

## Physico-chemical and sensory properties of *Plaa-som*, a Thai fermented fish product prepared by using low sodium chloride substitutes

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### Abstract

The purpose of this study was to investigate the partial replacement of sodium chloride (25, 50, and 75%, w/w) by potassium chloride and calcium chloride. The fermented fish were analyzed in terms of pH, total acidity, salt content, thiobarbituric acid (TBARS) value, water activity ( $a_w$ ), texture profile analysis, color values and sensory evaluation. During fermentation, the decrease of pH and  $a_w$  were observed in all samples ( $p < 0.05$ ). However, fish fermented with calcium chloride had lower pH than that with potassium chloride. This result was in accordance with the increase of total acidity. The samples contained lower sodium chloride content than the 100% NaCl (formulation I) ( $p < 0.05$ ). TBARS value was increased during fermentation, however, 100% NaCl (formulation I) had significantly higher TBARS value than the others samples ( $p < 0.05$ ). Differences in hardness, adhesiveness, springiness, cohesiveness, weight loss and color values were also observed during fermentation. Fish fermented using calcium chloride replacement showed higher hardness, adhesiveness and springiness than other samples ( $p < 0.05$ ). The result of sensory evaluation revealed that fish fermented with 25 and 50% potassium chloride had the highest overall acceptance scores compared with the calcium chloride replacement ( $p < 0.05$ ), however, no significant difference 100% NaCl (formulation I).

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### Introduction

Sodium chloride (NaCl) has been used since ancient times for the preservation of meat products and is one of the most commonly used ingredients in processed meat products. NaCl imparts a number of functional properties in meat products, contributing to the water-holding capacity, color, fat binding properties and flavor. Moreover, NaCl decreases water activity ( $a_w$ ) and this significantly affects the shelf-life (Sofos, 1984; Wirth, 1989). NaCl has a flavor enhancing effect in meat products with the perceived saltiness mainly due to the  $\text{Na}^+$  cation with the  $\text{Cl}^-$  anion modifying the perception (Miller and Barthoshuk, 1991; Ruusunen and Puolanne, 2005).

In recent year, the correlation between diet and health has been emphasized. The role for certain dietary components in the prevention of cardiovascular disease, some cancer, osteoporosis, inflammatory conditions and obesity has been established. NaCl intake exceeds the nutritional recommendations in several industrialized countries. Excessive intake of sodium has been linked to hypertension (Dahl, 1972; MacGregor and Sever, 1996). Hypertension is a major risk factor in the development of cardiovascular disease. Tuomilehto *et al.* (2001) found that high sodium intake correlated

with mortality and risk of coronary heart disease, independent of other cardiovascular risk factors, including blood pressure. These provide evidence of the harmful effects of high NaCl intake in the adult population. Recent reports for the Department of Health in Thailand have shown that the average NaCl consumption among Thai people was 10.8 g per day per person. The National Academy of Science in the USA recommended an upper level on higher than 6 g of salt per day by 2010. Thus, there is a need to cut down the excessive dietary intake of NaCl.

Fermentation was one of the first methods used to produce and preserve foods and has been practiced for thousands of years. It provides ways to preserve food products, enhance nutritive values, destroy undesirable factors, improve appearance and taste of some foods, salvage raw materials that otherwise are not usable for human consumption, and reduce energy used for cooking (Paredes-López and Harry, 1988). Fermented fish products contribute significantly to the protein intake of a large number of the World's population. The Thai fermentation fish product *Plaa-som* is produced using traditional family recipes in the north-eastern and central regions of Thailand (Adams *et al.* 1985; Saisithi, 1987; Ishige, 1993). They are typically composed of freshwater fish species, garlic, ginger, chilli, pepper and spices with salt, either

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raw, roasted, or steamed plain sticky rice, and sugar (Phithakpol *et al.* 1995; Paludan-Müller *et al.* 1999; Valyasevi and Rolle, 2002). Plaa-som can be served either as a main dish or as a snack with vegetable. The main preservation factors in fermented fish products are NaCl (reduced water activity) and acid (pH reduction). The salt concentration may range from 1 to 10% (w/w) in different types and batches of fermented fish (Anonymous, 1982; Saisithi, 1987). This is likely to have a pronounced influence on the microbial growth and the rate of fermentation, and thereby on the sensory quality and safety of the product.

One of the biggest barriers to salt replacement is the cost as salt is one of the cheapest food ingredients available. Also, consumers have grown accustomed to salt through processed foods so in some cases it is being difficult to remove as previously discussed. Another issue is that although there are alternatives to salt in term of functionality, some consumers and retailers may not be comfortable with these new ingredients on the label (Searby, 2006). Attempts to reduce the NaCl content of Plaa-som have been directed toward reduction of NaCl or its replacement by other chloride salts, mainly potassium chloride (KCl). KCl has similar properties to NaCl, but its addition to meat products is mainly limited by its bitter taste (Askar *et al.* 1994). In addition, potential NaCl reduction depends on aspects related to type of the product, composition, type of processing required and the preparation conditions. These factors determine the type of product that can be modified and the technological limitations of salt reduction. Other salt substitutes as calcium chloride can be used to reduce the sodium content in meat products (Pojedinec *et al.* 2011). However, with respect to fermented fish (*Plaa-som*), there has been very little research on the use of these salts on physico-chemical and sensory properties. Therefore, the objective of the study is aimed at investigating the physico-chemical and sensory properties of product prepared by using low sodium chloride substitutes.

## Materials and methods

### Chemicals

Trichloroacetic acid (TCA), sodium chloride, potassium chloride, calcium chloride, methanol, chloroform, silver nitrate and potassium chromate were purchased from Merck (Darmstadt, Germany). Thiobarbituric acid (TBA) was purchased from Sigma (St. Louis, MO, USA).

### Plaa-som preparation

Bighead carps (*Hypophthalmichthys molitrix* (Cuv.&Val)), caught from Phitsanulok Province and were placed in ice with a fish/ice of 1:2 (w/w) and transported to the Department of Agro-Industry, Naresuan University within 1 h. Upon arrival, fish were washed, scaled, gutted and filleted. The size of each fish fillet was selected to be in the range of 100-120 g. Fish fillets were divided into seven groups. Each group was salted, at room temperature for 7 days, using the following formulations: formulation I: 100% NaCl salt; formulation II: NaCl 75% and KCl 25%; formulation III: NaCl and KCl at 50% each; formulation IV: NaCl 25% and KCl 75%; formulation V: NaCl 75% and CaCl<sub>2</sub> 25%; formulation VI: NaCl and CaCl<sub>2</sub> 50% each; and formulation VII: NaCl 25% and CaCl<sub>2</sub> 75%. After 7 days, each group mixed thoroughly with minced garlic 20% and steamed sticky rice 20%. The mixture referred to as 'Plaa-som raw mix' was then stuffed into a Nylon/polyethylene bag. Samples were vacuum sealed and incubated at room temperature. Samples were taken every 12 hr of fermentation for analyses. Prior to analyses, the Nylon/polyethylene bags were removed. Samples were cut and ground in a meat grinder (model DPA1, Moulinex, France) for 2 min and kept in ice for further analyses.

### Determination of pH, total acidity and moisture contents

The pH, total acidity and moisture contents of sample were determined according to the AOAC (2002). The pH was determined by homogenizing 10 g of each sample with distilled water in a 1:10 ratio in triplicate. The homogenate was subjected to pH meter (CyberScan 510, Singapore) for five minutes while the pH readings were performed. The homogenate was then centrifuged at 3,000 g for 15 min at room temperature. The supernatant was filtered through a filter paper (Whatman No.4). The filtrate was titrated with the standardized 0.1 mol/l NaOH using phenolphthalein as an indicator. The total acidity was calculated as lactic acid and expressed as percentage (w/w). Moisture content was determined by drying 3-5 g of sample at 100-102°C to a constant weight.

### Determination of Salt

NaCl content in samples were measured by the method of AOAC (2002). Sample (1 g) was treated with 10 ml of 0.1N AgNO<sub>3</sub> and 10 ml of HNO<sub>3</sub>. The mixture was boiled gently on hot plate until all solids except AgCl<sub>2</sub> 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 determined by 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 0.25 mol/l HCl). 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 5500 x g 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 expressed as mg malonaldehyde/kg sample.

#### Measurement of water activity ( $a_w$ )

The  $a_w$  was determined at 25°C using Novasina model AWC 200 water activity meter (Pfäffikon, Switzerland).

#### Determination of weight loss

Weight loss was determined as described by Nakano *et al.* (1991). Sample (100 g) was accurate weighed before fermentation using an analytical balance (Model B 3100P, Germany). During fermentation process, *Plaa-som* was taken and then reweighed. Difference in weight of *Plaa-som* before and after fermentation was referred to as 'weight loss'.

#### Determination of expressible water content

Expressible water content of samples was measured according to the method of Funami *et al.* (1998) as modified by Visessanguan *et al.* (2004). The expressible water content was determined as the weight loss after the compression of sample. Sample was cut into a cylinder form (2.0 cm height x 2.0 cm diameter), placed between double layer of filter paper (Whatman No.4) and subjected to compression using the QTS-25 Texture Analyser (Brookfield Engineering Labs., USA) with the cylindrical probe (12.7 mm diameter). The measurement was performed with crosshead speed 3 mm/s to 70% strain for 60 s. Samples were subjected to moisture analysis by AOAC method (AOAC, 2002). The expressible water content was calculated as the ratio of the apparent expressible water to the total moisture content of *Plaa-som* according to the following equation:

Expressible water (%)

$$= \frac{100 \times \text{Apparent expressible water content}}{\text{Total moisture content}},$$

Where apparent expressible water content =  $100 \times (W_{\text{before}} - W_{\text{after}}) / W_{\text{before}}$   
 $W_{\text{before}}$  = weight before compression;  $W_{\text{after}}$  = weight after compression.

#### Texture profile analysis (TPA)

A double compression test known as Texture Profile Analysis (TPA) and a penetration test were performed on a Textural analysis by using a QTS-25 Texture Analyser (Brookfield Engineering Labs., USA) equipped with a load cell of 20 kg. The penetration test was equipped with cylindrical probe (12.7 mm diameter). Three slices of sample (30 mm thick and 20 mm diameter) were allowed to 50% of original height. TPA textural parameters were measured at room temperature with the following testing conditions: crosshead speed 5.0 mm/s, surface sensing force 99.0 g, threshold 30.0 g, and time of 1 s was allowed to elapse between the two compression cycles. Hardness, springiness, cohesiveness, and adhesiveness were used to collect and process the data using Texture pro software, version 2.0 (Brookfield Engineering Labs., USA). Data was calculated from the force-time curves generated for each sample (Bourne, 1978).

#### Objective 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

The fermented *Plaa-som* was evaluated for acceptance by an untrained 40-member panel. The panelists were undergraduate students in Department of Agro-Industry of age range between 20-22 years, Faculty of Agriculture, Natural Resource and Environment, Naresuan University. Panelists had sensorial acquaintance with *Plaa-som*. 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 *et al.*, 1990). Samples were sliced perpendicular to the long axis to obtain the length of 2.0 cm. Acceptance evaluation was performed on frying with 140°C 5 min. *Plaa-som* samples were place on dishes (diameter 3.0 cm)

and the samples were cover with aluminum foil. The samples were allowed to stand at room temperature for at least 30 min prior to analysis. Samples were randomly selected and coded with three-digit random number and presented to the panelists at room temperature. During evaluation, the panelists were situated in private booths. Room temperature water was given to rinse the mouth between samples. The panelists evaluated each sample for appearance, color, texture, taste, flavor, 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 pH, total acidity and moisture contents

Changes in moisture contents of *Plaa-som* with the partial replacement of NaCl during fermentation are depicted in Figure 1.

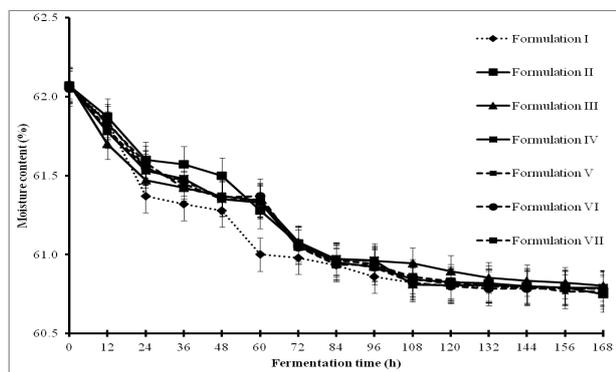


Figure 1. Changes in moisture contents of *Plaa-som* with the partial replacement of sodium chloride during fermentation. Formulation I, 100% NaCl; formulation II, NaCl 75% KCl 25%; Formulation III, NaCl 50% KCl 50%; Formulation IV, NaCl 25% KCl 75%; Formulation V, NaCl 75% CaCl<sub>2</sub> 25%; Formulation VI, NaCl 50% CaCl<sub>2</sub> 50%; Formulation VII, NaCl 25% CaCl<sub>2</sub> 75%.

At the beginning, the moisture content of all samples ranged from  $62.05 \pm 0.20$  to  $62.09 \pm 0.30\%$  and the final moisture content on 168 hr were from  $60.76 \pm 0.20$  to  $60.80 \pm 0.20\%$  ( $p < 0.05$ ). The rate of decrease increased as the fermentation time increased. This was probably related to decline of pH to a value close to isoelectric point of fish proteins, resulting in lower water-holding capacity of the *Plaa-som* (Huff-Lonergan and Lonergan, 2005). No significant differences ( $p < 0.05$ ) in the moisture contents were observed all of samples at any of fermentation times.

Changes in pH and total acidity of *Plaa-som* with the partial replacement of NaCl during fermentation are shown in Figure 2 and 3, respectively.

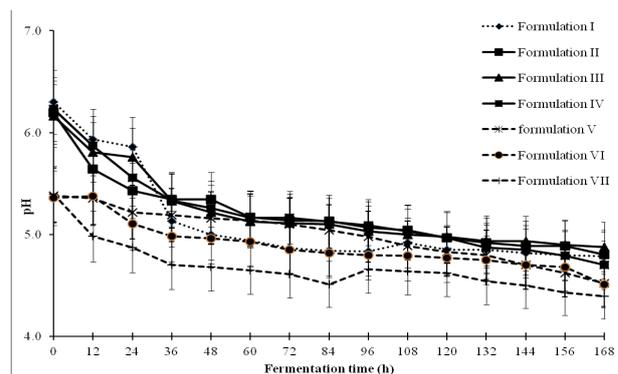


Figure 2. Changes in pH contents of of *Plaa-som* with the partial replacement of sodium chloride during fermentation. Formulation I, 100% NaCl; Formulation II, NaCl 75% KCl 25%; Formulation III, NaCl 50% KCl 50%; Formulation IV, NaCl 25% KCl 75%; Formulation V, NaCl 75% CaCl<sub>2</sub> 25%; Formulation VI, NaCl 50% CaCl<sub>2</sub> 50%; Formulation VII, NaCl 25% CaCl<sub>2</sub> 75%

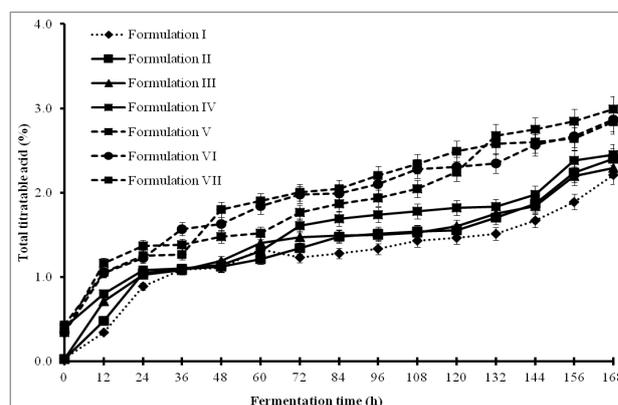


Figure 3. Changes in total acidity contents of of *Plaa-som* with the partial replacement of sodium chloride during fermentation. Formulation I, 100% NaCl; Formulation II, NaCl 75% KCl 25%; Formulation III, NaCl 50% KCl 50%; Formulation IV, NaCl 25% KCl 75%; Formulation V, NaCl 75% CaCl<sub>2</sub> 25%; Formulation VI, NaCl 50% CaCl<sub>2</sub> 50%; Formulation VII, NaCl 25% CaCl<sub>2</sub> 75%.

The pH values of *Plaa-som* with the partial replacement of NaCl, depended on the type and concentration of ions. The initial pH of all samples tested were ranged between  $5.36 \pm 0.02$  and  $6.30 \pm 0.36$ . As the fermentation proceeded, samples with 75% CaCl<sub>2</sub> (formulation VII) exhibited lower pH and higher total acidity than those of formulation I (100% NaCl) ( $p < 0.05$ ). The pH gradually decreased to  $4.39 \pm 0.07$  within 168 hr. with sample using 25% NaCl-75% CaCl<sub>2</sub> (formulation VII). Similar results were found in fermented meat products where the replacement of sodium by CaCl<sub>2</sub> decreased the pH of the final product (Gimeno *et al.* 1999; Horita *et al.* 2011). There were also some reports pointed out the relation between divalent cations (calcium) with

decrease pH (Terrell *et al.* 1981; Terrell and Olson, 1981; Hand *et al.* 1982).

For total acidity, samples fermented with  $\text{CaCl}_2$  had significantly higher than other samples ( $p < 0.05$ ). The higher acidification could caused a greater denaturation process, decreasing the binding capacity of proteins. The addition of  $\text{CaCl}_2$  to these type of products has been related to the decreased in pH values (Terrell *et al.* 1981).

#### Changes in sodium contents

NaCl intake exceeds the nutritional recommendations in most industrialized countries becoming one concern for public health. In the present study, the percentage of NaCl used in the modified products gave rise to significant reduction in the sodium contents. The percentage of reduction in relation to control were 12.0%, 32.0%, 37.0%, 10.0%, 30.0% and 38.0% for formulation II, III, IV, V, VI and VII, respectively (Figure 4).

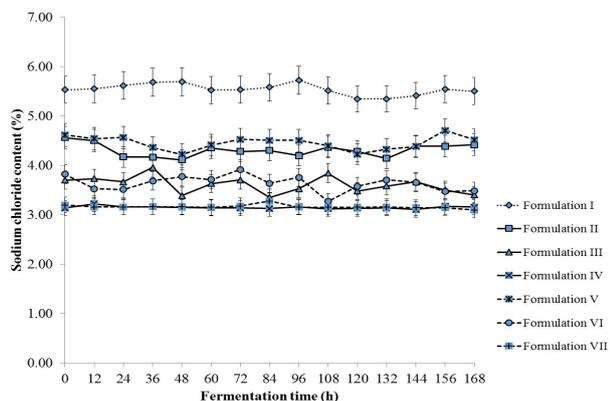


Figure 4. Changes in sodium chloride content of *Plaa-som* with the partial replacement of sodium chloride during fermentation. Formulation I, 100% NaCl; Formulation II, NaCl 75% KCl 25%; Formulation III, NaCl 50% KCl 50%; Formulation IV, NaCl 25% KCl 75%; Formulation V, NaCl 75%  $\text{CaCl}_2$  25%; Formulation VI, NaCl 50%  $\text{CaCl}_2$  50%; Formulation VII, NaCl 25%  $\text{CaCl}_2$  75%

No significant differences ( $p < 0.05$ ) was found in the NaCl contents in each formulation during fermentation times. These results showed a significant decrease of NaCl in the modified products which would imply nutritional benefits because the reduction of sodium intake in the diet is indicated as a way to reduce the risk factors of hypertension (Antonios and Macgregor, 1997). In addition, the increase in the potassium content can also give benefits, as epidemiological studies suggest that potassium intake is inversely correlated with the level of blood pressure and hypertension prevalence (Kawano *et al.* 1998).

#### Changes in thiobarbituric acid reactive substances (TBARS)

TBARS has been used to measure the concentration of relatively polar secondary reaction products, especially aldehydes (Nawar, 1996). The increase in TBARS indicated the formation of secondary lipid oxidation products (Kolakowska, 2002). Soon after processing (time zero), there were no difference among the formulations ( $p < 0.05$ ). As expected, TBARS values of all samples increased as fermentation time increased ( $p < 0.05$ ; Figure 5).

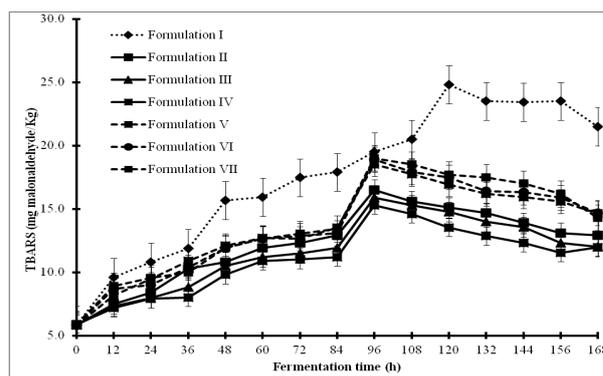


Figure 5. Changes in thiobarbituric acid reactive substances of *Plaa-som* with the partial replacement of sodium chloride during fermentation. Formulation I, 100% NaCl; Formulation II, NaCl 75% KCl 25%; Formulation III, NaCl 50% KCl 50%; Formulation IV, NaCl 25% KCl 75%; Formulation V, NaCl 75%  $\text{CaCl}_2$  25%; Formulation VI, NaCl 50%  $\text{CaCl}_2$  50%; Formulation VII, NaCl 25%  $\text{CaCl}_2$  75%.

The product with 100% NaCl showed the highest TBARS at 168 hr compared to the other groups with significant differences ( $p < 0.05$ ), indicating that KCl and  $\text{CaCl}_2$  had the lowest pro-oxidant action. Despite the significant differences ( $p < 0.05$ ) observed throughout fermentation time, the values for KCl and  $\text{CaCl}_2$  containing samples were lower than those of the 100% NaCl formulation. Similarly, Rhee *et al.* (1983) reported that KCl had lower pro-oxidant activity in meat than NaCl. NaCl, or table salt, is an important ingredient in meat industry. It acts generally as pro-oxidant, but sometimes also as an antioxidant (Kanner and Kinsella, 1983). In comminuted meat samples, under different processing conditions, NaCl did not act as an antioxidant, but its neutral or pro-oxidant effects were clearly demonstrated (Wettasinghe and Shahidi, 1996). Kanner *et al.* (1991) have demonstrated the pro-oxidant effect of NaCl in a comminuted muscle system and suggested it may promote the displacement of iron from binding sites of heme compound by interfering with iron-protein interactions. The free iron ions so formed may catalyze lipid peroxidation. Recently, Wettasinghe and Shahidi (1996) reported that NaCl, KCl and  $\text{CaCl}_2$  exhibited pro-oxidant activities in cooked

meat model system. This result showed that NaCl exhibited more pro-oxidant activities than  $\text{CaCl}_2$  and KCl, respectively. Thus, all the salt combinations studied could be used to replace NaCl in *Plaa-som*. Previous studies during storage in refrigeration of salted pork patties (Cheng *et al.* 2007) and fresh pork meat (Hernández *et al.* 2002) showed that 50% NaCl replacement by KCl had significantly lower TBARS values. Similarly, Rhee *et al.* (1983) reported KCl had lower pro-oxidant activity in meat than NaCl.

#### Changes in the $a_w$

Changes in the  $a_w$  of *Plaa-som* with the partial replacement of NaCl during fermentation are shown in Figure 6.

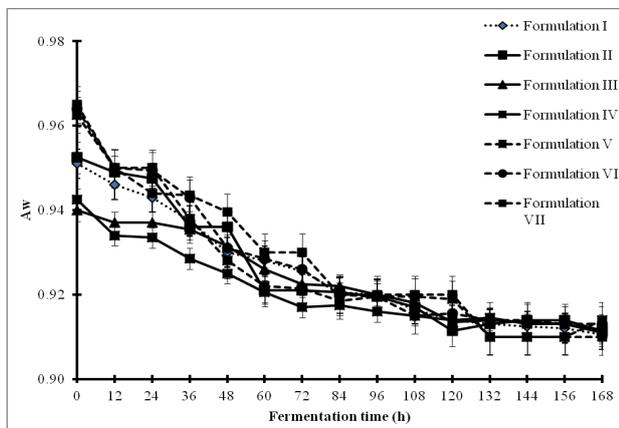


Figure 6. Changes in water activity of *Plaa-som* with the partial replacement of sodium chloride during fermentation. Formulation I, 100% NaCl; Formulation II, NaCl 75% KCl 25%; Formulation III, NaCl 50% KCl 50%; Formulation IV, NaCl 25% KCl 75%; Formulation V, NaCl 75%  $\text{CaCl}_2$  25%; Formulation VI, NaCl 50%  $\text{CaCl}_2$  50%; Formulation VII, NaCl 25%  $\text{CaCl}_2$  75%

From the result, the  $a_w$  slightly decreased with fermentation time in all samples ( $p < 0.05$ ). However, no significant differences were found between formulation I in and the partial replacement of NaCl during fermentation time. At the beginning, the  $a_w$  of every samples were in the ranged between  $0.94 \pm 0.01$  and  $0.97 \pm 0.01$  and the final  $a_w$  on 168 hr were between  $0.91 \pm 0.02$  to  $0.92 \pm 0.01$  ( $p < 0.05$ ).

#### Changes in weight loss

Weight losses were significantly affected by the partial replacement of sodium chloride and fermentation time ( $p < 0.05$ ; Figure 7). From the result, the weight loss increased with the increasing fermentation time in every samples ( $p < 0.05$ ). The higher of weight loss was found in fermented products which using the replacement of NaCl by  $\text{CaCl}_2$ . Weight loss varied from  $0.14 \pm 0.01$  to  $4.63 \pm 0.13\%$ , a significant difference was not being

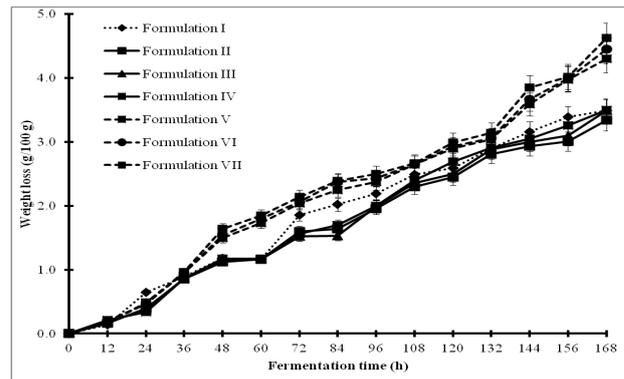


Figure 7. Changes in weight loss of *Plaa-som* with the partial replacement of sodium chloride during fermentation. Formulation I, 100% NaCl; Formulation II, NaCl 75% KCl 25%; Formulation III, NaCl 50% KCl 50%; Formulation IV, NaCl 25% KCl 75%; Formulation V, NaCl 75%  $\text{CaCl}_2$  25%; Formulation VI, NaCl 50%  $\text{CaCl}_2$  50%; Formulation VII, NaCl 25%  $\text{CaCl}_2$  75%

observed between the formulation I (100% NaCl) and the modified products (KCl) ( $p < 0.05$ ). Agreeing with these results, Guàrdia *et al.* (2008) did not find alterations in weight loss when replacing 50% of NaCl content by KCl in dry-fermented sausages. Weight losses depend on many factors including the temperature and relative humidity of the ripening room, the air movement and the ripening time (Stiebing and Roedel, 1987).

#### Changes in expressible water

According to Figure 8, the initial expressible water of all samples tested were ranged between  $1.18 \pm 0.56$  and  $1.32 \pm 0.25$  g/100 g.

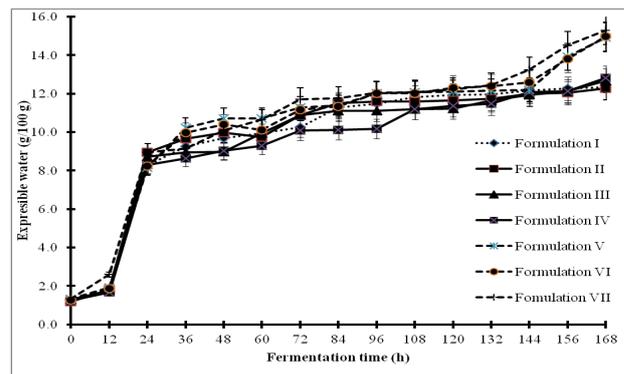


Figure 8. Changes in expressible water of *Plaa-som* with the partial replacement of sodium chloride during fermentation. Formulation I, 100% NaCl; Formulation II, NaCl 75% KCl 25%; Formulation III, NaCl 50% KCl 50%; Formulation IV, NaCl 25% KCl 75%; Formulation V, NaCl 75%  $\text{CaCl}_2$  25%; Formulation VI, NaCl 50%  $\text{CaCl}_2$  50%; Formulation VII, NaCl 25%  $\text{CaCl}_2$  75%

The amount of expressible water increased as the fermentation time increased. During fermentation, *Plaa-som* with 75%  $\text{CaCl}_2$  (formulation VII) had the highest expressible water compared to the

other groups ( $p < 0.05$ ). This could be attributed to the presence of divalent cations ( $Ca^{++}$ ), which contribute to reduced myofibrillar protein extraction (Barbut, 1995). Furthermore, Visessanguan *et al.* (2004) reported the amount of expressible water was presumable caused by denaturation of muscle proteins during fermentation.

*Changes in color*

The color of *Plaa-som* with the partial replacement of NaCl during fermentation, obtaining  $L^*$ ,  $a^*$  and  $b^*$ -values are shown in Table 1.

Table 1. Changes in color of *Plaa-som* with the partial replacement of sodium chloride at 0 and 168 h during fermentation

Formulation	Fermentation time (h)	$L^*$	$a^*$	$b^*$
I	0	45.92±0.89 <sup>c</sup>	8.01±0.01 <sup>A</sup>	8.41±0.12 <sup>A</sup>
	168	59.14±0.05 <sup>b</sup>	12.65±0.00 <sup>b</sup>	11.23±0.05 <sup>b</sup>
II	0	47.81±0.10 <sup>c</sup>	8.30±0.01 <sup>A</sup>	8.41±0.10 <sup>A</sup>
	168	59.01±0.20 <sup>b</sup>	12.27±0.00 <sup>b</sup>	11.44±0.02 <sup>b</sup>
III	0	48.13±0.02 <sup>c</sup>	8.45±0.01 <sup>A</sup>	8.48±0.05 <sup>A</sup>
	168	59.95±0.14 <sup>b</sup>	12.73±0.01 <sup>b</sup>	11.61±0.05 <sup>b</sup>
IV	0	46.78±0.19	8.14±0.02 <sup>A</sup>	8.44±0.02 <sup>A</sup>
	168	59.96±0.11 <sup>b</sup>	12.01±0.03 <sup>b</sup>	11.95±0.02 <sup>b</sup>
V	0	54.11±0.17 <sup>b</sup>	8.40±0.00 <sup>A</sup>	7.88±0.04 <sup>B</sup>
	168	66.11±0.01 <sup>a</sup>	13.30±0.01 <sup>a</sup>	13.58±0.08 <sup>a</sup>
VI	0	56.04±0.10 <sup>A</sup>	8.63±0.01 <sup>A</sup>	7.48±0.12 <sup>B</sup>
	168	65.63±0.21 <sup>a</sup>	13.62±0.02 <sup>a</sup>	13.58±0.20 <sup>a</sup>
VII	0	54.32±0.09 <sup>b</sup>	8.26±0.01 <sup>A</sup>	7.60±0.09 <sup>B</sup>
	168	65.60±0.15 <sup>a</sup>	13.78±0.03 <sup>a</sup>	13.27±0.14 <sup>a</sup>

Mean ± SD from five determinations.

Different superscripts in the same column under the same fermentation time indicate significant differences ( $p < 0.05$ ). I, 100% NaCl; II, NaCl 75% KCl 25%; III, NaCl 50% KCl 50%; IV, NaCl 25% KCl 75%; V, NaCl 75%  $CaCl_2$  25%; VI, NaCl 50%  $CaCl_2$  50%; VII, NaCl 25%  $CaCl_2$  75%.

Significant differences were found in every analyzed parameter as determined by the CIE Lab system. In the initiation time, lightness of every samples tested were ranged between 45.92±0.89 and 56.04±0.10.  $L^*$ -values of all samples increased as fermentation time increased ( $p < 0.05$ ). The samples which replaced of NaCl by  $CaCl_2$  had a higher  $L^*$  values than those of other samples ( $p < 0.05$ ). However, no significant difference were found in the samples with KCl replacement compared with formulation I (100% NaCl). Boyle *et al.* (1994) found that calcium supplementation lightened internal color of frankfurters. In addition, the degree of lightening increased as amount of calcium incorporation increased. The difference may be explained by the different physicochemical characteristics (pH, aw and moisture) of the meat products. Riebroy *et al.* (2008) studied the effect of inoculation of different lactic acid bacteria on the fermentation and quality of Som-fug, found that  $L^*$ ,  $a^*$  and  $b^*$ -values increased during fermentation. The increase in  $L^*$ -value might be a result of protein denaturation induced by acid formed. The increases in  $a^*$  and  $b^*$ -value were possible due

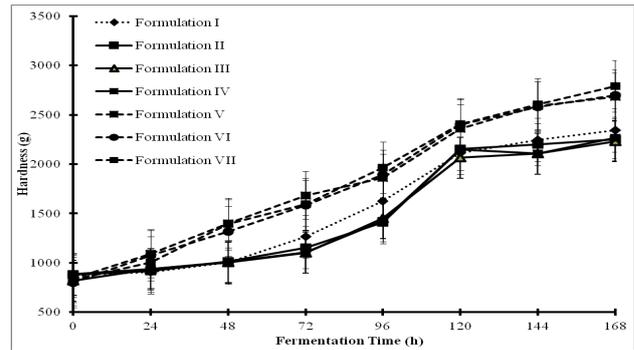


Figure 9. Changes in hardness of *Plaa-som* with the partial replacement of sodium chloride during fermentation. Formulation I, 100% NaCl; Formulation II, NaCl 75% KCl 25%; Formulation III, NaCl 50% KCl 50%; Formulation IV, NaCl 25% KCl 75%; Formulation V, NaCl 75%  $CaCl_2$  25%; Formulation VI, NaCl 50%  $CaCl_2$  50%; Formulation VII, NaCl 25%  $CaCl_2$  75%

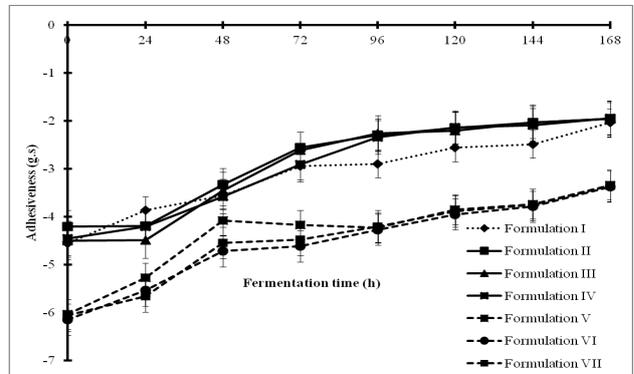


Figure 10. Changes in adhesiveness of *Plaa-som* with the partial replacement of sodium chloride during fermentation. Formulation I, 100% NaCl; Formulation II, NaCl 75% KCl 25%; Formulation III, NaCl 50% KCl 50%; Formulation IV, NaCl 25% KCl 75%; Formulation V, NaCl 75%  $CaCl_2$  25%; Formulation VI, NaCl 50%  $CaCl_2$  50%; Formulation VII, NaCl 25%  $CaCl_2$  75%

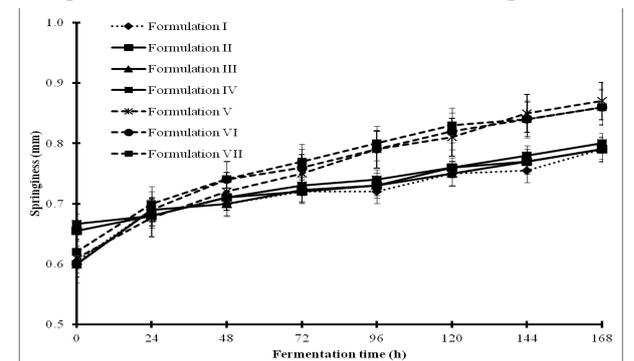


Figure 11. Changes in springiness of *Plaa-som* with the partial replacement of sodium chloride during fermentation. Formulation I, 100% NaCl; Formulation II, NaCl 75% KCl 25%; Formulation III, NaCl 50% KCl 50%; Formulation IV, NaCl 25% KCl 75%; Formulation V, NaCl 75%  $CaCl_2$  25%; Formulation VI, NaCl 50%  $CaCl_2$  50%; Formulation VII, NaCl 25%  $CaCl_2$  75%

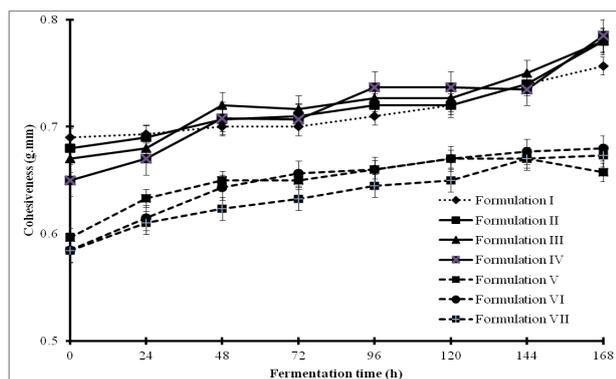


Figure 12. Changes in cohesiveness of *Plaa-som* with the partial replacement of sodium chloride during fermentation. Formulation I, 100% NaCl; Formulation II, NaCl 75% KCl 25%; Formulation III, NaCl 50% KCl 50%; Formulation IV, NaCl 25% KCl 75%; Formulation V, NaCl 75% CaCl<sub>2</sub> 25%; Formulation VI, NaCl 50% CaCl<sub>2</sub> 50%; Formulation VII, NaCl 25% CaCl<sub>2</sub> 75%

to the non-enzymatic browning reaction as the free amino acid group as well as lipid oxidation products increased, particularly with increasing fermentation time. Maillard reaction between carbonyl compound and amino acids is the major non-enzymatic browning in foods (Sikorski, 2001).

#### Changes in texture profile analysis (TPA)

As shown in Figure 9-12, the replacement of NaCl with KCl and CaCl<sub>2</sub> had a great influence on the textural properties of *Plaa-som*. During fermentation, the samples with CaCl<sub>2</sub> had a higher hardness, adhesiveness and springiness than those of the other samples ( $p < 0.05$ ), whilst no significant differences in the samples with KCl replacement compared to the formulation I (100% NaCl) ( $p < 0.05$ ). At the end of fermentation, *Plaa-som* with the partial replacement of NaCl showed a marked increase in all TPA textural attributes, compared to the samples before fermentation ( $p < 0.05$ ). The development of firmness is related to pH reduction and moisture removal (Baumgartner, 1980). Riebroy *et al.* (2008) also reported that texture formation of fermented fish mince was closely associated to the rapid decline in pH, which induced conformational changes of gelation. Higher acidity in the *Plaa-som* with the partial replacement of NaCl resulted in superior organoleptic properties. The obtained results are in accordance with results of Totosaus *et al.* (2004), found that sausages with reduced salt and fat contents also had higher hardness values with 0.5% CaCl<sub>2</sub>, explained by the compact protein matrix formation of gel.

#### Acceptability test

The results obtained for the acceptability of color,

flavor, texture, saltiness, sourness and overall liking of *Plaa-som* with the partial replacement of NaCl are presented in Table 2. In the present study the panel scores a significant higher color, flavor, texture and overall liking values ( $p < 0.05$ ) in the samples with 25 and 50% KCl replacement (Formulation II and III), however no significant differences ( $p < 0.05$ ) when compared with formulation I (100% NaCl). Lower acceptance in all attributes was observed in the samples using CaCl<sub>2</sub> replacement. Color acceptability was significantly reduced in CaCl<sub>2</sub> replacement compared to the formulation I, which may be related to the higher values of  $L^*$ ,  $a^*$  and  $b^*$ -values found in this treatment (Table. 2).

Table 2. Acceptance score of *Plaa-som* with the partial replacement of sodium chloride

Formulation	Attributes					
	Color	Flavor	Texture	Saltiness	Sourness	Overall liking
I	8.50±0.54 <sup>a</sup>	8.60±0.87 <sup>a</sup>	8.50±0.89 <sup>a</sup>	8.00±0.36 <sup>a</sup>	8.40±0.71 <sup>a</sup>	8.50±0.63 <sup>a</sup>
II	8.80±0.65 <sup>a</sup>	8.70±0.47 <sup>a</sup>	8.60±0.65 <sup>a</sup>	8.10±0.63 <sup>a</sup>	8.50±0.72 <sup>a</sup>	8.50±0.53 <sup>a</sup>
III	8.70±0.39 <sup>a</sup>	8.70±0.32 <sup>a</sup>	8.60±0.25 <sup>a</sup>	8.30±0.72 <sup>a</sup>	8.30±0.79 <sup>a</sup>	8.60±0.47 <sup>a</sup>
IV	7.20±0.91 <sup>b</sup>	7.30±0.89 <sup>ab</sup>	7.30±0.45 <sup>b</sup>	6.90±0.89 <sup>b</sup>	7.00±0.58 <sup>b</sup>	6.80±0.72 <sup>b</sup>
V	6.60±0.81 <sup>b</sup>	6.90±0.12 <sup>b</sup>	6.70±0.25 <sup>b</sup>	6.10±0.24 <sup>b</sup>	6.70±0.36 <sup>b</sup>	6.20±0.89 <sup>b</sup>
VI	5.30±0.25 <sup>c</sup>	5.30±0.35 <sup>c</sup>	4.50±0.48 <sup>c</sup>	4.70±0.28 <sup>c</sup>	6.70±0.89 <sup>b</sup>	4.30±0.25 <sup>c</sup>
VII	3.00±0.45 <sup>d</sup>	3.20±0.47 <sup>d</sup>	3.20±0.98 <sup>d</sup>	3.60±0.15 <sup>d</sup>	4.70±0.15 <sup>c</sup>	3.00±0.22 <sup>d</sup>

Mean ± SD from 25 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, NaCl 25% KCl 75%; V, NaCl 75% CaCl<sub>2</sub> 25%; VI, NaCl 50% CaCl<sub>2</sub> 50%; VII, NaCl 25% CaCl<sub>2</sub> 75%.

The obtained results are in accordance with results of Askar *et al.* (1994) which 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, according to Gelabert *et al.* (2003) and Desmond (2006). In addition, Olson and Terrell (1981) and Terrell and Olson (1981) reported that KCl could be substituted for NaCl without loss of functionality. However, at substitution levels greater than 50% of the NaCl, bitter or metallic off-flavor reportedly become objectionable. Several researchers have reported that replacement of NaCl with KCl at 30-50% levels can be accomplished without any loss functionality (Hand *et al.* 1982; Keeton, 1984). Some of the characteristics of CaCl<sub>2</sub> are bitterness, sourness and sweetness (Lawless *et al.* 2003). Santos *et al.* (2013) reported the salami produced with 50% CaCl<sub>2</sub> was less accepted by consumers, presenting rancid aroma, rancid flavor, and bitterness as more prominent attributes.

## Conclusions

In conclusion, the results of present study have indicated that the partial replacement of NaCl by KCl in *Plaa-som* during fermentation can be performed to develop healthier products with reduce NaCl contents. The partial replacement of NaCl by KCl to *Plaa-som* could retard lipid oxidation while decreasing the consumption of NaCl. The product with the best sensory acceptance resulted by substituting of 25 and 50% KCl (formulation II and III). The obtained results led us to conclude that use of KCl would not imply significant problems in the technological process.

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