and transferred into 3 L plastic container with the volume up to the brim. The paste was packed in the plastic box and the plastic box was then covered entirely with a polyethylene sheet. The samples were left for fermentation at room temperature (30-35°C) for 3 months. Samples were taken every weeks of fermentation for analyses.

Determination of NaCl

NaCl content in samples were measured by the method of **AOAC (2002)**. Sample (1 g) was treated with 10 ml of 0.1N $AgNO_3$ and 10 ml of HNO_3. The mixture was boiled gently on hot plate until all solid except $AgCl_2$ were dissolved (usually 10 min). The mixture was then cooled using running water and 50 ml distilled water and 5 ml of ferric alum indicator were added. The mixture was titrated with standardized 0.1N KSCN until the solution became permanent brownish-red. The salt content was expressed as % NaCl.

Determination of thiobarbituric acid reactive substances (TBARS)

TBARS was determined according to the method of **Buege and Aust (1978)**. Sample (5 g) was homogenized with 25 ml of TBARS solution (TBA (0.375 g/100 ml), TCA (15 g/100 ml), and HCl 0.25 mol/l). The mixture was heated for 10 min in boiling water (95-100°C) to develop a pink color. Then the mixture was cooled with running water and centrifuge at 5500g for 25 min. The absorbance of the supernatant was measured at 532 nm using a spectrophotometer. TBARS value was calculated from a standard curve of malonaldehyde and express as mg malonaldehyde/kg sample.

Measurement of antioxidant activity by ABTS assay

Antioxidant activity was estimated using the ABTS assay following a modified method of **Jeong *et al*. (2010)**. Briefly, 2.45 mM $K_2S_2O_8$ solution was added into 7 mM ABTS (1:1, v/v) and kept for 16-12 h at room temperature in the dark to make ABTS solution. The ABTS solution was diluted with ethanol to an absorbance of less than 0.700±0.02 at 734 nm before analysis. The Kapi extracts were diluted with ethanol (500, 1,000, 1,500, 2,000 and 2,500 mg/L) and 1.0 ml of the diluted sample was mixed with 2.9 ml of the diluted ABTS solution. After 20 min, the absorbance was measured at 734 nm using the spectrophotometer. The calculation of the percent inhibition of antioxidant activity was as follows.

$$\% \text{ inhibition} = \frac{(Ac-As)\times 100}{Ac}$$

Where Ac is the absorbance of control and As is the absorbance of sample.

Measurement of antioxidant activity by DPPH assay

Antioxidant activity was estimated using the DPPH (1, 1-diphenyl-2-picryl hydrazine) assay described previously **(Wu *et al*., 2003)** with some modification. The DPPH solution 0.15 mM in 95% ethanol was prepared and 1.5 mL of this solution was added to 1.5 ml of extract solution (water extract from salted shrimp pastes). The mixture was then mixed and allowed to stand for 30 min in the dark at room temperature. The absorbance was measured at 517 nm using the spectrophotometer. The blank was prepared in the same manner except the distilled water was used instead of the sample.

Measurement of water activity (Aw)

The Aw was determined at 25°C using Aw box (Novasina Awc 200, Switzerland).
Physical analyses

Determination of weight loss

Weight loss was determined as described by **Nakao *et al*. (1991)**. Treated sample (100 g) was accurate weighed during fermentation using an analytical balance (Model B 3100P, Germany). Difference in weight of Kapi before and after fermentation was referred to as 'weight loss'.

Color measurement

Color was measured using a Hunter Lab colorimeter (DP 9000, Hunter Associates Laboratory, Reston, VA, USA) with the angle 10° and a D65 illuminant standard observed. Color evaluation was made through the CIE L*, a*, b* system. CIE L*, a*, b* values were determined as indicators of lightness, redness/greenness, and yellowness/blueness, respectively.

Acceptability test

Sensory properties of Kapi were provided by Kapi producers. Kapi was evaluated for acceptance by an untrained 50-member panels. The panellists were undergraduate students in Department of Agro-Industry, Faculty of Agriculture, Natural Resource and Environment, Naresuan University of age ranged between 20-22 years. Panellists had sensorial acquaintance with Kapi. A nine-point hedonic scale, in which a score of 1= dislike extremely, 5= neither like nor dislike and 9 = like extremely, was used for evaluation **(Meilgarrd, Civille and Carr, 1990)**. A formulation for producing Thai shrimp paste chili sauce (Nam Prik Kapi) was developed in the laboratory. The ingredients used in preparing the shrimp paste chili were fermented shrimp paste, garlic, fresh red, palm sugar, lime juice and fish sauce. Samples were randomly selected and coded with three-digit random number and presented to the panellists at room temperature. During evaluation, the panelists were situated in private booths. Room temperature water was given to rinse the mouth between samples. The panellists evaluated each sample for **color, flavor, taste, and overall likings.**

Statistical analysis

Microsoft Excel 5.0 (Microsoft Co., Washington, USA) was used for all statistical analyses. Data were analyzed using one-way ANOVA, and means were compared using Duncan's multiple range test. Differences were considered to be significant at P≤0.05.

RESULTS AND DISCUSSION

Changes in NaCl contents

Recent reports for the Department of Health in Thailand have shown that the average NaCl consumption among Thai people was 10.8g per day per person. NaCl intake exceeds the nutritional recommendation in most industrialized countries becoming one concern for public health. Agreement with **Pongsetkul *et al*. (2014)** reported salt content of salted shrimp paste produced in Thailand contained 22.77-35.47% NaCl. In the present study, the percentage of NaCl used in the modified products gave rise to significant reduction in sodium chloride content. Salt content of salted shrimp paste was in the range of 10.89±0.85 and 22.89±1.05% NaCl. The percentage of reduction in relation to 10.80%, 32.25%, 11.0% and 30.85% for II, III, IV and V, respectively (Figure 1). No significant difference (P≥0.05) was found in the salt contents in each formulation during fermentation period. Similarly, **Jittrepotch *et al*. (2015)** reported that no significant difference of NaCl content in Plaa –som, a Thai fermented fish product prepared by using low sodium chloride substitutes during fermentation time.

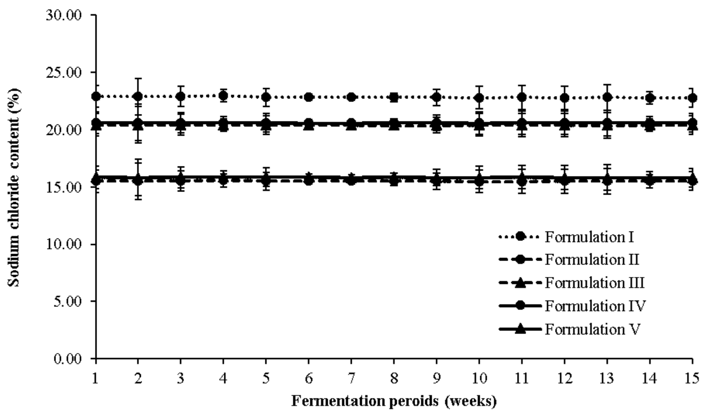

Figure 1 Changes in sodium chloride content of fermented shrimp paste with partial replacement of sodium chloride during fermentation period. Formulation I: 100% NaCl salt, formulation II: NaCl and KCl at 50% each, formulation III NaCl 75% and KCl at 25%, formulation IV NaCl and $CaCl_2$ at 50% each and formulation V NaCl 75% and $CaCl_2$ 25 %

Changes in Aw

Changes in Aw of all samples during fermentation period are shown in Figure 2. No significant difference (P≥0.05) was found in Aw atthe beginning. Aw of all samples was between 0.68±0.01 to 0.72±0.01 At the final,Aw of all samples was between 0.66±0.01 to 0.71±0.01 (P≤0.05). Formulation II, NaCl and KCl at 50% each showed lower Aw in comparison with other groups (P≤0.05). Similar results were found by **MoraGallego *et al.* (2016)**, in fermented sausages added with KCl. The partial replacement of NaCl by KCl might influence Aw because potassium ion has higher polarity compared to sodium, which could enable it to bind to a greater number of water molecules, leading to a lower Aw. In addition, **Pongsetkul *et al.* (2014)** reported that the Aw of salted shrimp paste was in the range of 0.695-0.774.

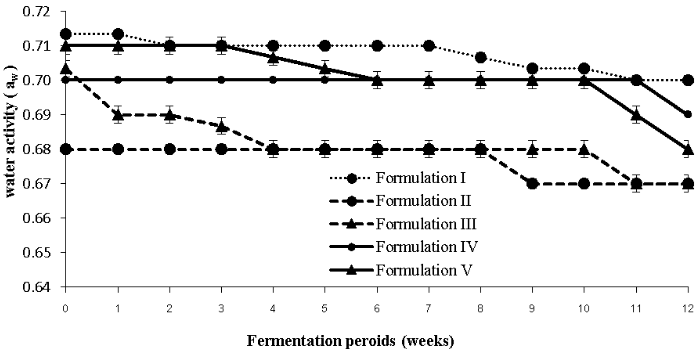

Figure 2 Changes in water activity of fermented shrimp paste with partial replacement of sodium chloride during fermentation period. Formulation I: 100% NaCl salt, formulation II: NaCl and KCl at 50% each, formulation III NaCl 75% and KCl at 25%, formulation IV NaCl and $CaCl_2$ at 50% each and formulation V NaCl 75% and $CaCl_2$ 25 %

Change in TBARS

Lipid oxidation is one of the main phenomena responsible for the reduced shelf life and sensory quality of fermented meat products. TBARS has been used to measure the concentration of relatively polar secondary reaction products, especially aldehydes **(Nawar, 1996)**. Figure 3 shows the effect on lipid oxidation (TBARS) of reducing or replacing NaCl with KCl and/or $CaCl_2$ during the fermentation period of shrimp paste. At the beginning of fermentation period (Day 0), the measured TBARS amounts were not significantly different among the treatments. On the 2nd weeks of fermentation, only formulation I (100% NaCl) presented TBARS values higher than other groups ($P<0.05$). At the end of the process (12 weeks), the treatments with 100% NaCl (formulation I) showed significantly higher TBARS compared to the other groups. The data reported agree with previous measurements of TBARS in fermented fish products **(Jittrepotch, *et al.*, 2015)**. There are many postulations as how sodium chloride acts as a prooxidant. **Kanner and Rosenthal** (1992) argued that NaCl acts as a prooxidant by displacing the iron ions with sodium in the heme pigments of the muscle tissue, whereas others recognize the chloride ion acting upon the lipid as the source **(Ellis *et al.*, 1970)**. According to **Rhee and Ziprin (2001)**, the addition of 0.5–2.5% NaCl in meat products is enough to provide a prooxidant effect, thus increasing oxidative reactions during the processing and storage stages of products.

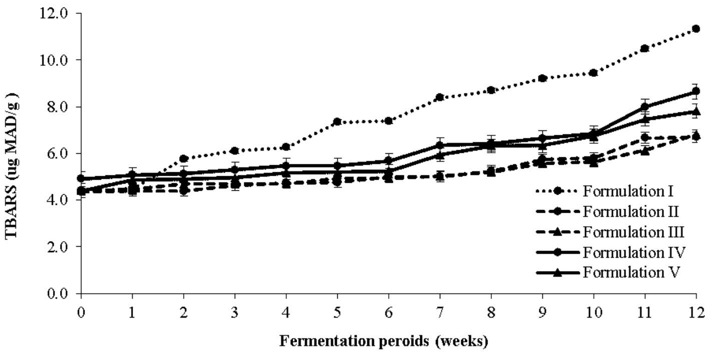

Figure 3 Changes in TBARS of fermented shrimp paste with partial replacement of sodium chloride during fermentation period. Formulation I: 100% NaCl salt, formulation II: NaCl and KCl at 50% each, formulation III NaCl 75% and KCl at 25%, formulation IV NaCl and $CaCl_2$ at 50% each and formulation V NaCl 75% and $CaCl_2$ 25 %

Change in antioxidant activities

Change in percentage of inhibition antioxidant activities of fermented shrimp paste during fermentation periods as analyzed by DPPH and ABTS radical scavenging activity are shown in Figure 4. The percentages of inhibition DPPH and ABTS radical scavenging activity were evaluated in fermented shrimp paste collected every week for 12 weeks. The results show that the percentage inhibition of DPPH and ABTS radical scavenging activity significantly increased (P≤0.05) with increasing fermentation period, however there was no significant difference between treatments (Figure 4). The increase of percentage inhibition in both DPPH and ABTS radical scavenging activity might be due to the active breakdown of protein to peptides and amino acids by the initial bacterial load in shrimp paste. Similar to previous studies reporting that peptides, amino acids and maillard reaction products in the fermented products possessed the antioxidant activity **(Parelta, *et al.*, 2008; Prapasuwannakul, Suwannahong and Saksri, 2014)**. This indicated that peptides or free amino acids in fermented products possessed the ability to donate the hydrogen atom to free radicals, in which the propagation process could be retarded.

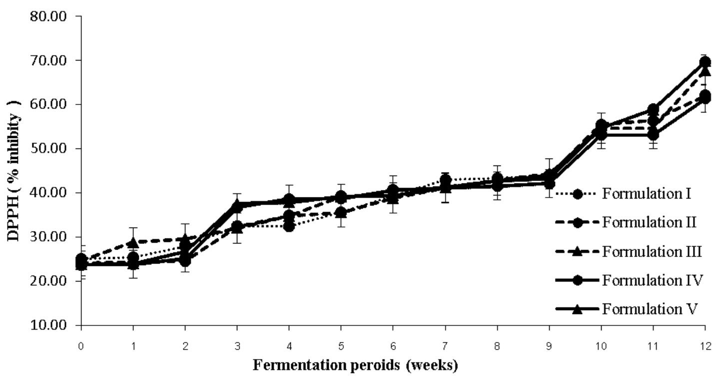

Figure 4 Changes in DPPH of fermented shrimp paste with partial replacement of sodium chloride during fermentation period. Formulation I: 100% NaCl salt, formulation II: NaCl and KCl at 50% each, formulation III NaCl 75% and KCl at 25%, formulation IV NaCl and $CaCl_2$ at 50% each and formulation V NaCl 75% and $CaCl_2$ 25 %

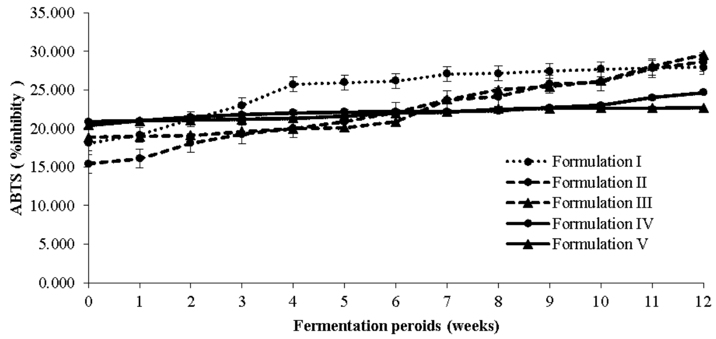

Figure 5 Changes in ABTS of fermented shrimp paste with partial replacement of sodium chloride during fermentation period. Formulation I: 100% NaCl salt, formulation II: NaCl and KCl at 50% each, formulation III NaCl 75% and KCl at 25%, formulation IV NaCl and $CaCl_2$ at 50% each and formulation V NaCl 75% and $CaCl_2$ 25 %

Change in weight loss

The weight loss tended to increase with the increasing fermentation period in every samples (P≤0.05). Weight loss in all samples were in the range 2.49±0.02 to 2.77±0.05% (Figure 5). During fermentation, shrimp paste with 100% NaCl (formulation I) had the highest weight loss compared to the other groups (P≤0.05). According to **Sgarbiere (1998)**, this difference occurs because the addition of salt to meat products increases the ionic strength, improving the solubility and consequently the functionality of the myofibrillar proteins. Weight loss depend on many factors including temperature and relative humidity of ripening room, air movement and ripening time **(Stebing and Roedel, 1987)**.

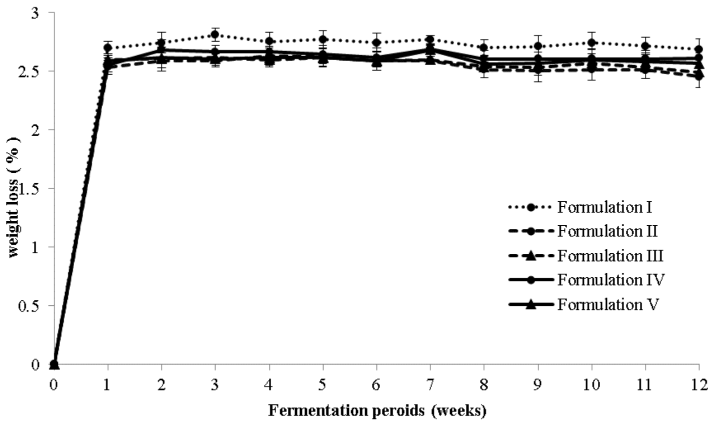

Figure 6 Changes in weight loss content of fermented shrimp paste with partial replacement of sodium chloride during fermentation period. Formulation I: 100% NaCl salt, formulation II: NaCl and KCl at 50% each, formulation III NaCl 75% and KCl at 25%, formulation IV NaCl and $CaCl_2$ at 50% each and formulation V NaCl 75% and $CaCl_2$ 25 %

Change in color

Color is the first quality attribute of food evaluated by consumers, and is therefore an important component of food quality relevant to market acceptance. The color of shrimp paste with partial replacement of NaCl during fermentation periods, obtaining *L**, *a** and *b** values are shown in table 1.

Table 1 Changes in color of shrimps paste with the partial replacement of sodium chloride during fermentation periods

Formulation	Fermentation time (week)	*L**	*a**	*b**
I	0	39.59±0.06[a]	7.09±0.08[b]	7.81±0.05[d]
	12	33.77±0.10[cd]	9.39±0.04[a]	12.03±0.02[a]
II	0	39.27±0.04[a]	6.38±0.02[c]	7.73±0.01[d]
	12	34.09±0.03[c]	8.93±0.10[a]	10.15±0.12[b]
III	0	37.79±0.05[a]	6.62±0.04[c]	7.36±0.04[d]
	12	32.09±0.08[d]	8.93±0.05[a]	10.08±0.08[b]
IV	0	40.36±0.04[a]	4.68±0.08[d]	6.58±0.06[f]
	12	31.72±0.07[b]	7.89±0.07[b]	9.39±0.03[bc]
V	0	39.94±0.05[a]	4.84±0.07[d]	6.62±0.03[f]
	12	32.62±0.09[d]	8.15±0.10[ab]	9.24±0.04[bc]

Mean ± SD from determinations.
Different superscripts in the same column indicate significant differences (P≤0.05).
I, 100% NaCl; II, NaCl 75% KCl 25%; III, NaCl 50% KCl 50%; IV, 75% NaCl 25$CaCl_2$; V, 50% NaCl 50 %$CaCl_2$

During fermentation, significance difference was found in every analyzed parameter as determined by the CIE Lab system. The *L** values which were decreasing significantly that showing the lightness of the samples were from lighter to darker as fermentation progressed until 12 weeks (P≤0.05). The lightness of samples tested were ranged between 31.72±0.07 and 40.36±0.04. However, no significant difference was found in the samples with KCl replacement compared with formulation I (100% NaCl). Agreement with **Faithong and Benjakul (2012)** studied the physicochemical properties of Kapi, a fermented shrimp paste, during fermentation, found that *L** values decreased during fermentation period. On the other hand, *a** values and *b** values gradually increased with the increasing fermentation period (P≤0.05). The results indicated that the color of shrimps paste was developed extensively as fermentation period. During extended fermentation, the autolysis might cause the increased release of carotenoids from carotenoprotein. Astaxanthin, protein-pigment complexes in shrimp, has a red-orange in color via denaturation of protein due to the separation of pigment from protein moiety **(Schiedt *et al*., 1993)**.

Table 2 Acceptability score of fermented shrimp paste with the partial replacement of sodium chloride

Formulation	Attributes				
	Color	Flavor	Saltiness	Bitterness	Overall liking
I	8.57±0.25[a]	8.60±0.36[a]	8.00±0.02[a]	8.41±0.15[a]	8.52±0.02[a]
II	8.65±0.14[a]	8.64±0.22[a]	8.12±0.04[a]	8.02±0.14[a]	8.75±0.06[a]
III	8.35±0.17[a]	8.65±0.14[a]	8.09±0.08[a]	8.00±0.11[a]	8.42±0.08[a]
IV	6.53±0.09[b]	6.86±0.28[b]	6.67±0.15[b]	6.73±0.09[b]	6.53±0.07[b]
V	6.40±0.25[b]	6.13±0.25[b]	6.04±0.16[b]	5.20±0.08[c]	5.93±0.12[c]

Means ±SD from 25 determinations.
I, 100% NaCl; II, NaCl 75% KCl 25%; III, NaCl 50% KCl 50%; IV, 75% NaCl 25$CaCl_2$; V, 50% NaCl 50 %$CaCl_2$
Different superscripts in the same column indicate significant differences (P≤0.05).

Acceptability test

The fermented shrimp paste were analyzed by an untrained 50-member panel. The results obtained for sensory evaluation for color, flavor, saltiness, bitterness and overall liking of samples with the partial replacement of NaCl are shown in Table 2. A significant higher scores in color, flavor, saltiness, bitterness and overall liking values (P≤0.05) in the samples obtained from 25 and 50 KCl replacement (Formulation II and III) however there was no significant difference (P≥0.05) when compared with formulation I (100% NaCl). **Jittrepotch *et al.* (2015)** reported the replacement NaCl with 25 and 50% KCl in fermented fish had the highest overall scores compared with the $CaCl_2$ replacement, however, no significant difference from 100% NaCl. **Askar *et al*. (1994)** found out that the replacement is possible in these percentages without significant influence on the taste. At 60% of replacement by KCl the bitter taste in products is developed.

CONCLUSION

The results indicated that a reduction of NaCl by using KCl in fermented shrimp paste during fermentation period can be performed to develop healthier products with reduce NaCl contents. The reduction of NaCl by KCl in the fermented shrimp paste could be reduced lipid oxidation while decreasing the consumption of NaCl. All samples possessed antioxidant activity, which could be an important source of natural antioxidants. Furthermore, replacement of 25 and 50% KCl had the best sensory acceptance. Thus, from the point of view of the oxidative rancidity and antioxidant activities, we conclude that the addition of KCl is a good alternative to reduce NaCl content in fermented shrimp paste.

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