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Effect of strong alkaline solutions on yield and characteristics of Pacific white shrimp *(Litopenaeus vannamei)*

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Abstract

Effects of sodium hydroxide (NaOH) and potassium hydroxide (KOH) solutions with different concentrations on yield and characteristics of Pacific white shrimps *(Litopenaeus vannamei)* meat were studied. Peeled and deveined shrimps were soaked in both solutions having concentrations of 0.125, 0.25, 0.50 and 0.75% (w/v) in the presence of 2.5% NaCl. The increases in weight gain and cooking yield were observed with increasing concentration of both solutions ($P<0.05$). The coincidental decrease in cooking loss was noticeable. pH of soaked shrimp muscle (6.84-9.44) increased as the concentrations of alkaline solutions increased ($P<0.05$). When protein patterns of soaking solutions were determined, higher degradation of proteins was found as the concentration of both solutions increased. Although the treatment of shrimp with 0.75% NaOH + 2.5% NaCl showed the highest cooking yield, it resulted in the increased a^* value, the decreased shear force and lower likeness score for all attributes. Generally, shrimps treated with mixed phosphates or sodium bicarbonate possessed the superior characteristics to those with alkaline treatments.

Introduction

Shrimp is the important fishery product, accounting for 15.4% of the total fishery products traded internationally in 2008 (FAO, 2010). Pacific white shrimp *(Litopenaeus vannamei)* is nowadays the most important shrimp for aquaculture, replacing *Penaeus monodon* and *Peneaus chinensis* (FAO, 2009). Shrimp processing contains various steps, such as cooking and freezing, which can affect textural and physicochemical properties as well as weight loss. A loss in moisture is caused by the decreased capacity of proteins in holding water due to denaturation or aggregation of proteins (Carneiro *et al*., 2013). To tackle this problem, some additives have been used. Phosphate and bicarbonate in conjunction with NaCl have been employed due to their synergistic action (Murphy and Zerby, 2004; Chantarasuwan *et al*., 2011b). Those additives have been used for increasing water retention in flesh, reducing the thaw loss in frozen fish, modifying the texture, yielding the better color and reducing cooking loss (Chang and Regenstein, 1997; Rattanasatheirn *et al.*, 2008; Chantarasuwan *et al*., 2011b; Manheem *et al*., 2012; Carneiro *et al*., 2013). The increases in interaction between protein and water molecules is due to increased pH and ionic strength, and the reduced interactions among proteins (Martin *et al*., 2002; Ünal *et al*., 2006; Erdogdu *et al*., 2007; Damodaran *et al*., 2008). Similarly, the effectiveness of bicarbonate is owing to the ability to partially solubilize myofibrillar proteins and the increased electrostatic repulsion by pH raising (Chantarasuwan *et al*., 2011b). The treatment using phosphate or bicarbonate therefore causes the swelling of myofibrils, permitting higher water absorption and retention.

Recently, the strict regulation for the uses of phosphate and bicarbonate as the processing aid in shrimp and shrimp products has been implemented. Thus, other alternatives, especially alkaline compounds, can be considered as the agent with the equivalent efficacy in improving quality of shrimp. However, the conditions for treatment and some factors could determine their effectiveness in quality improvement as well as the characteristics of resulting shrimps. Thus, the objective of this study was to investigate the effect of strong alkaline solutions as phosphate and bicarbonate replacers, on the yield and characteristics of Pacific white shrimp.

Materials and Methods

Collection and preparation of shrimp

Pacific white shrimp *(Litopeneaeus vannamei)* (55-60 shrimp/kg) were purchased from a local market in Hat Yai, Songkhla, Thailand. Shrimp with

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storage time less than 6 h after capture were stored in the insulated box containing the crushed ice using a shrimp/ice ratio of 1:2 (w/w). The samples were transported to the Department of Food Technology, Prince of Songkla University within 2 h. Upon arrival, shrimp were cleaned using tap water. Shrimp were peeled and deveined manually. During preparation, shrimp were kept on ice. Prepared shrimp were placed in polyethylene bag and stored in ice until used.

Effects of NaOH and KOH at various concentrations on yield and characteristics of Pacific white shrimp

Preparation of shrimp treated with alkaline solutions

Shrimp (peeled and deveined) were mixed with different soaking solutions. Those included 2.5% NaCl containing 0.125, 0.25, 0.5 and 0.75% (w/v) NaOH with the pHs of 12.40, 12.60, 12.91 and 13.04, respectively and 2.5% NaCl comprising 0.125, 0.25, 0.5 and 0.75% (w/v) KOH, which had the pHs of 12.22, 12.52, 12.83 and 12.98, respectively. Shrimp were mixed with solutions at a ratio of 1:2 (w/v). The mixtures were stirred gently for 30 min at 4°C and allowed to stand at 4°C for 30 min. After removal from the solutions, the shrimp were placed on the plastic screen for 5 min (4°C) to drain off solution. Sample soaked in 2.5% NaCl containing 3% mixed phosphates (sodium tripolyphosphate + tetrasodium pyrophosphate; 1:2, w/w) (pH 9.45) (Manheem, 2012) and in 2.5% NaCl containing 2% $NaHCO_3$ (pH 8.5) (Chantarasuwan *et al*., 2011b) were used as the positive controls. Sample without soaking was referred to as the control. After each treatment, those shrimp were divided to two portions. The first portion was used as raw shrimp and another portion was subjected to cooking to obtain cooked samples. To prepare the cooked shrimp, the treated shrimp were heated by steaming until the core temperature of the second segment of shrimp reached 85°C. The samples were cooled rapidly in iced water for 1 min and then the prepared samples were drained on a screen for 5 min at 4°C. Both raw and cooked shrimp were subjected to analyses.

Determination of weight gain

Weight gain was determined by weighing the shrimps before and after soaking in the solutions. Weight gain was calculated as follows:

$$\text{Weight gain (\%)} = [(B-A)/A] \times 100$$

where: A = initial weight (before soaking)
B = weight after soaking, followed by draining

Determination of cooking loss and cooking yield

Cooking loss and cooking yield were measured by weighing the shrimps before and after heating according to method of Manheem *et al.* (2012). Cooking yield and cooking loss were calculated by the following equations:

$$\text{Cooking loss (\%)} = [(B-C)/B] \times 100$$

$$\text{Cooking yield (\%)} = (C/A) \times 100$$

where : A = initial weight (without soaking and steaming)
B = weight after soaking, followed by draining
C = weight after steaming, followed by cooling in iced water

Determination of pH

pH of raw shrimp without and with treatments was measured by the method of Martínez-Álvarez *et al*. (2005) with a slight modification. Approximately 2 g of shrimp meat was homogenized with 10 ml of deionized water for 1 min at a speed of 1,000 rpm (IKA labortechnik, Selangor, Malaysia). The homogenate was kept at room temperature for 5 min. The pH was determined using a pH-meter.

Determination of NaCl content

NaCl content of both raw and cooked shrimp was determined as per the method of AOAC (2000). NaCl content was expressed as % (dry weight basis).

Determination of color

Color of raw and cooked shrimp were determined and expressed as L^* (lightness), a^* (greenness/ redness) and b^* (yellowness/ blueness). The second segment of shrimp was subjected to measurement using a Hunterlab colorimeter (Hunter Associates Laboratory, Inc., Reston, VA, USA), using a CIE Lab scale (Young and Whittle, 1985).

Determination of shear force

Shear force of raw and cooked shrimp was measured using the TA-XT2i texture analyzer (Stable Micro Systems, Surrey, England) equipped with a Warner-Bratzler shear apparatus (Brauer *et al*., 2003). The operating parameters consisted of a cross head speed of 10 mm /s and a 25 kg load cell. The shear force, perpendicular to the axis of the second segment muscle fibers, was measured.

Determination of protein pattern of soaking solutions

After being soaked, the resulting soaking solutions were subjected SDS-PAGE to determine the

patterns of proteins leached out from shrimp muscle into solutions.SDS-PAGE was performed using 10% separating and 4% stacking gels as described by Leammli (1970). Soaking solution (20 ml) was mixed with 10 ml of 10% (w/v) SDS solution. The mixture was then homogenized at 11,000 rpm for 1 min. The homogenate was incubated at 85°C for 1 h to dissolve total proteins. The sample was then centrifuged at 7,500 x g for 15 min to remove undissolved debris. Protein concentration of the supernatant was determined by the Biuret method (Robinson and Hogden, 1940). Sample (10 µg protein) was loaded onto the gel consisting of 4% stacking gel and 10% separating gel. Separation was performed by electrophoresis apparatus (Mini-Protein III, Bio-Rad Laboratories, Inc., Richmond, CA, USA) using 30 mA. Protein was fixed and stained for 3 h in 1.25% Coomassie Brilliant Blue R-250 in 40% methanol and 10% glacial acetic acid. Gels were destained for 15 min with destaining solution I (50% methanol and 7.5% glacial acetic acid) and with the destaining solution II (5% methanol and 7.5% gracial acetic acid) for 3 h. Wide range molecular weight standards were used and the molecular weight of proteins was estimated.

Sensory evaluation

The cooked samples with the highest cooking yield, the control (without treatment) and positive controls (treated with mixed phosphates and with bicarbonate) were subjected to sensory analysis. The samples were evaluated by 30 panelists from the Department of Food Technology with the age of 25-35, using the 9-point hedonic scale, where 9 = like extremely; 7= like moderately; 5 = neither like or not dislike; 3 = dislike moderately; 1 = dislike extremely (Meilgaard et al., 1990). Panelists were acquainted with shrimp consumption and had no allergies to shrimp. All panelists were asked to evaluate for appearance, color, flavor, texture, taste and overall likeness. Samples were presented in the plates coded with three-digit random numbers.

Determination of microstructure

Microstructure was analyzed as described by Ratanasatein *et al*. (2008). Samples were immersed in liquid nitrogen and were allowed to stand at room temperature for 5 min. Thereafter, the prepared samples were then cut into a cube (4 x 4 x 4 mm) with a razor blade. The prepared samples were fixed with 2.5% glutaraldehyde in 0.2 M phosphate buffer, pH 7.2, at room temperature for 2 h. All specimens were washed three times with deionized water for 15 min each and dehydrated with a serial concentration of 20, 50, 60, 70, 80, 90 and 100% ethanol for 15 min each. All specimens were coated with 100% gold (Sputter coater SPI-Mpdule, PA, USA). The microstructure was visualized using a scanning electron microscope (JEOL, JSM-5800 LV, Tokyo, Japan). Magnification of 10,000X and 5,000X were used for longitudinal section and cross section, respectively.

Statistical analysis

A completely randomized design (CRD) was used for the entire experiments. Experiments were run in triplicate using three different lots of shrimp. Data were subjected to analysis of variance and mean comparison was carried out using Duncan's multiple range test (Steel and Torrie, 1980). Statistical analyses were performed using the Statistical Package for Social Science (SPSS 11.0 for windows, SPSS Inc., Chicago, IL, USA).

Results and Discussion

pH

pH of shrimp meat (6.60-6.70) generally increased when treated with alkaline soaking solutions (Figure 1a). pH of meat varied, depending on the solutions used. Generally, the pHs of treated shrimp meat (6.75-9.44) were much lower than those of solutions (12.20-13.30). NaOH and KOH are strong alkali and can be completely ionized in water. In the present study, pH of $NaHCO_3$ solution was adjusted to 8.5, which was the optimal pH for shrimp treatment as suggested by Chantarasuwan *et al.* (2011b). The mixed phosphates showed slightly alkaline pH (9.4-9.5), reflecting alkaline nature of those phosphates. The difference in pH between shrimp muscle and solution might be explained by the buffering capacity of muscle proteins toward alkaline compounds. At the same concentration of alkaline solution used, it was noted that the pH of shrimp meat treated with NaOH solution was higher than that found in shrimp soaked in KOH solution ($P<0.05$). This was in agreement well with the higher pH of NaOH solution. For shrimp treated with bicarbonate, the similar pH was noticeable, compared with those treated with 0.125% KOH or 0.125% NaOH ($P>0.05$). The pH changes of shrimp meat more likely determined the changes in muscle, particularly the modification of charge as well as conformation of proteins (Chantarasuwan *et al*., 2011b).

Weight gain, cooking loss and cooking yield

Weight gain, cooking yield and cooking loss of Pacific white shrimp soaked in NaOH and KOH solutions at various concentrations in the presence of

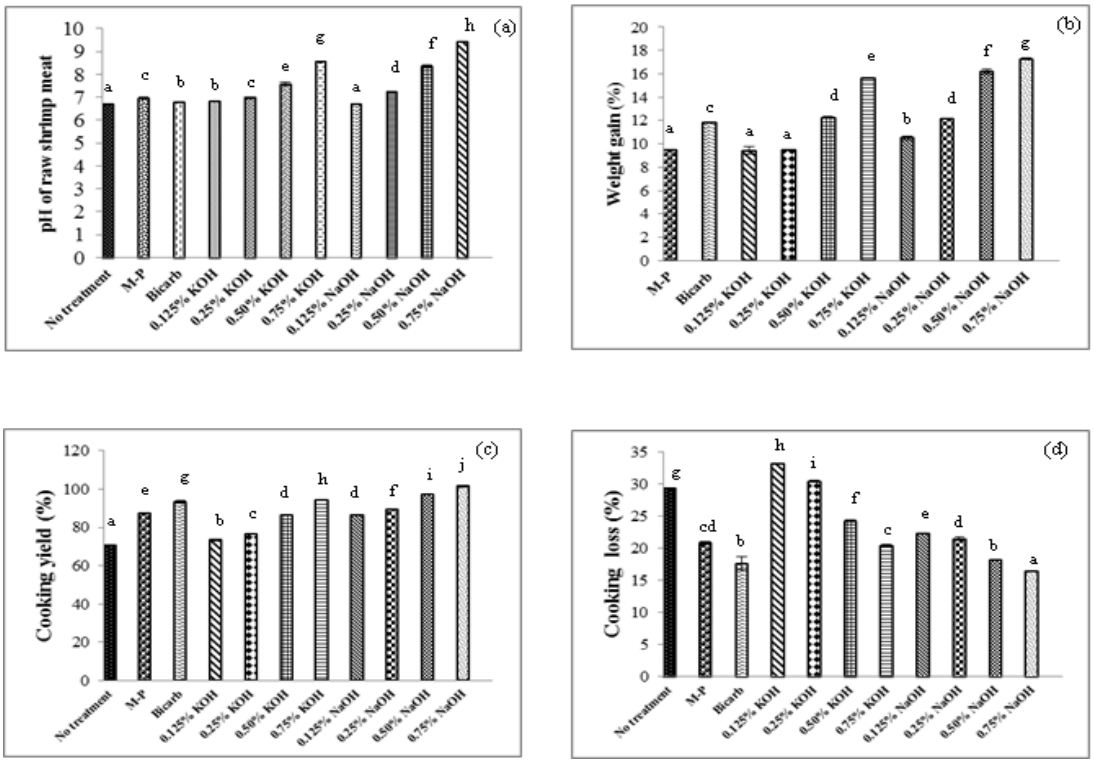

Figure 1. pH (a), weight gain (b), cooking yield (c) and cooking loss (d) of Pacific white shrimp after soaking in KOH and NaOH solutions at different concentrations in the presence of 2.5% NaCl. Note: M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1 (w/w)).; Bicarb: solution containing 2.5% NaCl and 2.5% sodium bicarbonate. Different lowercase letters on the bars indicate significant differences ($P<0.05$). Bars represent the standard deviation (n=3)

2.5% NaCl are shown in Figure 1. Weight gain (Figure 1b) and cooking yield (Figure 1c) of the treated shrimp increased, while cooking loss decreased when the concentration of solutions increased, regardless of types of solutions ($P<0.05$). When pH was far away from pI, particularly in alkaline pH range, the proteins became negatively charged, in which protein molecules repulsed each other. This resulted in the swollen muscle fibers, which could facilitate water uptake into muscle structure (Zayas, 1997). At the same concentration of solution, shrimp treated with NaOH solution showed the higher weight gain and cooking yield than those soaked in KOH solution ($P<0.05$). This coincided with the higher pH of the former. The highest weight gain and cooking yield were observed in shrimp soaked with 0.75% NaOH ($P<0.05$) Apart from higher pH, alkaline solution with higher concentration might be related with higher ionic strength. Ionic interaction between water molecule and protein structure could be regulated by ionic strength (Rattanasatheirn *et al*., 2008). Chatarasuwan *et al.* (2011b) found that different weight gain of shrimp treated with sodium carbonate and sodium bicarbonate was caused by the differences in ionic strength of the solution used.

Cooking loss of shrimp soaked in NaOH and KOH solutions at various concentrations in the presence of 2.5% NaCl is shown in Figure 1d. When comparing with the control (no treatment), all samples had the lower cooking loss, except for the samples treated with 0.125% and 0.25% KOH solutions, which showed the increased cooking loss. The lower cooking loss with higher cooking yield of the shrimp treated with alkaline solution indicated that the shrimp muscle had a higher water holding capacity even after cooking (Rattanasatheirn *et al*., 2008). When heat was applied, proteins underwent aggregation, resulting in the loss of water holding capacity. Furthermore, the water was probably lost, associated with heat induced denaturation of proteins. As a whole, less water was entrapped within the protein structures held by capillary forces (Aaslyng *et al*., 2003). For the samples treated with KOH at low concentration, the water uptaken into the muscle might be located loosely in the muscle structure, which could be expelled with ease when heat was introduced. This was evidenced by the high cooking loss. The lowest cooking loss was found in shrimp treated with 0.75% NaOH ($P<0.05$), followed by those treated with 0.75% KOH. During the soaking process, the negatively charged domains of muscle proteins might bind with water, thereby enhancing

Table 1. Color and shear force of raw and cooked Pacific white shrimp after soaking in KOH and NaOH solutions at different concentrations in the presence of 2.5% NaCl

Sample	Treatment	*L**	*a**	*b**	Shear force (g)
Raw	No treatment	46.15±1.98 ^{f,cdef}	-1.30±0.50 ^{ab}	0.31±0.40 ^{de}	1913±238 ^{abc}
	M-P	44.23±1.65 ^{b}	-1.77±0.49 ^{a}	-3.98±1.04 ^{a}	2083±346 ^{bcd}
	Bicarb	46.42±1.33 ^{def}	-1.74±0.50 ^{a}	-2.01±1.46 ^{b}	1997±255 ^{bc}
	0.125% KOH	44.42±1.09 ^{b}	-0.76±0.55 ^{b}	-1.12±1.02 ^{bc}	2293±183 ^{cd}
	0.25% KOH	45.65±1.27 ^{bcde}	1.24±0.75 ^{c}	1.34±0.84 ^{ef}	2078±66 ^{bcd}
	0.50% KOH	46.87±1.46 ^{efg}	2.11±0.51 ^{cd}	1.78±1.00 ^{f}	1927±150 ^{bc}
	0.75% KOH	48.11±1.25 ^{g}	2.20±0.82 ^{d}	1.38±0.93 ^{ef}	1877±174 ^{ab}
	0.125% NaOH	41.66±1.04 ^{a}	1.08±0.49 ^{c}	-1.07±1.04 ^{bc}	2418±382 ^{cd}
	0.25% NaOH	44.53±1.48 ^{bc}	1.21±0.70 ^{c}	-0.38±1.29 ^{g}	2186±229 ^{bd}
	0.50% NaOH	44.75±1.52 ^{bcd}	2.14±1.04 ^{d}	-0.26±0.92 ^{cd}	1787±229 ^{ab}
	0.75% NaOH	47.74±1.27 ^{fg}	2.97±1.59 ^{d}	-0.18±1.15 ^{cd}	1550±238 ^{a}
Cooked	No treatment	72.34±1.82 ^{g}	16.41±2.04 ^{d}	19.33±0.94 ^{f}	2260±394 ^{b}
	M-P	66.71±2.74 ^{de}	9.06±2.92 ^{c}	15.79±3.26 ^{de}	1492±166 ^{a}
	Bicarb	66.18±2.21 ^{de}	8.52±2.54 ^{bc}	14.28±3.42 ^{cde}	1395±213 ^{a}
	0.125% KOH	71.78±1.35 ^{g}	9.12±2.72 ^{c}	13.93±3.91 ^{bcde}	1642±263 ^{a}
	0.25% KOH	67.80±1.21 ^{ef}	8.25±1.81 ^{c}	16.92±2.04 ^{ef}	1538±138 ^{a}
	0.50% KOH	63.46±2.00 ^{c}	6.20±1.43 ^{ab}	12.89±3.38 ^{bcd}	1496±148 ^{a}
	0.75% KOH	57.59±1.59 ^{a}	5.95±2.10 ^{a}	12.01±2.22 ^{abc}	1470±188 ^{a}
	0.125% NaOH	69.20±1.61 ^{f}	8.64±2.23 ^{b}	11.20±2.68 ^{ab}	1620±153 ^{a}
	0.25% NaOH	65.11±1.99 ^{cd}	6.38±1.73 ^{ab}	14.96±3.13 ^{cde}	1430±40 ^{a}
	0.50% NaOH	60.76±1.33 ^{b}	5.02±1.69 ^{a}	11.10±2.01 ^{ab}	1395±108 ^{a}
	0.75% NaOH	58.68±1.41 ^{a}	5.04±1.06 ^{a}	9.83±1.94 ^{a}	1384±61 ^{a}

† Mean±SD (n=3).
M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1 (w/w)).; Bicarb: solution containing 2.5% NaCl and 2.5% sodium bicarbonate. Different lowercase superscripts in the same column under the same state of sample indicate the significant differences ($P<0.05$).

water binding capacity (Chatarasuwan *et al*., 2011b), leading to the increased weight gain with coincidental decrease in cooking loss.

Salt content

Similar NaCl contents were found in all treated samples ($P>0.05$). Raw and cooked samples had the salt content of 6.94-7.21% and 6.18-6.66%, respectively. For the control (without treatment), NaCl contents of 2.00 and 1.22% were observed in the raw and cooked samples, respectively (data not shown). It was noteworthy that all solutions contained 2.5% NaCl. During soaking, NaCl could penetrate into the meat, regardless of alkaline compounds. Salt generally shows synergistic effect on shrimp quality improvement of phosphates (Manheem *et al.*, 2013). NaCl is added to meat products to improve their binding and water holding properties. Chloride ions tend to penetrate into the myofilaments, causing them to swell (Hamm, 1970), and the sodium ions form an ion cloud around the filaments (Offer and Tringick, 1983). Salt content in Pacific white shrimp soaked in either sodium carbonate or sodium bicarbonate solution increased when pH of solutions increased from 5.5 to 11.5 (Chantarasuwan et al., 2011b). At physiological pH (6.4-7.0), which was slightly higher than pI of muscle proteins, the negatively charged domains were present. When NaCl underwent dissociation, Cl^- could get into muscle, thus neutralizing the positive charge. As a consequence, the ionic interactions between filaments were lowered. This could augment the migration of alkaline and water into the muscle compartment. Simultaneously, NaCl could get into muscle as indicated by the increase in NaCl content in the treated shrimp.

Color

Color of raw and cooked shrimp after soaking in alkaline solutions (KOH and NaOH) at different concentrations in the presence of 2.5% NaCl is illustrated in Table 1. Color is one of the quality attributes of shrimp *(Litopenaeus vannamei)*, determining the consumer acceptability

(Chantarasataporn *et al*., 2013). For raw shrimp, L^* value increased when the concentrations of solution increased (P<0.05). During soaking, proteins became more charged under the alkaline conditions. This led to the increasing water uptake into muscle. The water distributed in the muscle compartment might be related with the swollen structure, in which the light could pass through and was associated with increased transparency. Shrimp treated with bicarbonate showed the higher L^*-value than those treated with mixed phosphate. Rattanasatherin (2008) reported the translucence of shrimp after being treated with phosphate depended on pH of solution.

Shrimp turned to be red after being treated with both alkaline solutions. This was coincidental with the increase in a^* value (Table 1). At alkaline pH, the denaturation of carotenoid proteins was induced, leading to the appearance of red color caused by free carotenoids (Chantarasuwan *et al.*, 2011b). Astaxanthin is a pigment commonly found in crustacean, providing the tissue with red-orange pigmentation (Okawa *et al*., 1994). With increasing concentrations of solutions used for shrimp treatment, the resulting shrimp had more redness as indicated by the increased a^*-value. For b^* value, the similar trend was obtained, in comparison with a^*-value. However, b^*-value of shrimp treated with KOH solution was higher than those observed in shrimp soaked in NaOH solution (P<0.05). Thus, the reaction related with color changes induced by both alkaline solutions might be varied. NaOH, which is strong alkali, might leach out the pigment from shrimp meat to a higher extent. This resulted in the fader color of shrimp treated with NaOH solution (data not shown).

For cooked shrimp, L^* value was higher than those found in raw shrimp. L^*-value decreased when the concentration of solution increased (P<0.05), regardless of types of solution. When the concentration of solution increased, those shrimp could bind more water, thereby preventing the aggregation of protein during cooking. The higher aggregated proteins generally became more turbid or opaque, as indicated by increased L^* value. Chantarasuwan *et al.* (2011b) found that L^* value of cooked shrimp treated with sodium carbonate and sodium bicarbonate in the presence of 2.5% NaCl at different pHs was decreased gradually as pH of solutions increased (P<0.05). Fading of red color in cooked shrimp were more pronounced when concentrations of solution increased (data not shown). This was in accordance with the decreases in both a^* and b^* values (Table 1). Carotenoproteins in shrimp were postulated to leach out during soaking to a higher extent as the alkaline solution with higher concentrations was used for treatment.

For the control, the highest a^*, b^* and L^* value were found. The control with the lowest cooking yield and the highest protein aggregation with negligible loss of carotenoprotein more likely had the highest content of pigments retained. Dense structure caused by the intense aggregations of muscle proteins and less water retained also result in the turbidity and high redness (Table 1).

Shear force

Shear force of raw and cooked shrimp soaked in KOH and NaOH solutions at different concentrations in the presence of 2.5% NaCl is shown in Table 1. The shear force of the raw and cooked shrimps is a good indicator to evaluate the texture, particularly the tenderness (Chantarasataporn *et al.*, 2013). Shear force of raw shrimp decreased when the concentration of solutions increased (P<0.05). Under the alkaline condition, the repulsion between protein molecules was more pronounced, resulting in the loosen structure, which became less resistant to the force applied (Chantarasuwan *et al*., 2011b). The swelling of muscle and retained water most likely weakened the muscle structures as evidenced by the lower shear force of treated shrimp (Kaewmanee *et al*., 2009). However, the shear force of all treated samples was not different from the control. It was noted that similar shear force was observed between shrimp treated with bicarbonate and those treated with the mixed phosphate (P>0.05). For cooked shrimp treated with alkaline solutions or mixed phosphates or bicarbonate, the lower shear force was observed, in comparison with the control (P<0.05). In general, there was no difference in shear force among all samples subjected to difficult treatments (P>0.05). During cooking, protein underwent denaturation and some weak bonds might be disrupted. Those unfolded proteins might undergo aggregation, leading to the toughening of texture along with the loss in water. Shrimp meat showed higher firmness or solidity by heat processing and gets too solid when its inner temperature is above 100 °C (Mizuta *et al*., 1999). When the proteins underwent the thermal denaturation, the water was less imbibed or bound in their structure. The release of water from protein molecules might facilitate the muscle fiber to align closely, leading to the more compact structure (Rattanasatherin *et al*., 2008). For treated shrimp, the repelled proteins could not form the excessive aggregate, especially in the presence of water held in the compartment. Therefore, the treatment of shrimp using alkaline solutions lowered the shear force of cooked shrimp.

Table 2. Likeness score of cooked Pacific white shrimp with different treatments

Attributes	No treatment	M-P	Bicarb	0.75% NaOH +2.5% NaCl
Appearance	6.93±1.14^{b}	7.97±0.49^{c}	7.70±0.65^{c}	5.70±1.51^{a}
Color	7.43±0.63^{b}	7.93±0.64^{b}	7.73±0.78^{b}	5.60±1.45^{a}
Flavor	7.27±0.94^{b}	7.72±0.64^{b}	7.70±0.65^{b}	3.70±1.82^{a}
Texture	6.73±1.28^{b}	7.72±0.64^{c}	7.93±0.64^{c}	4.40±1.52^{a}
Taste	7.27±1.23^{b}	7.76±0.73^{bc}	7.87±0.73^{c}	3.67±1.35^{a}
Overall	6.87±1.07^{b}	7.79±0.61^{c}	7.70±0.65^{c}	4.47±1.14^{a}

Mean±SD (n=3).
M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1 (w/w)).; Bicarb: solution containing 2.5% NaCl and 2.5% sodium bicarbonate. Different lowercase superscripts in the same row indicate the significant differences (P<0.05).

Protein pattern of soaking solution

Protein patterns of different alkaline solutions containing 2.5% NaCl after soaking with shrimp are shown in Figure 2. Myosin heavy chains (MHC) and actin were noticeable in solutions of mixed phosphate or bicarbonate. Both proteins were also found in NaOH or KOH solutions at concentrations of 0.125 and 0.25%. At concentration higher than 0.25%, MHC and actin band disappeared in both solutions. MHC and actin were plausibly hydrolyzed under the strong alkaline solution. Chinabhark *et al.* (2007) found that MHC and actin bands of film-forming solutions from bigeye snapper *(Priacanthus tayenus)* surimi were degraded to a higher extent at alkaline pH, leading to the decreased peptide chain length. With increased degradation of protein chains, the muscle compartments became loosen and could absorb water more effectively. This was coincidental with the increased weight gain (Figure 1b). Moreover, NaCl was able to solubilize muscle protein in conjunction with alkaline solution (Rattanasatheirn *et al*., 2008). In general, similar protein patterns were observed between solution of mixed phosphates and bicarbonate after shrimp soaking. This was in agreement with the similar efficacy in water uptake of both treatments. The results indicated that muscle proteins underwent degradation to a higher degree as the concentration of alkaline solution increased. Less compact structure could enhance water absorption of shrimp muscle.

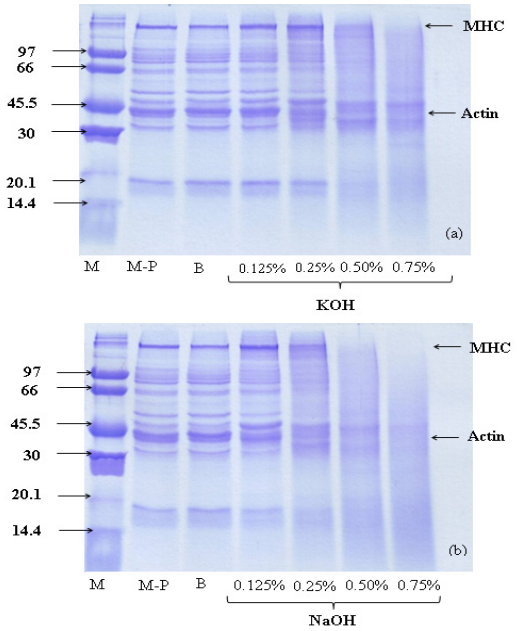

Figure 2. Protein pattern of KOH solution (a) and NaOH solution (b) containing 2.5% NaCl after soaking with shrimp. M, standard marker; M-P: soaking solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1 (w/w)).; B: solution containing 2.5% NaCl and 2.5% sodium bicarbonate

Characteristics of Pacific white shrimp treated with the selected alkaline solution

Sensory property

Likeness score of cooked shrimp treated with 0.75% NaOH containg 2.5% NaCl in comparison with other treatments is shown in Table 2. Lower likeness score in all attribute was found in the sample soaked in 0.75% NaOH with 2.5% NaCl, compared with other samples (P<0.05). This was due to the slimy surface associated with the excessive solubilization of protein at very alkaline pH. Strong offensive alkaline odor was also obtained. The lower color likeness score was coincidentally with the lower L^* and a^* (Table 1). Generally, shrimp treated with 2.5% NaCl containing mixed phosphate or bicarbonate showed the higher score for all attributes (P<0.05). For texture likeness, those treated with 2.5% NaCl containing mixed phosphate or bicarbonate had the higher score than the control (P<0.05). The water uptaken in muscle compartment resulted in the juiciness and more tenderness of shrimp. Based on sensory evaluation, the use of NaOH solution

for shrimp treatment showed the negative effect on sensory property of shrimp.

Microstructure

Microstructures of Pacific white shrimp muscle treated with 0.75% NaOH in the presence of 2.5% NaCl in comparison with those of samples treated with 2.5% NaCl containing mixed phosphates or bicarbonate and the control (without treatment) are illustrated in Figure 3. For longitudinal section, after being soaked in 2.5% NaCl comprising mixed phosphates, bicarbonate and 0.75% NaOH, myofibrils became larger in size, compared with those observed in the control. However, myofibrils were less attached as indicated by gaping. Disintegration of M-line was clearly observed in shrimps treated with mixed phosphates or bicarbonate and 0.75% NaOH after cooking. The result was in accordance with Rattanasatheirn *et al.* (2008) who reported the degradation of M-line in cooked Pacific white shrimp after phosphate treatment. The disappearance of M-line was more likely related with the increased translucence of treated samples.

For the transverse sections (Figure 3), the dense structure was noticeable in the control shrimps. When the proteins underwent the thermal denaturation, the water was less imbibed or bound in the muscle. The release of water from protein molecules might facilitate the myofibrils to align closely, leading to the more compact structure. Treated shrimps with 2.5% NaCl containing mixed phosphate, bicarbonate and 0.75% NaOH had the loosen structure than the control (without treatment). The result confirmed that the 0.75% NaOH containing 2.5% NaCl could enhance the repulsion of myofilaments and maintain the water in the compartment, after cooking. This would lead to the higher cooking yield with lower cooking loss in shrimp treated with alkaline solution.

Conclusion

The alkaline (NaOH or KOH) treatment had potential to improve water holding capacity of shrimp. The increases in weight gain and cooking yield with the lowered cooking loss were obtained with increasing alkaline concentrations. The efficiency of alkaline treatment in improving the quality was governed by pH, which determined solubilization and disruption of the muscle compartments. Shrimp soaked in 0.75% NaOH containing 2.5% NaCl solution (pH 13.04) showed the highest weight gain and cooking yield, with the lowest cooking loss. However, such a treatment caused the increased a^* value, the decreased shear force and lower likeness score for all attributes. Thus, further improvement for the use of strong alkali for shrimp treatment is still required.

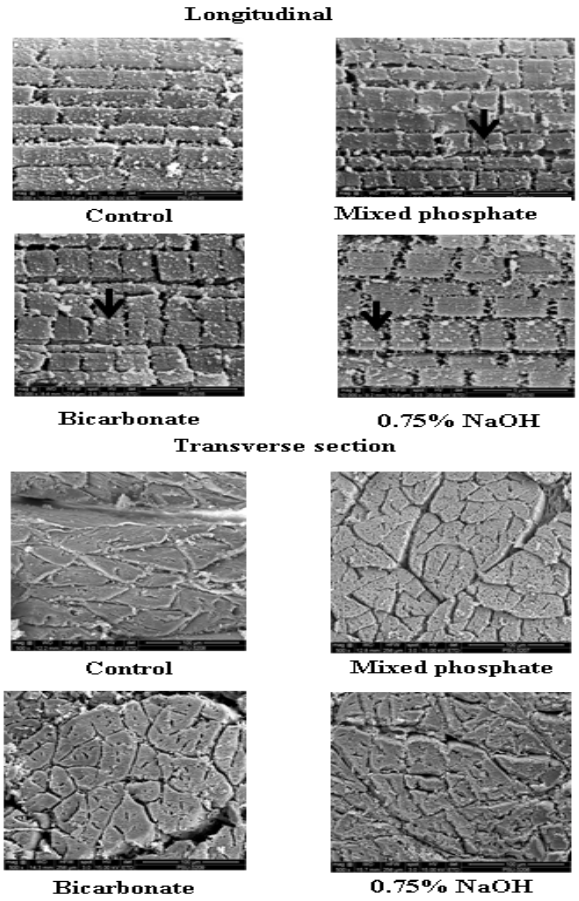

Figure 3. SEM micrographs of longitudinal and transverse sections of cooked shrimp muscle with different treatments. Magnification = 10,000 x and 5,000 x for longitudinal and transverse section, respectively. *Arrow indicates M-line

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