รายงานวิจัยฉบับสมบูรณ์

ผลของสารละลายด่างที่ไม่ใช่ฟอสเฟตต่อคุณภาพและสมบัติทางเคมีกายภาพของกล้ามเนื้อกุ้งขาว

Impact of non-phosphate alkaline solution on the quality and physicochemical properties of Pacific white shrimp muscle

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กิตติกรรมประกาศ

ขอขอบพระคุณมหาวิทยาลัยสงขลานครินทร์ในการสนับสนุนทุนโครงการวิจัยประเภททั่วไป ประจำปีงบประมาณ 2555 นอกจากนี้ขอขอบคุณ คุณจักรวรรดิ์ จันทรสุวรรณ ที่ช่วยให้งานวิจัยนี้สำเร็จได้ด้วยดี

บทคัดย่อ

จากการศึกษาผลของโซเดียมคาร์บอเนต (Na_2CO_3) และโซเดียมไบคาร์บอเนต ($NaHCO_3$) ต่อผลผลิตและคุณลักษณะของกุ้งขาวแวนนาไมพบว่า การแช่กุ้งในสารละลายโซเดียมคลอไรด์ร้อยละ 2.5 ซึ่งมีส่วนผสมของสารประกอบทั้งสองที่ระดับพีเอชต่างๆ (5.5, 7, 8.5, 10 และ 11.5) ให้น้ำหนักที่เพิ่มขึ้นและผลผลิตภายหลังการให้ความร้อนสูงขึ้นพร้อมทั้งมีการสูญเสียจากการให้ความร้อนลดลงเมื่อพีเอชของสารละลายสูงขึ้น (p<0.05) พีเอชและปริมาณเกลือในเนื้อกุ้งที่สูงขึ้นสัมพันธ์กับพีเอชที่เพิ่มขึ้น (p<0.05) พีเอชของสารละลายที่ใช้แช่กุ้งที่สูงขึ้นมีผลละลายโปรตีนและแคโรทีโนโปรตีนในกล้ามเนื้อบางส่วน พีเอชที่มากกว่า 8.5 มีผลต่อการชะล้างเม็ดสีซึ่งสัมพันธ์กับค่าสีแดงของกุ้งที่ผ่านการให้ความร้อนที่ลดลง ค่าแรงเฉือนของกุ้งดิบและกุ้งที่ผ่านการให้ความร้อนลดลงอย่างต่อเนื่องเมื่อพีเอชของสารละลายเพิ่มขึ้น (p<0.05) สารละลายที่ประกอบด้วยโซเดียมคลอไรด์ร้อยละ 2.5 และ $NaHCO_3$ร้อยละ 2.0 (พีเอช 8.5) สามารถใช้ทดแทนสารประกอบฟอสเฟตที่สามารถเพิ่มผลผลิตและลดการสูญเสียน้ำหนักโดยไม่มีผลต่อสมบัติทางประสาทสัมผัส

เมื่อศึกษาการเปลี่ยนแปลงแอกโตไมโอซิน (NAM) ของกุ้งขาวแวนนาไมในสารละลาย $NaHCO_3$ ที่ระดับความเข้มข้นต่างๆ (0 ถึง 1 โมลาร์) ในสภาวะที่มีและไม่มีโซเดียมคลอไรด์ร้อยละ 2.5 พบว่า ความขุ่นของ NAM ลดลงพร้อมกับมีการละลายที่เพิ่มขึ้น เมื่อความเข้มข้นของ $NaHCO_3$เพิ่มขึ้น ไฮโดรโฟบิกซิตีบริเวณพื้นผิว (SoANS) และปริมาณหมู่ซัลฟ์ไฮดริลทั้งหมดของ NAM เพิ่มขึ้น เมื่อความเข้มข้น $NaHCO_3$เพิ่มขึ้นกิจกรรม Ca^{2+}- และ Mg^{2+}-ATPase ของ NAM ลดลงมากขึ้นเมื่อความเข้มข้นของ $NaHCO_3$เพิ่มขึ้น บ่งชี้ถึงการสูญเสียธรรมชาติของบริเวณส่วนหัวของไมโอซินและการแตกตัวของแอกโตไมโอซินเชิงซ้อน จากการวิเคราะห์ศักย์ซีตา พบว่า บริเวณผิวหน้าของ NAM มีประจุลบเพิ่มขึ้น (-12.12 ถึง -26.56) เมื่อความเข้มข้นของ $NaHCO_3$เพิ่มขึ้น การเปลี่ยนแปลงดังกล่าวเพิ่มขึ้นในสภาวะที่มีโซเดียมคลอไรด์ร้อยละ 2.5 จากการศึกษาโครงสร้างโดยใช้กล้องจุลทรรศน์แบบส่องผ่าน พบว่า โครงสร้างของแอกโตไมโอซินมีการแตกตัวเพิ่มขึ้นและสูญเสียโครงสร้างเส้นใยเมื่อ $NaHCO_3$ มีระดับความเข้มข้นสูงขึ้น

ABSTRACT

Effects of sodium carbonate (Na_2CO_3) and sodium bicarbonate ($NaHCO_3$) on yield and characteristics of Pacific white shrimp(*Litopenaeusvannamei*) were studied. Shrimp soaked in 2.5% NaCl containing both compounds at different pH (5.5, 7, 8.5, 10 and 11.5) showed an increase in the weight gain and cooking yield and a reduced cooking loss as pH of solutions increased (p<0.05). The coincidental increases in pH and salt content in soaked shrimp muscle were obtained with increasing pH (p<0.05). Higher pH of soaking solution partially solubilized proteins in the muscle as well as carotenoproteins. pH of solutions above 8.5 led to the pronounced leaching of pigments, associated with the lowered redness of cooked shrimp. Shear force of raw and cooked shrimp continuously decreased as pH of solution increased (p<0.05). Solution containing 2.5% NaCl and 2.0% $NaHCO_3$ (pH 8.5) was recommended for treatment of white shrimp as a promising alternative for phosphates to increase the yield and to lower cooking loss without any negative effect on sensory property.

Changes in natural actomyosin (NAM) from Pacific white shrimp(*Litopenaeusvannamei*) treated with sodium bicarbonate ($NaHCO_3$) at different concentrations (0-1 M) in the absence or the presence of 2.5% NaCl were studied. Turbidity of NAM solutions decreased with coincidental increase in solubility was observed as the concentration of $NaHCO_3$ increased. Surface hydrophobicity (S_oANS) and total sulfhydryl content of NAM also increased when $NaHCO_3$ concentration increased. Greater decreases in Ca^{2+}- and Mg^{2+}-ATPase activity were found in all NAM as $NaHCO_3$ concentration increased, suggesting the denaturation of myosin head and the dissociation of actomyosin complex. The zeta potential (ζ) analysis suggested that the surface of NAM became more negatively charged (-12.12 to -26.98) as

$NaHCO_3$concentration increased. Those changes were more intense in the presence of 2.5% NaCl. Transmission electron microscopy showed that the structure of actomyosin was more dissociated and lost the filamental structure when $NaHCO_3$ at higher levels was used.

CHAPTER 1

INTRODUCTION

Seafood of Thailand is well known for its long standing excellent reputation worldwide, owing to its outstanding quality. To maintain the quality of seafoods, some additives have been widely used. The ability of muscle to absorb the added water during processing and capacity of retaining the water after cooking and freezing are the important factors governing the quality of seafood and seafood products. Moisture content generally influences meat juiciness, tenderness and mouthfeel (Ogawa et al., 1994).

Salt and phosphates are commonly used in combination to exploit their synergistic action (Murphy and Zerby, 2004). Those additives have been used in fish and seafood to improve the functional properties of seafood products, especially 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). The mechanism responsible for the increased tenderness and juiciness is connected with higher water holding and swelling of myofibrils (Bouton et al., 1973). However, small peeled and deveined shrimp can be over-treated by those compounds (Henson and Kowalewski, 1992). Over-treatment generally results in the formation of a translucent and slimy texture. Due to the strict regulation for the limit of the residual phosphate in fish (0.5% for EU and Japan) and frozen seafood (0.5% for EU and 0.2% for Japan) (Department of Fisheries, 2004), the processors have to search for other potential alternative, which have the ability in improving the quality and yield of seafood products.

To increase water holding capacity of meat or seafoods, phosphate compounds have been intensively used (Rattanasatheirn et al., 2008). Due to the strict regulation of using phosphates in seafoods, especially shrimp, other additives with the similar properties in increasing the yield have been paid increasing attention.Non-phosphate additives, particularly sodium bicarbonate, have been reported to be effective in improving the water-holding capacity, color, and organoleptic properties of fresh meats, beef, pork and poultry (Kauffman et al., 2000).Bicarbonate has been also used to minimize the problem of pale, soft and exudative pork (Wynveen et al., 2001) and to mask the typical aroma and flavor in sow meat (Sindelar et al., 2003). Furthermore, salts, especially sodium chloride, have been often usedto modify the ionic strength of muscle. Salt can slightly stabilize or destabilize the proteins, depending on the nature of the specific charge distribution within the protein (Record et al., 1998).NaCl at a level of 2.5% was used in combination with 0.875% sodium acid pyrophosphate (SAPP) and 2.625% tetrasodium pyrophosphate (TSPP) to increase the yield of Pacific white shrimp (Rattanasatheirn et al., 2008). Therefore, the appropriate alkaline treatment can be of an alternative treatment to increase the yield and lower the cooking loss of Pacific white shrimp (*Litopenaeus vannamei*), an economically important species of Thailand.

Reviews of Literature

Phosphates have been widely accepted as the potential additives in fish and seafood to improve the functional properties of those products by increasing water retention in fresh fish and reducing the thaw loss in frozen fish (Chang and Regenstein, 1997). Phosphate is also added to surimi as a cryoprotectant with its function as a metal chelator and/or antioxidant. In addition, because of the strength of phosphate in raising pH, the water holding/binding of the gel can be improved and salt solubilization of myofibrillar proteins can be increased (Trout and Schmidt, 1983). The effectiveness of phosphates on water retention properties of meat products depends on the type of phosphates and the amount used (Shults et al., 1972; Trout and Schmidt, 1984, 1986; Lewis et al., 1986). Trout and Schmidt (1986) showed that the effectiveness of phosphates on prevention of cook loss of meat products was in the following order: pyrophosphate > tripolyphosphate > tetrapolyphosphate > hexametaphosphate. Xiong et al. (2000)reported that pyrophosphate and tripolyphosphate were able to promote protein extraction, leading to the improved hydration properties of chicken muscle. Overall, phosphates influenced the ultrastructure of myofibrils and extraction of their constituents in the order: PP ~ TPP > HMP > P ~ nonphosphate control (Xiong et al., 2000). The effects of phosphates on increasing water retention of muscle were summarized by Hamm (1971), involving the increases in pH and ionic strength, the binding of phosphates to meat proteins, and the dissociation of actomyosin into actin and myosin. Phosphate is normally added to surimi in combination with cryoprotectants such as sugar or sorbitol (Sultanbawa and Li-Chan, 2001). The raising pH caused by this compound results in the improved

water holding/binding of the gel as well as better solubilization of myofibrillar proteins (Park, 2000). Pyrophosphate has been reported to dissociate protein complex, leading to the improved gel forming ability (Matsukawa et al., 1995). Trout and Schmidt (1987) concluded that at high ionic strengths (>0.25), pyrophosphate affected hydrophobic interactions which stabilize the protein structure, and thus, the thermal stability of the protein. Yagi et al. (1985) confirmed that inorganic polyphosphate offered a high degree of protection (to carp myofibrils) from thermal denaturation. Water retention is correlated with increased pH and normally associated with the use of alkaline polyphosphates such as sodium tripolyphosphate. Orthophosphates have virtually no effect on water-binding (Offer and Trinick, 1983). NaCl has been used in combination with phosphate in order to obtain the synergistic effect on quality improvement. Nevertheless, phosphates did not increase water holding capacity (WHC) or functional properties of muscle when the NaCl concentration was below 0.8% (Bendall 1954). Froning and Sackett (1985) reported that use of salt in combination with phosphates had synergistic effect on tumbling turkey breast muscle to reduce cooking loss and expressible moisture. Xiong and Kupski (1999) found that salt would produce a synergism with phosphate to dissociate actomyosin in chicken filets. Use of mixed phosphates led to quality improvement of both fresh shrimp and ice-stored shrimp by lowering cooking loss and increasing cooking yield and weight gain. Rattanasatheirn et al. (2008) reported that the use of SAPP in combination with TSPP or STPP could decrease the translucence to some extent. However, the greater translucence was found in shrimp with lower freshness after phosphate treatment, in which M-line was disappeared after heating.

Sodium bicarbonate is widely used as a marinade in Chinese cookery (Skurray et al., 1986), but it has been largelyoverlooked in the West, where acidic marinades have received much greater attention (Gault, 1991). Bicarbonate has been used to minimize the problem of pale, soft and exudative (PSE) pork (Wynveen et al., 2001) and to mask the typical aroma and flavor in sow meat (Sindelar et al.,2003). Bicarbonate was shown to reduce drip loss and shear force of pork (Wynveen et al., 2001), presumably because of the improved water holding capacity at elevated pH (Bouton et al., 1973). The use of bicarbonate or other mild alkaline compound should be as alternative to improve the yield and quality of shrimp, both raw and cooked, and to reduce the phosphate residue in treated shrimp.

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Objectives

To determine the effects of sodium carbonate and sodium bicarbonate at various pH on yield and some characteristics of Pacific white shrimp.

To investigate the changes in biochemical properties and microstructure of natural actomyosin of Pacific white shrimp as affected by selected alkaline treatment.

CHPATER 2

Effects of sodium carbonate and sodium bicarbonate on yield and characteristics of Pacific white shrimp (*Litopenaeus vannamei*)

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ABSTRACT

Effects of sodium carbonate (Na_2CO_3) and sodium bicarbonate ($NaHCO_3$) on yield and characteristics of Pacific white shrimp (*Litopenaeus vannamei*) were studied. Shrimp soaked in 2.5% NaCl containing both compounds at different pH (5.5, 7, 8.5, 10 and 11.5) showed an increase in the weight gain and cooking yield and a reduced cooking loss as pH of solutions increased ($p<0.05$). The coincidental increases in pH and salt content in soaked shrimp muscle were obtained with increasing pH ($p<0.05$). Higher pH of soaking solution partially solubilized proteins in the muscle as well as carotenoproteins. pH of solutions above 8.5 led to the pronounced leaching of pigments, associated with the lowered redness of cooked shrimp. Shear force of raw and cooked shrimp continuously decreased as pH of solution increased ($p<0.05$). Solution containing 2.5% NaCl and 2.0% $NaHCO_3$ (pH 8.5) was recommended for treatment of white shrimp as a promising alternative for phosphates to increase the yield and to lower cooking loss without any negative effect on sensory property.

Keywords: Pacific white shrimp, sodium carbonate, sodium bicarbonate, yield, carotenoprotein

INTRODUCTION

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minimize the problem of pale, soft and exudative (PSE) pork (Wynveen et al., 2001) and to mask the typical aroma and flavor in sow meat (Sindelar et al., 2003). Bicarbonate was shown to reduce drip loss and shear force of pork (Wynveen et al., 2001), presumably because of the improved water holding capacity at elevated pH (Bouton et al., 1973). The use of bicarbonate or other mild alkaline compound should be as alternative to improve the yield and quality of shrimp, both raw and cooked, and to reduce the phosphate residue in treated shrimp. As a consequence, the exporting problem associated with the strict regulation of using phosphate can be alleviated. Nevertheless the little information regarding the use of bicarbonate as well as carbonate in shrimp exists. The mechanisms of those compounds in the improvement of yield and quality should be elucidated in order to have an effective application.

Thus, the objective of this investigation was to determine the effects of sodium carbonate and sodium bicarbonate at various pH on yield and some characteristics of Pacific white shrimp.

MATERIAL AND METHODS

Chemicals

Sodium carbonate (Na_2CO_3) and sodium bicarbonate ($NaHCO_3$) were obtained from Asahi chemical industry company Ltd. (Tokyo, Japan). Sodium chloride and Tris (hydroxymethyl) aminomethane were purchased from Fisher Scientific (Fair Lawn, NJ, U.S.A.). Coomassie Brilliant Blue R-250 and bovine serum albumin (BSA) were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). β-mercaptoethanol (βME), acrylamide, *N, N, N′ N′*-tetramethyl ethylene diamine (TEMED) and bis-acrylamide were purchased from Fluka (Buchs, Switzerland).

Sample preparation

Pacific white shrimp (*Litopenaeus vannamei*) with the size of 50 shrimps per kg were obtained from a farm in Songkhla province, Thailand. The shrimp were placed in ice with an ice/shrimp ratio of 2:1 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai, Songkhla within approximately 1 h. Upon the arrival, shrimp were washed with clean water and beheaded. The shell was then peeled off and the shrimp were subjected to manual deveining.

Effects of sodium bicarbonate and sodium carbonate at various pH on yield and characteristics of Pacific white shrimp

The prepared shrimp were soaked in 2.5% NaCl solution containing 2.0% sodium carbonate or 2.0% sodium bicarbonate, adjusted to various pH (5.5, 7, 8.5, 10 and 11.5) using either 6 mol equi/L NaOH or 6 mol equi/L HCl, for 4 h at 4^{o}C. Subsequently, the treated samples were drained for 5 min on the plastic screen at 4^{o}C. Both soaking solutions and resulting shrimp were subjected to analyses. All analyses were conducted in triplicate.

Determination of weight gain, cooking loss and cooking yield

Weight gain was determined by weighing the shrimps before and after soaking in the solutions. After soaking, the samples were drained on plastic screen for 5 min at 4°C. Weight gain was calculated as follows (Rattanasatheirn et al., 2008):

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

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

Cooking loss and cooking yield were measured by weighing the shrimps before and after cooking. Shrimp were cooked in boiling water for 2.5 min, immediately cooled in iced water for 2 min and drained for 5 min at 4°C. Cooking loss and cooking yield were calculated as follows:

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

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

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

Determination of salt content

Salt content was determined as per the method of AOAC (2000). Sample (1 g) was added with 10 mL of 0.1 mol equi/L $AgNO_3$ and 10 mL of conc. HNO_3. The mixture was boiled gently on a hot plate until all samples except $AgCl_2$ were dissolved. The mixture was then cooled using running water. Then 50 mL of distilled water and 5 mL of 50 g/L ferric alum ($FeNH_4(SO_4)_2.12H_2O$) indicator were added. The mixture was filtered with Whatman No. 1 filter paper (Whatman International Ltd, Maidstone, U.K.) and adjusted to 100 mL with distilled water. The mixture was titrated with standardized 0.1 mol equi/L KSCN until the solution became permanent brownish red. The salt content was then calculated as follows:

$$\text{Salt (\%)} = 5.8 \times [(V_1 \times N_1) - (V_2 \times N_2)]/W$$

where: V_1 = volume of $AgNO_3$ (mL); N_1 = concentration of $AgNO_3$ (mol equi/L);

V_2 = volume of KSCN (mL); N_2 = concentration of KSCN (mol equi/L);

W = weight of sample (g).

Determination of pH of shrimp meat and soaking solution

The pH of the shrimp meat was determined as described by Benjakul et al. (1997). The samples were added with distilled water at a ratio of 1 : 5 (w/v) and the mixture was homogenized at a speed of 10,000 rpm for 2 min using a Polytron (PT-MR 3,000, Littau, Switzerland). The homogenates were subjected to pH measurement using a combined glass electrode pH meter (Sartorious model PB-20, Goettingen, Germany). Soaking solution was directly determined using a pH meter.

Measurement of total carotenoid content and released protein content of soaking solutions

Total carotenoid content was determined according to the method of Simpson and Haard (1985) with a slight modification. The soaking solution (5 mL) was homogenized in 25 mL of cold acetone (-20^{o}C) using a homogenizer at a speed of 20,000 rpm for 2 min. The homogenate was filtered through a Whatman No. 1 filter paper under vacuum. The filtrate was placed in a separatory funnel and was partitioned with 25 mL of petroleum ether. The separatory funnel and contents were shaken gently and were left to stand at room temperature (25^{o}C) for 10 min. The lower layer was drawn off. The top layer was washed twice with 25 mL of distilled water. The petroleum ether layer obtained was dried by occasional shaking with 15 g of anhydrous sodium sulfate for 30 min. The dried material was filtered through a coarse sintered glass funnel. The residual sodium sulfate was then washed with small volumes of petroleum ether for several times to remove all pigments. The washings were pooled with the filtrate and then evaporated under vacuum at 50^{o}C using a rotary evaporator (Rotavapor-R, Brinkmann, Switzerland). The residue was dissolved in petroleum ether and made up to a final volume of 10 mL. The absorbance of the

extract, appropriately diluted, was measured at 468 nm. The concentration (C) of carotenoid in the extract was calculated using the equation given by Saito and Regier (1971):

$$C\ (\mu g/g\ sample) = \frac{A_{468} \times vol\ of\ extract \times dilution}{0.2 \times weight\ of\ sample\ used\ in\ grams}$$

where: 0.2 is the A_{468} of 1 µg/mL standard canthaxanthin.

Released protein concentration in soaking solution was determined by the Biuret method (Robinson and Hogden, 1940) using bovine serum albumin as standard. Prior to analysis, soaking solution were centrifuged at 3500 × g for 20 min using a refrigerated centrifuge (Avanti® J-E, Beckman Coulter, Inc., Palo Alto, CA, U.S.A) to remove undissolved debris. The supernatant was determined for protein content.

Determination of protein patterns

Protein patterns of soaking solutions were determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using 10% running gel and 4% stacking gel as described by Laemmli (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 samples were centrifuged at 3500 × g for 20 min to remove undissolved debris. Protein concentration was determined by the Biuret method (Robinson and Hogden, 1940). Sample (10 µg protein) was loaded onto the gel. Separation was performed by electrophoresis apparatus (Mini-Protein II, Bio-Rad, U.S.A.) using 50V and 150V. Proteins were 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% glacial acetic acid) for 3 h. Wide range molecular weight standards were used.

Determination of shear force

Shear force of soaked shrimp, raw and cooked, 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 in 6 replicates for each sample. The peak of the shear force profile was regarded as the shear force value.

Sensory analysis

Raw and cooked shrimp samples with different treatments were evaluated by 30 panelists, who were the graduate students in Food Science and Technology program and were acquainted with shrimp consumption. The samples were served on a white paper plate at room temperature. All samples were coded with three digit random numbers and presented at the same time in randomized order. The panelists were asked to assess samples for color, opaqueness, slime, texture, taste, and overall liking using a 9-point hedonic scale (Mailgaad et al., 1999), in which: 1: dislike extremely; 2: dislike very much; 3: dislike moderately; 4: dislike slightly; 5: neither like nor dislike; 6: like slightly; 7: like moderately; 8: like very much; 9: like extremely. Panelists were instructed to rinse their mouths with water before starting and between sample evaluations. Evaluations were made in individual sensory

evaluation booths under fluorescent white light. The numerical scores of each sensory attribute were collected for statistical analysis.

Statistical analysis

Experiments were run in triplicate. Data were subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan's multiple range test (DMRT) (Steel and Torrie, 1980). Statistical analyses were performed using the Statistical Package for Social Science (SPSS 11.0 for windows, SPSS Inc., Chicago, IL, U.S.A.).

RESULTS AND DISCUSSION

Effects of sodium bicarbonate and sodium carbonate at various pH on weight gain, cooking yield and cooking loss of Pacific white shrimp

Weight gain, cooking yield and cooking loss of Pacific white shrimp soaked in 2.5% NaCl containing 2.0% sodium bicarbonate or 2.0% sodium carbonate with various pH are shown in Figure 1. Weight gain and cooking yield increased when pH increased, regardless of types of solutions ($p<0.05$). At the same pH used, the higher weight gain was observed in shrimp treated with sodium carbonate, compared with those treated with sodium bicarbonate ($p<0.05$), except at pH 11.5, where no difference was found ($p>0.05$). At a pH above pI or very alkaline pH, proteins have a negative charge, in which protein molecules repulse each other, resulting in the swollen muscle structure (Zayas, 1997). As a consequence, water could be more uptaken. Increases in weight gain corresponded to the higher moisture content (data not shown), indicating a higher mobility of water into the muscle. At the pH lower than 11.5, some differences in weight gain of shrimp treated with both chemicals

might be caused by the differences in ionic strength of the solution (sodium bicarbonate: 0.666 mol/L; sodium carbornate: 0.995 mol/L). The increasing ionic strength generally weakened the structural integrity of myofibrils to a greater extent (Wu and Smith, 1987). Additionally, CO_3^{2-}, a divalent ion, could provide the net negative charge for those proteins. For HCO_3^-, it mostly neutralized the positive charge of NH_3^+. Therefore, the former resulted in the higher repulsion between negatively charged protein molecules.

Cooking loss and cooking yield of shrimp treated with 2.5% NaCl containing 2.0% sodium carbonate or 2.0% sodium bicarbonate at different pH are depicted in Figure 1B and 1C, respectively. Lower cooking loss with the concomitant increased cooking yield was found in shrimp treated with both solutions having the higher pH (p<0.05). The highest cooking loss was found in shrimp treated with both chemicals at pH 5.5, whereas the lowest cooking yield was obtained at the same pH used (p<0.05). At pH 5.5 which was close to pI of muscle proteins, the proteins had the net charge of zero and the loss in water holding capacity of proteins was enhanced. As a result, the lowest cooking yield was noticeable at this pH. During the soaking process, the carbonate ion could penetrate into the muscle and water molecules might be bound tightly with those ion or proteins via ionic interaction. It was reported that alkaline pH of soaking solution could increase the solubilization of muscle proteins via the dissociation of actomyosin complex into actin and myosin (Wu and Smith, 1987). Generally water can be entrapped within the protein structures held by capillary forces (Aaslyng et al., 2003). Due to the increased water uptake of samples soaked in both solutions at high pH, the water was more retained after heating. No differences in both cooking yield and cooking loss were obtained between shrimp soaked in both solutions when pH was higher than 7.0 (p<0.05). Bicarbonate was reported to reduce

drip loss and shear force (Wynveen et al., 2001), presumably because of improved water holding at elevated pH (Bouton et al., 1973). Kauffman et al. (1998) found that the injection of sodium bicarbonate containing sodium chloride to the post-mortem pork resulted in higher ultimate pH, improved color and reduced drip loss. The improvement of water holding capacity, color and sensory properties of beef, pork and poultry by the injection of sodium bicarbonate solution into the carcass was also reported (Kauffman et al., 2000). pH of brine containing sodium carbonate or sodium bicarbonate had the influence on weight gain, cooking yield and cooking loss of Pacific white shrimp.

Effects of sodium bicarbonate and sodium carbonate at various pH on salt content of Pacific white shrimp

Salt content of both raw and cooked shrimp soaked in 2.5% NaCl containing 2.0% sodium carbonate or 2.0% sodium bicarbonate with different pH is shown in Figure 2. The increase in salt content was noticeable in shrimp as the pH of solutions increased, regardless of types of solutions ($p<0.05$). It was noted that the muscle of unsoaked shrimps contained NaCl at levels of 1.13 $\pm$ 0.05% and 0.80 $\pm$ 0.04% (dry basis) for raw and cooked sample, respectively. At pH 8.5, 10 and 11.5, the higher salt content was found in shrimp soaked in brine containing sodium carbonate, in comparison with that found in those soaked in brine comprising sodium bicarbonate ($p<0.05$). Generally the increased chloride uptake by the muscle was reported when the brine with alkaline-pH was used (Stefansson and Hultin, 1994). As salt is taken up, and the ionic strength increases. As anions bind to the filaments, this raises the negative charge and the repellent forces, thus increasing the space between filaments. This could induce the higher penetration of salt (Offer and Trinick, 1983). Increased

salt content of raw shrimp after soaking in both brines related with the increasing weight gain (Figure 1A) and moisture content (data not shown).

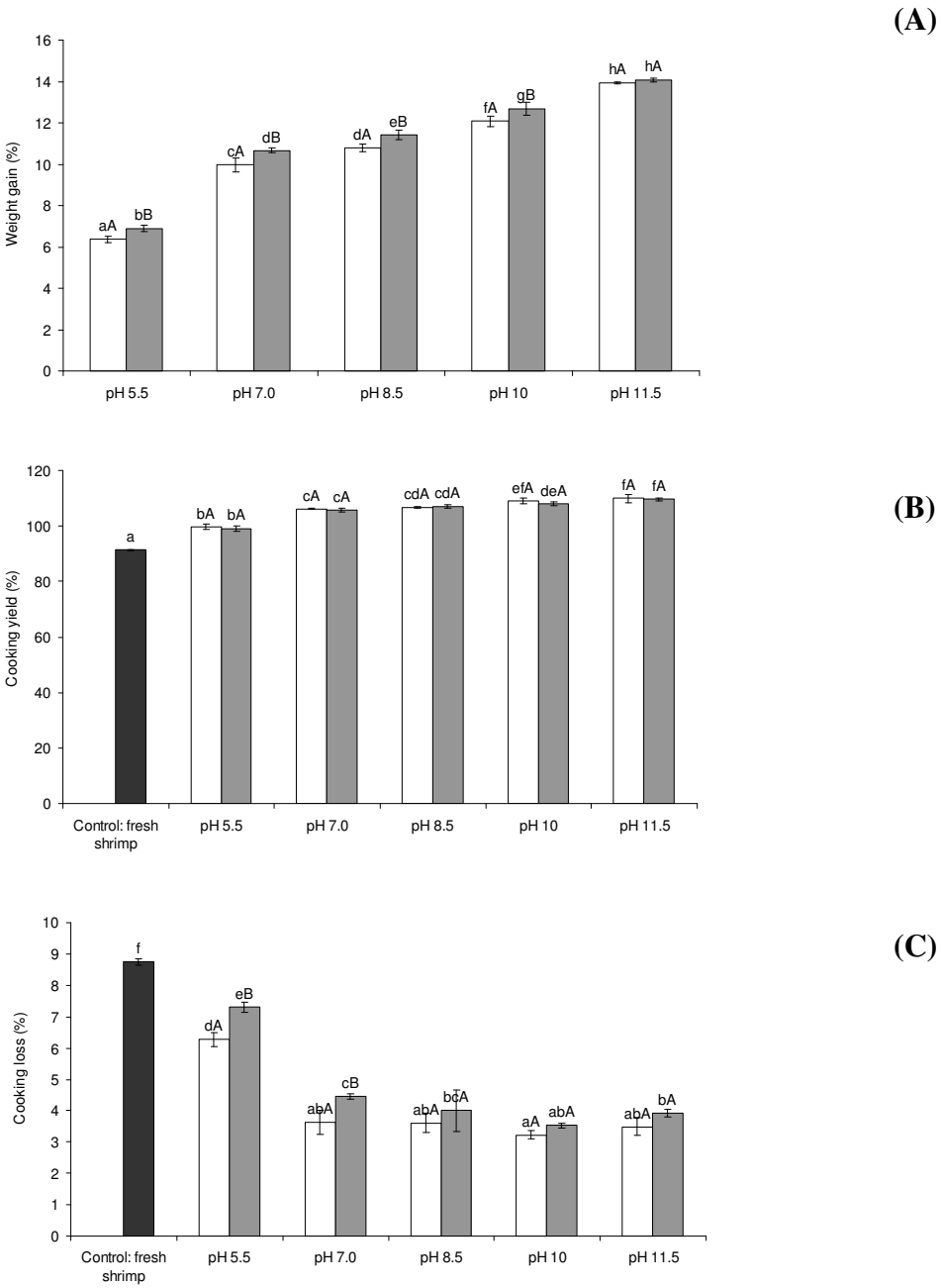

Figure 1 Weight gain (A), cooking yield (B) and cooking loss (C) of Pacific white shrimp after soaking in solutions containing 2.5% NaCl and 2.0% $NaHCO_3$ or 2.0% Na_2CO_3 with different pH. Bars represent the standard deviation (n =3). The different letters within the same solution indicated significant difference (p<0.05).The different capital letters within the same pH indicated significant difference (p<0.05).

□ : 2.5% NaCl + 2.0% $NaHCO_3$, ▭ : 2.5% NaCl + 2.0% Na_2CO_3

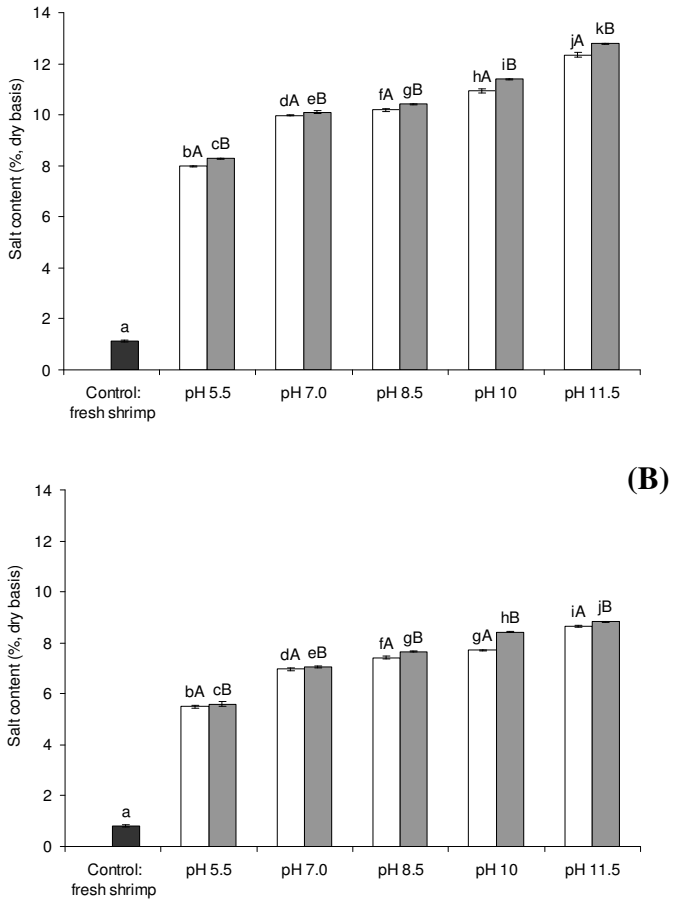

Figure 2 Salt content of raw (A) and cooked (B) Pacific white shrimp after soaking in solutions containing 2.5% NaCl and 2.0% $NaHCO_3$ or 2.0% Na_2CO_3 with different pH. Bars represent the standard deviation (n =3). The different letters within the same solution indicated significant difference (p<0.05).The different capital letters within the same pH indicated significant difference (p<0.05).

□ : 2.5% NaCl + 2.0% $NaHCO_3$, ▭ : 2.5% NaCl + 2.0% Na_2CO_3

Effects of sodium bicarbonate and sodium carbonate at various pH on the released protein and carotenoid content of soaking solutions.

As shown in Table 1, the protein release was more pronounced in soaking solution with the higher pH (p<0.05). At the same pH, the higher content of protein released was obtained in brine containing sodium carbonate (p<0.05). The higher protein solubilization in brine containing sodium carbonate was coincidental with the

high NaCl content in shrimp meat (Figure 2). The increase in NaCl in the muscle might enhance the solubilization of myofibrillar proteins in shrimp meat. The increase in protein solubilization at very alkaline pH was reported by Stefansson and Hultin (1994). Also, the chlorides clearly had a major effect on muscle protein solubility and on water holding properties (Fennema, 1977). Protein solubility refers to the amount of total muscle protein that goes into solution under specified conditions (Zayas, 1997) and depends on protein structure, pH, concentration of salt, temperature, duration of extraction and many other intrinsic factors (Mohan et al., 2007). High ionic strength has been shown to decrease actin-myosin interactions in the muscle (Brenner et al., 1984). The differences in pH can markedly alter solubility of sarcoplasmic and myofibrillar proteins (Stefansson and Hultin, 1994). Wu and Smith (1987) reported that the solubilzation of myofibrillar protein was lower at pH 5.5 than at pH 7.0. However, the solubility in the isoelectric region might be altered by salt that causes salting-in and salting-out effects (Skaara and Regenstein, 1990).

Total carotenoid content of soaking solution was increased when pH of solutions containing sodium carbonate or sodium bicarbonate increased (Table 1) (p<0.05). At the same pH, brine containing sodium carbonate had more influence in leaching free carotenoids and carotenoprotein from shrimp meat, thus leading to the fading in color of cooked sample. The higher release of carotenoids was in accordance with the higher solubilization of protein as evidenced by the increased amount of proteins released in soaking solution (Table 1). Astaxanthin and its esters have been found to be the major carotenoids in marine crustaceans (Yanar et al., 2004). The total amount of proteins extracted with 1 mol/L NaCl solution was found to correlate well with the amount of extracted carotenoid. Birkeland et al. (2004) reported that salting affected the surface discoloration of salmon smoking product. The salt concentration

had the influence on the leaching of protein-carotenoid complex from salmon (Birkeland et al., 2004).

Table 1 Released protein and total carotenoid contents of soaking solutions with different pH after soaking with Pacific white shrimp.

Treatments	pH	Released protein content (mg/mL)*	Total carotenoid content (μg/mL)
2.5% NaCl + 2.0%$NaHCO_3$	5.5	$3.90 \pm 0.23^{b**}$	0.19 ± 0.01^{a}
	7	3.66 ± 0.27^{ab}	0.22 ± 0.01^{a}
	8.5	3.58 ± 0.06^{a}	0.30 ± 0.03^{b}
	10	4.94 ± 0.07^{d}	0.42 ± 0.01^{c}
	11.5	5.48 ± 0.10^{e}	0.47 ± 0.02^{c}
2.5% NaCl + 2.0% Na_2CO_3	5.5	4.25 ± 0.19^{c}	0.95 ± 0.03^{d}
	7	4.24 ± 0.08^{c}	1.14 ± 0.01^{e}
	8.5	4.23 ± 0.06^{c}	1.28 ± 0.05^{f}
	10	4.96 ± 0.23^{d}	1.50 ± 0.05^{g}
	11.5	5.98 ± 0.10^{f}	1.56 ± 0.01^{g}

*Mean $\pm$ SD (n = 3)

**Different subscripts in the same column indicate the significant differences (p<0.05).

Effects of sodium bicarbonate and sodium carbonate at various pH on protein patterns of soaking solutions

Figure 3 shows the electrophoretic profiles of the proteins released from shrimp into the soaking solution, brines containing sodium bicarbonate (Figure 3A) and sodium carbonate (Figure 3B), after 4 h of soaking. The increases in band intensity of myosin heavy chains (MHC) were noticeable as pH of both solutions increased, which was concomitant with the increased leaching of proteins into soaking solutions (Table 1). During salting, instability of MHC might be occurred via the

aggregation (Morrissey et al., 1987) and degradation (Thorarinsdottir et al., 2002) of proteins. Band intensity of MHC in soaking solution was more pronounced at pH higher than 8.5. Coincidentally, actin band was detectable in soaking solution with pH greater than 8.5. Proteins with molecular weight of 85.19, 81.02, 52.17 and 34.16 kDa were found in all solutions, regardless of pH. Those proteins were leached out from the muscle, resulting in some loss in muscle proteins.

Effects of sodium bicarbonate and sodium carbonate at various pH on pH and shear force of Pacific white shrimp.

pH of raw and cooked shrimp soaked in different solutions, compared with control (fresh shrimp) is depicted in Figure 4A and 4B, respectively. The pH of shrimp meat increased as pH of soaking solutions increased (p<0.05). After soaking, alkaline solutions were penetrated into the shrimp meat. The pH changes of shrimp meat more likely determined the changes in muscle, particularly the modification of charge as well as conformation of proteins. At the same pH of soaking solutions, shrimp meat soaked in brine containing sodium bicarbonate had the slightly higher pH, compared to those soaked in brine containing sodium carbonate. The difference in pH of shrimp muscle might be explained by the different buffering capacity of muscle proteins toward different alkaline compounds.

Shear forces of raw and cooked shrimp after being soaked in brine containing sodium carbonate or sodium bicarbonate are shown in Figure 4. Generally, shear force of raw and cooked shrimp decreased when pH of soaking solutions used increased (p<0.05). At the same pH of soaking solution, the higher shear force was noticeable with shrimp soaked in brine containing sodium bicarbonate (p<0.05). CO_3^{2-} and HCO_3^- might have the impact on the proteins in muscle differently.

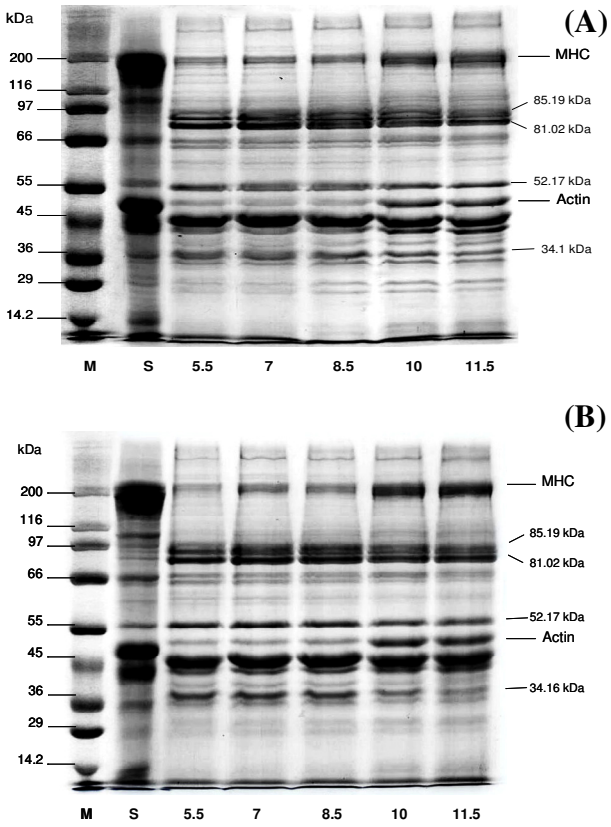

Figure 3 Protein patterns of soaking solutions with different pH. Solution containing 2.5% NaCl and 2.0% $NaHCO_3$ (A) and 2.0% Na_2CO_3 (B) with different pH were used. M: Standard marker; S: shrimp muscle; MHC: myosin heavy chain. Numbers denote pH of soaking solutions.

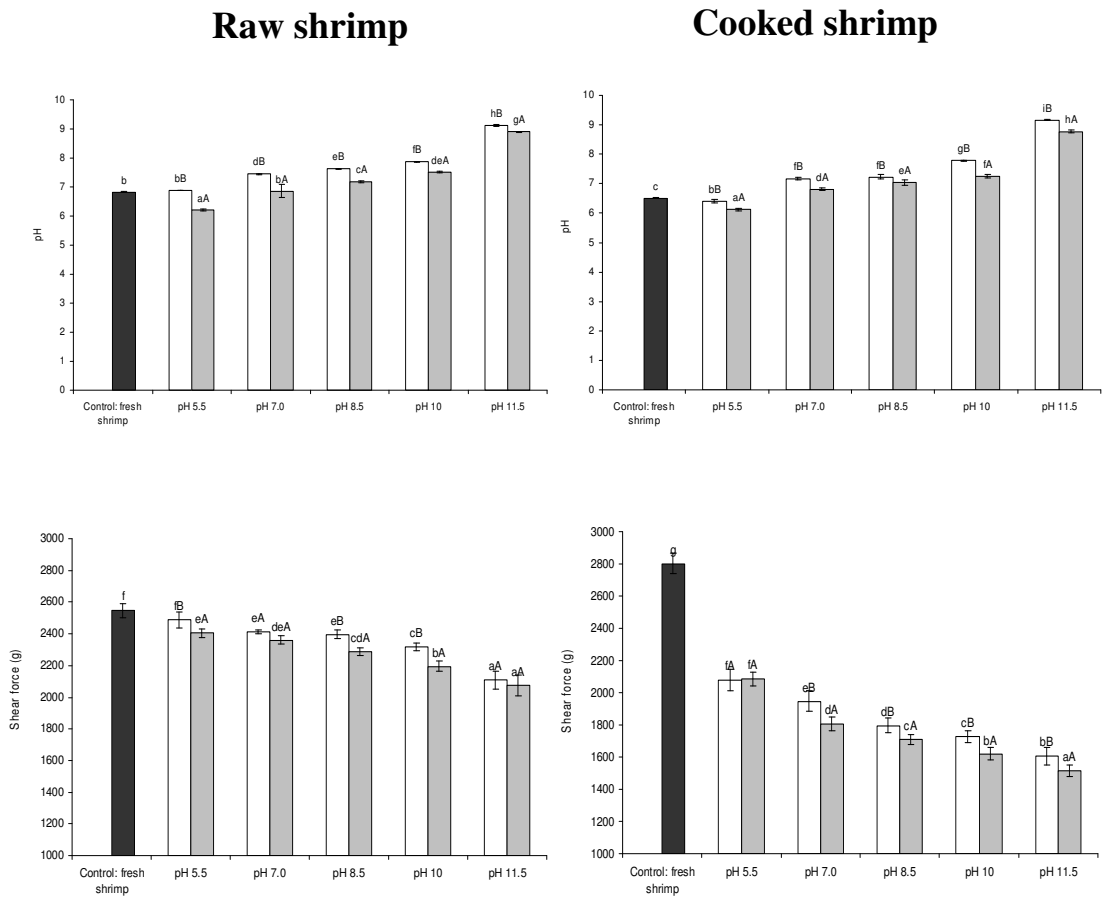

Figure 4 pH and shear force of raw and cooked Pacific white shrimp after soaking in solutions containing 2.5% NaCl and 2.0% $NaHCO_3$ or 2.0% Na_2CO_3 with different pH. Bars represent the standard deviation (pH: n = 3; shear force: n = 6). The different letters within the same solution indicated significant difference (p<0.05). The different capital letters within the same pH indicated significant difference (p<0.05). □ : 2.5% NaCl + 2.0% $NaHCO_3$, ▒ : 2.5% NaCl + 2.0% Na_2CO_3

Under the alkaline condition, the repulsion between protein molecules was more pronounced, resulting in the loosen structure, which became less resistant to shear force applied.

For cooked shrimp soaked in all soaking solutions, shear force was lower than that found in raw shrimp (p<0.05). During cooking, muscle proteins underwent denaturation and some weak bonds might be disrupted. Additionally, collagen in the

connective tissue of shrimp muscle might be solubilized by heat treatment, leading to the softening of meat. For shrimp soaked in solution with very alkaline pH, some partial hydrolysis might take place. The cleavage of peptides could lower the firmness of shrimp meat. Therefore, soaking of shrimp in both solutions had the effect on texture of treated shrimp, mostly governed by the pH of soaking solutions. Moeller et al. (2010) reported that a decreasing shear force of pork meat was obtained as the pH increased.

Effects of sodium bicarbonate and sodium carbonate at various pH on color of Pacific white shrimp

The color (L*, a*, and b* - value) of raw and cooked shrimp soaked in 2.5% NaCl containing 2.0% sodium carbonate or 2.0% sodium bicarbonate with various pH is shown in Table 2. For raw shrimp, L*-value increased when pH increased (p<0.05). At the same pH of soaking solution, the sample soaked in brine containing sodium carbonate had the higher L*-value than those soaked in brine having sodium bicarbonate (p<0.05). The a*-value generally increased as pH increased and the marked increases were obtained at pH 10 and 11.5. Shrimp turned to be reddish when soaked in the solutions with pH of 10 or 11.5. At very alkaline pH, carotenoprotein could be solubilized as indicated by the increase in carotenoid content in the soaking solution (Table 1). Simultaneously, an extremely alkaline pH could induce the denaturation of carotenoproteins, leading to the appearance of red color caused by free carotenoids, especially astaxanthin. Astaxanthin has been reported as the dominant carotenoid in shrimp (Yanar et al., 2004). The b*-values decreased as pH increased up to pH 8.5. With increasing pH (10 and 11.5), b*-value decreased (p<0.05).

Table 2 Color of raw and cooked Pacific white shrimp soaked in 2.5% NaCl containing 2.0% $NaHCO_3$ or 2.0% Na_2CO_3 with different pH

State of sample	Treatment	pH	Color*		
			L*	**a***	**b***
Raw	Control		41.85 ± 1.14^{ef}**	0.21 ± 0.04^{a}	-2.35 ± 0.07^{cd}
	2.5% NaCl + 2.0% $NaHCO_3$	5.5	40.25 ± 1.00^{a}	0.18 ± 0.03^{a}	-1.19 ± 0.07^{g}
		7	40.36 ± 0.81^{ab}	0.19 ± 0.04^{a}	-2.39 ± 0.16^{c}
		8.5	40.88 ± 0.57^{abc}	0.22 ± 0.67^{a}	-3.36 ± 0.32^{a}
		10	41.16 ± 0.57^{cde}	2.12 ± 0.21^{c}	-2.71 ± 0.44^{b}
		11.5	41.26 ± 0.44^{cde}	3.64 ± 0.45^{f}	-1.93 ± 0.32^{e}
	2.5% NaCl + 2.0% Na_2CO_3	5.5	40.25 ± 1.44^{a}	0.18 ± 0.04^{a}	-1.19 ± 0.07^{g}
		7	41.03 ± 0.69^{bcd}	0.22 ± 0.03^{a}	-1.93 ± 0.17^{e}
		8.5	41.69 ± 1.59^{def}	0.46 ± 0.09^{b}	-2.21 ± 0.20^{d}
		10	42.37 ± 1.03^{f}	2.52 ± 0.28^{d}	-1.73 ± 0.21^{f}
		11.5	43.34 ± 1.47^{g}	3.27 ± 0.74^{e}	-1.75 ± 0.16^{f}
Cooked	Control		70.80 ± 1.34^{i}	15.44 ± 1.12^{i}	15.71 ± 1.08^{h}
	2.5% NaCl + 2.0% $NaHCO_3$	5.5	67.74 ± 1.75^{g}	14.80 ± 0.36^{h}	14.53 ± 0.43^{g}
		7	64.44 ± 1.50^{de}	11.03 ± 0.38^{f}	11.00 ± 0.47^{de}
		8.5	63.05 ± 1.19^{c}	10.59 ± 0.42^{e}	10.77 ± 0.54^{d}
		10	61.46 ± 1.64^{b}	7.94 ± 0.38^{c}	8.72 ± 0.33^{a}
		11.5	57.98 ± 1.30^{a}	6.93 ± 0.31^{a}	8.73 ± 0.41^{a}
	2.5% NaCl + 2.0% Na_2CO_3	5.5	69.00 ± 1.29^{h}	13.30 ± 0.46^{g}	12.96 ± 0.35^{f}
		7	65.89 ± 1.59^{f}	11.28 ± 0.61^{f}	11.25 ± 0.41^{e}
		8.5	65.17 ± 0.97^{ef}	10.12 ± 0.65^{d}	10.31 ± 0.45^{c}
		10	63.55 ± 1.07^{cd}	7.87 ± 0.50^{c}	8.80 ± 0.24^{ab}
		11.5	61.78 ± 1.74^{b}	7.38 ± 0.35^{b}	9.09 ± 0.30^{a}

*Mean ± SD (n = 30)

**Different subscripts in the same column under the same state of sample indicate the significant differences (p<0.05).

For cooked shrimp, L*, a*, and b* - values were much higher than those of raw shrimp. Higher L*-value representing lightness of cooked shrimp was mainly associated with the heat denaturation of muscle protein. L*-value decreased gradually as pH of solutions increased ($p<0.05$). After cooking, a*- and b*- values of cooked shrimp decreased when pH of solution increased ($p<0.05$). This was due to the decrease of the carotenoprotein content in shrimp soaked in solutions with higher pHs, as indicated by the increased carotenoid content in soaking solutions. Astaxanthin being a pigment commonly found in crustacean provides the tissue with red-orange pigmentation (Okada et al., 1994). Although the weight gain and cooking yield of shrimp treated with brine containing sodium carbonate or sodium bicarbonate at pH 10 or 11.5 markedly increased, those treatments caused the decrease in redness of cooked shrimp. Therefore, soaking solution with pH of 8.5, which was close to the original pH of sodium bicarbonate (8.4-8.5), was selected for soaking shrimp.

Effects of sodium bicarbonate and sodium carbonate at various pH on sensory properties of Pacific white shrimp

The likeness scores of raw and cooked shrimp soaked in brine containing sodium carbonate or sodium bicarbonate at different pHs are shown in Table 3. For raw shrimp, no differences in color likeness were found between the control (fresh shrimp) and those soaked in brine containing 2.0% sodium bicarbonate, pH 8.5 ($p>0.05$). Nevertheless, lower color likeness was found in sample soaked in brine containing 2.0% sodium carbonate ($p<0.05$). The lower likeness of surface compactness was found in the samples soaked in both solutions ($p<0.05$). This was due to the slimy surface associated with the excessive solubilization of proteins at

alkaline pH. No differences in opaqueness, texture and overall likeness were observed among all samples (p>0.05).

Table 3 Sensory scores of raw and cooked Pacific white shrimp soaked in 2.5% NaCl containing 2.0% $NaHCO_3$ or 2.0% Na_2CO_3 (pH 8.5)

State of sample	Attributes	Control*	2.5% NaCl + 2.0% $NaHCO_3$; pH: 8.5	2.5% NaCl + 2.0%Na_2CO_3; pH: 8.5
Raw				
	Color	$8.00 \pm 0.91^{b**}$	7.23 ± 0.68^{b}	6.80 ± 0.71^{a}
	Opaqueness	7.50 ± 1.22^{a}	7.27 ± 1.08^{a}	7.17 ± 0.99^{a}
	Surface compactness	7.43 ± 0.73^{b}	6.80 ± 0.71^{a}	6.67 ± 0.84^{a}
	Texture	7.50 ± 1.25^{a}	7.27 ± 0.98^{a}	7.17 ± 1.23^{a}
	Overall	7.73 ± 0.94^{b}	7.47 ± 0.86^{b}	7.33 ± 0.80^{b}
Cooked				
	Color	7.37 ± 0.93^{a}	6.87 ± 1.11^{a}	6.87 ± 1.04^{a}
	Opaqueness	7.23 ± 0.50^{b}	6.73 ± 0.58^{a}	6.73 ± 1.11^{a}
	Texture	7.43 ± 1.14^{b}	7.17 ± 1.21^{ab}	6.77 ± 0.94^{a}
	Taste	7.10 ± 0.80^{a}	7.37 ± 0.85^{a}	7.00 ± 0.69^{a}
	Overall	7.13 ± 1.20^{a}	7.10 ± 1.16^{a}	6.93 ± 0.94^{a}

*Mean $\pm$ SD (n = 30)

**Different subscripts in the same row indicate the significant differences (p<0.05).

Linking score = 1: dislike extremely; 5: neither like nor dislike and 9: like extremely.

Similar results were found in cooked sample. Cooked shrimp with the treatments had the lower likeness in opaqueness (p<0.05), suggesting the higher translucence of treated samples. It was noted that the sample soaked in brine containing 2.0% sodium carbonate (pH 8.5) had the lower likeness score fore texture, compared to the control and those soaked in brine containing sodium bicarbonate (p<0.05). The disturbance of muscle structure induced by the repulsion of muscle fiber in shrimp treated with sodium carbonate might cause the lowering of firmness, which was associated with the mushy or too soft texture of cooked shrimp.

CONCLUSIONS

To improve the water retention and lower the cooking loss of white shrimp, soaking the shrimp in 2.5% NaCl containing 2.0% sodium bicarbonate (pH 8.5) could be an effective means with no negative effect on sensory property for both raw and cooked sample. The action of sodium bicarbonate in improving the quality was governed by pH, which caused partial solubilization and disturbance of the muscle structure. Therefore, sodium bicarbonate could be used as the alternative additive to phosphate compounds, which have become more strict for application in shrimp and other seafoods.

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CHAPTER 3

The effects of sodium bicarbonate on conformational changes of natural actomyosin from Pacific white shrimp (*Litopenaeus vannamei*)

Food Chemistry

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ABSTRACT

Changes in natural actomyosin (NAM) from Pacific white shrimp (*Litopenaeus vannamei*) treated with sodium bicarbonate ($NaHCO_3$) at different concentrations (0-1 M) in the absence or the presence of 2.5% NaCl were studied. Turbidity of NAM solutions decreased with coincidental increase in solubility was observed as the concentration of $NaHCO_3$ increased. Surface hydrophobicity (S_oANS) and total sulfhydryl content of NAM also increased when $NaHCO_3$ concentration increased. Greater decreases in Ca^{2+}- and Mg^{2+}-ATPase activity were found in all NAM as $NaHCO_3$ concentration increased, suggesting the denaturation of myosin head and the dissociation of actomyosin complex. The zeta potential (ζ) analysis suggested that the surface of NAM became more negatively charged (-12.12 to -26.98) as $NaHCO_3$ concentration increased. Those changes were more intense in the presence of 2.5% NaCl. Transmission electron microscopy showed that the structure of actomyosin was more dissociated and lost the filamental structure when $NaHCO_3$ at higher levels was used.

Keywords: Physicochemical changes; natural actomyosin; sodium bicarbonate; Pacific white shrimp; muscle protein

INTRODUCTION

Functional properties of muscle protein are closely associated with the integrity of proteins. Denaturation and degradation of fish muscle proteins mainly contribute to the loss of those functionalities (Montecchia et al., 1997). Protein-protein interactions termed association, aggregation and polymerization, are dependent upon many factors such as temperature, pH, etc (Zayas, 1997). Since protein-protein interactions lead to changes in the secondary and tertiary structures of the protein molecules, these changes could affect fat and water-binding affinities of these molecules (He et al., 2010). The ability of muscle to absorb the added water during processing and capacity of retaining the water after cooking and freezing are the important factors governing the quality of seafood and seafood products (Ogawa et al., 1994). Textural changes of meat or seafoods are due to protein denaturation and aggregation and are associated with water holding capacity (WHC) (Xiong et al., 2007)

To increase water holding capacity of meat or seafoods, phosphate compounds have been intensively used (Rattanasatheirn et al., 2008). Due to the strict regulation of using phosphates in seafoods, especially shrimp, other additives with the similar properties in increasing the yield have been paid increasing attention.

Non-phosphate additives, particularly sodium bicarbonate, have been reported to be effective in improving the water-holding capacity, colour, and organoleptic properties of fresh meats, beef, pork and poultry (Kauffman et al., 2000). Bicarbonate has been also used to minimise the problem of pale, soft and exudative pork (Wynveen et al., 2001) and to mask the typical aroma and flavour in sow meat (Sindelar et al., 2003). Furthermore, salts, especially sodium chloride, have been often used to modify the ionic strength of muscle. Salt can slightly stabilise or destabilise the proteins, depending on the nature of the specific charge distribution within the protein (Record et al., 1998). NaCl at a level of 2.5% was used in combination with 0.875% sodium acid pyrophosphate (SAPP) and 2.625% tetrasodium pyrophosphate (TSPP) to

increase the yield of Pacific white shrimp (Rattanasatheirn et al., 2008). Recently, Chantarasuwan et al. (2011) reported the increase in water uptake by 11.7% when white shrimp were soaked in 2.0% $NaHCO_3$ for 4 h. However, no information regarding the role of sodium bicarbonate in muscle proteins of Pacific white shrimp (*Litopenaeus vannamei*), an economically important species of Thailand, has been reported. The objectives of this study were to investigate the changes in biochemical properties and microstructure of natural actomyosin of Pacific white shrimp as affected by sodium bicarbonate at different concentrations in combination with and without 2.5% NaCl.

MATERIALS AND METHODS

Chemicals

Adenosine-5´-triphosphate (ATP), 8-anilino-1-naphthalenesulphonic acid (ANS), guanidine thiocyanate, sodium hydrogen sulphite, guanidine thiocyanate and Tris-maleate were procured from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Potassium chloride, sodium chloride, calcium chloride, trichloroacetic acid, potassium dihydrogen phosphate and ammonium molybdate were purchased from Merck (Darmstadt, Germany). 5,5´-Dithiobis (2-nitrobenzoic acid) (DTNB) was obtained from Wako Pure Chemical Industries (Tokyo, Japan). Bovine serum albumin (BSA) was purchased from Fluka (Buchs, Switzerland). Sodium bicarbonate ($NaHCO_3$) was obtained from Asahi chemical industry company Ltd. (Tokyo, Japan).

Sample preparation

Pacific white shrimp (*Litopenaeus vannamei*) with the weight of 20-22.5 g and the length of 12.5-13.5 cm were obtained from a farm in Songkhla province, Thailand. The shrimp were placed in ice with an ice/shrimp ratio of 2:1 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai, Songkhla within approximately 1 h. Upon

the arrival, shrimp were washed with clean water and beheaded, peeled, deveined and the meat was collected. The meat was then finely chopped, stored in ice until use.

Preparation of natural actomyosin (NAM)

NAM was prepared according to the method of Benjakul et al. (2001) with a slight modification. Shrimp mince (50 g) was homogenised in 10 volumes of chilled 0.6 M KCl, pH 7.0 at a ratio of 1:10 (w/v) at a speed of 10,000 rpm using a homogeniser (IKA, Labortechnik, Selangor, Malaysia). To avoid over heating, the sample was placed in ice and homogenised for 20 s, followed by a 20 s rest interval for a total extraction time of 4 min. The extract was centrifuged at 5000*g* for 30 min at 4 ^{o}C using a refrigerated centrifuge (Avanti® J-E, Beckman Coulter, Inc., Palo Alto, CA, USA). Three volumes of chilled deionised water were added to precipitate NAM. The NAM was collected by centrifuging at 5000*g* for 20 min at 4 ^{o}C. The NAM pellet was stored in ice until use.

Effects of sodium bicarbonate in the presence or absence of 2.5% NaCl on the changes of NAM from Pacific white shrimp

NAM pellet was suspended in chilled sodium bicarbonate solution with different ionic strength (0, 0.2, 0.4, 0.6, 0.8 and 1.0 M) containing 0 and 2.5% NaCl (pH 8.5). The mixtures were stirred gently for 10 min in ice. Thereafter the mixtures were allowed to stand at 4 ^{o}C for 30 min prior to analysis. Samples were taken for analyses. The concentration of NAM solution was adjusted to the concentration of 4.5 mg protein/mL.

Analyses

Measurement of turbidity and solubility

NAM solutions (4.5 mg protein/mL) with different treatments were placed in a cuvette (path length of 1 cm). Turbidity was determined by measuring the absorbance at 660 nm against the blanks using a UV1601 UV-vis spectrophotometer (Shimadzu, Tokyo, Japan) (Sano et al., 1994). To determine the solubility, NAM solutions with different treatments were subjected to centrifugation at 20000*g* for 30 min. The obtained supernatants were determined for soluble protein content using the Biuret assay (Robinson and Hogden, 1940). To determine total protein in the pellet, the exact amount of pellet was completely solubilised using 0.5 M NaOH. Solubility was expressed as that found in the supernatant relative to that obtained in the pellet.

Determination of surface hydrophobicity

Surface hydrophobicity was measured according to the method of Benjakul et al. (2001) using 8-anilo-1-naphthalenesulfonic acid (ANS) as a probe. Treated NAM solutions were diluted to 0.125, 0.25, 0.5 and 1 mg/mL using the same buffer. To 2.0 mL of diluted NAM solution, 10 mL of 10 mM ANS dissolved in 50 mM potassium phosphate buffer (pH 7.0) was added and the mixtures were mixed thoroughly. Sample blanks of each protein concentration were prepared in the same manner, except the same volume of 50 mM potassium phosphate buffer (pH 7.0) was used instead of ANS solution. Fluorescence intensity was measured using a RF-1501 spectrofluorometer (Shimadzu, Kyoto, Japan) at the excitation and emission wavelength of 374 and 485 nm, respectively. Surface hydrophobicity was calculated from the initial slope of the plot of fluorescence intensity against protein concentrations and was referred to as 'S_0ANS'.

Determination of total sulfhydryl and disulphide bond contents

Total sulfhydryl (SH) content was measured according to the method of Ellman (1959) as modified by Benjakul et al. (1997). To 1 mL of NAM solutions (4.5 mg protein/mL), 9 mL of 0.2 M Tris-HCl buffer (pH 6.8) containing 8 M urea, 2% sodium dodecyl sulphate (SDS) and 10

mM EDTA were added. After incubation with 0.4 mL of 0.1% DTNB in 0.2 M Tris-HCl buffer, (pH 6.8) at 40 ^{o}C for 25 min, the absorbance at 412 nm was measured using a spectrophotometer (UV-1601, Shimadzu, Kyoto, Japan). Reagent blank was prepared by replacing the sample with 50 mM potassium phosphate buffer (pH 7.0) containing 0.5 M NaCl. For the sample blank, the reaction was run in the same manner except that 0.2 M Tris-HCl buffer (pH 6.8) was used instead of DTNB solution. The total SH content was calculated using a molar extinction coefficient of 13,600 M^{-1} cm^{-1}.

Disulphide bond content in samples was determined using the 2-nitro-5-thiosulphobenzoate (NTSB) assay as described by Thannhauser, Konishi and Scheraga (1987). To 2 mL of NAM solution (4.5 mg protein/mL), 3 mL of freshly prepared NTSB assay solution were added. The mixtures were incubated in the dark at room temperature (25-27 ^{o}C) for 25 min. Absorbance was then measured at 412 nm. The disulphide bond content was calculated using a molar extinction coefficient of 13,900 M^{-1} cm^{-1}.

Assay of Ca^{2+}-ATPase and Mg^{2+}-ATPase activities

Ca^{2+}-ATPase and Mg^{2+}-ATPase activities were determined as described by Benjakul et al. (1997). To 1 mL of NAM solutions (4.5 mg protein/mL), 0.6 mL of 0.5 M Tris-maleate, pH 7.0, was added. $CaCl_2$ or $MgCl_2$ solutions were added to the system, with the total volume of 9.5 mL, to obtain final concentrations of 10 mM and 2 mM for Ca^{2+}-ATPase and Mg^{2+}-ATPase activity assays, respectively. To each assay solution, 0.5 mL of 20 mM ATP was added to initiate the reaction. The reaction was conducted for 10 min at 25 ^{o}C and stopped by addition of 5 mL chilled 15% (w/v) trichloroacetic acid. The reaction mixture was subjected to centrifugation at 6500*g* for 5 min. The inorganic phosphate liberated in the supernatant was measured by the method of Fiske and Subbarow (1925). Specific activity was expressed as µmoles inorganic

phosphate (Pi) released/mg protein/min. A blank was performed by adding the chilled trichloroacetic acid prior to the addition of ATP.

Transmission electron microscopy

NAM solutions (4.5 mg protein/mL) were diluted to 0.2 mg protein/mL with the corresponding solutions. A drop of sample was fixed for 5 min on a carbon-coated grid, negatively stained with 4% uranyl acetate for 5 min and washed with distilled water until the grid was cleaned. The specimens were visualised using a JEOL JEM-2010 transmission electron microscope (JEOL Ltd., Tokyo, Japan) (25,000×) at an accelerating voltage of 160 kV.

Measurement of zeta potential

The NAM solutions (4.5 mg protein/mL) with different treatments were stirred gently for 10 min in ice. Thereafter the mixtures were allowed to stand at 4 ^{o}C for 30 min prior to analysis. The zeta (ζ) potential of NAM solutions was measured using a ZetaPALs analyser (Brookhaven Instruments Co., Holtsville, NY, USA) at room temperature.

Protein determination

Protein content was measured using the Biuret method (Robinson and Hogden, 1940). Bovine serum albumin was used as a standard.

Statistical analysis

All experiments were run in triplicate and completely randomized design was used throughout the study. Data were subjected to analysis of variance (ANOVA) and mean comparisons were carried out by using Duncan's Multiple Range Test. For pair comparison, T-

test was used (Steel and Torrie, 1980). Statistical analysis was performed using the statistical Package for Social Sciences (SPSS for windows: SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

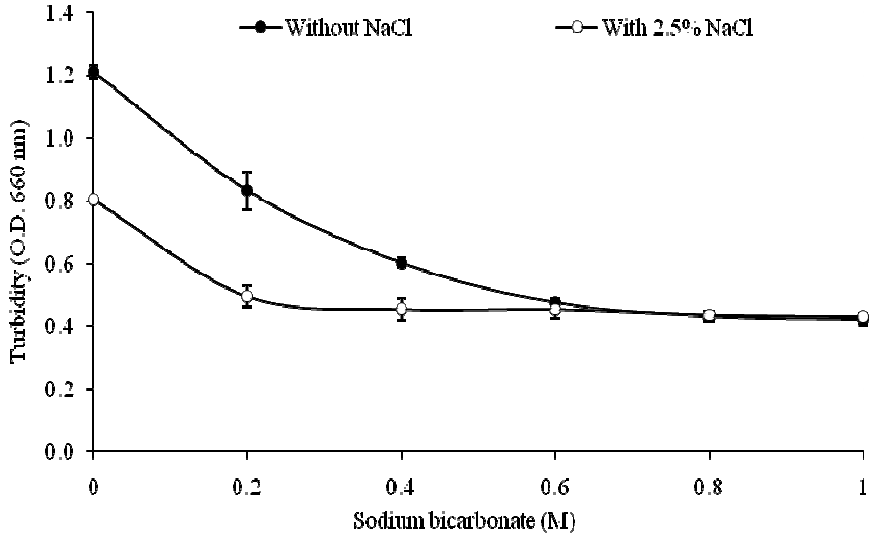
Turbidity and solubility of NAM from Pacific white shrimp as affected by sodium bicarbonate at different concentrations

Changes in turbidity and solubility of NAM from Pacific white shrimp treated with $NaHCO_3$ at different concentrations (0, 0.2, 0.4, 0.6, 0.8 and 1 M) in the presence or absence of 2.5% NaCl are shown in Figure. 1. Turbidity of NAM solutions decreased when the concentrations of $NaHCO_3$ increased up to 0.6 M ($P < 0.05$) (Figure. 1 A). No further changes in turbidity were noticeable when $NaHCO_3$ increased up to 1 M ($P > 0.05$). In the presence of 2.5% NaCl, the sharp decrease in turbidity was obtained when $NaHCO_3$ concentration was 0.2 M. No changes in turbidity were found thereafter ($P > 0.05$). In the presence of $NaHCO_3$ at the concentration range of 0-0.6 M, turbidity was lower when 2.5% NaCl was incorporated, in comparison with samples without NaCl ($P < 0.05$). Thus, NaCl might exhibit the synergistic effect on dissociation of NAM in conjunction with $NaHCO_3$ at the sufficient concentration. Higher ionic strength of sample containing 2.5% NaCl might lower the ionic interaction of NAM more effectively. Protein solubility is a complex function of the physiochemical nature of the proteins, which depends on pH, temperature and the concentration of the salt used. It also depends on whether the salt is Kosomtropic (stabilizes water structure) or Chaotropic (disrupts water structure). High ionic strength was shown to decrease actin-myosin interactions in the relaxed and activated muscle (Zayas, 1997). Additionally Wu and Smith (1987) reported that the increasing ionic strength or increasing incubation time decreased the turbidity of the bovine longissimus myofibrillar proteins and increased the solubility.

Furthermore, the chlorides clearly had a major effect on muscle protein solubility and on water holding properties (Kauffman et al., 2000). $NaHCO_3$ at concentration greater than 0.2 M had the less impact on solubility in the presence of 2.5% NaCl. Protein solubility depends on protein structure, pH, concentration of salt, temperature, duration of extraction and other intrinsic factors (Zayas, 1997). The degree of protein solubility in an aqueous medium is the result of electrostatic and hydrophobic interactions between protein molecules, and proteins are extracted when electrostatic repulsion between proteins is greater than hydrophobic interactions (Zayas, 1997). Wu et al. (1987) reported a marked decrease in the turbidity and the increase in solubility of bovine longissimus myofibrillar protein treated with KCl or NaCl having 0.10 to 0.35 M ionic strength. At $NaHCO_3$ concentration greater than 0.2 M, 2.5% NaCl had no synergistic effect on lowering turbidity of NAM ($P > 0.05$). Thus NaCl might not be required when $NaHCO_3$ concentration was higher than 0.2 M.

For solubility (Figure 1B), high solubility of NAM was obtained in the presence of 2.5% NaCl with the range of 89.9-98.0%. Slight increase in solubility was noticeable as the concentration of $NaHCO_3$ increased ($P < 0.05$). In the absence of 2.5% NaCl, the solubility of 94.4% was obtained when $NaHCO_3$ at a level of 0.2 M was used as solubility medium. At the concentration above 0.2 M, the gradual increase in solubility was obtained up to 1.0 M. NaCl has been added to solubilise myofibrilar proteins, including NAM (He et al., 2010). However, NAM could be more solubilised by only $NaHCO_3$ when the sufficient concentrations were implemented.

a)



b)

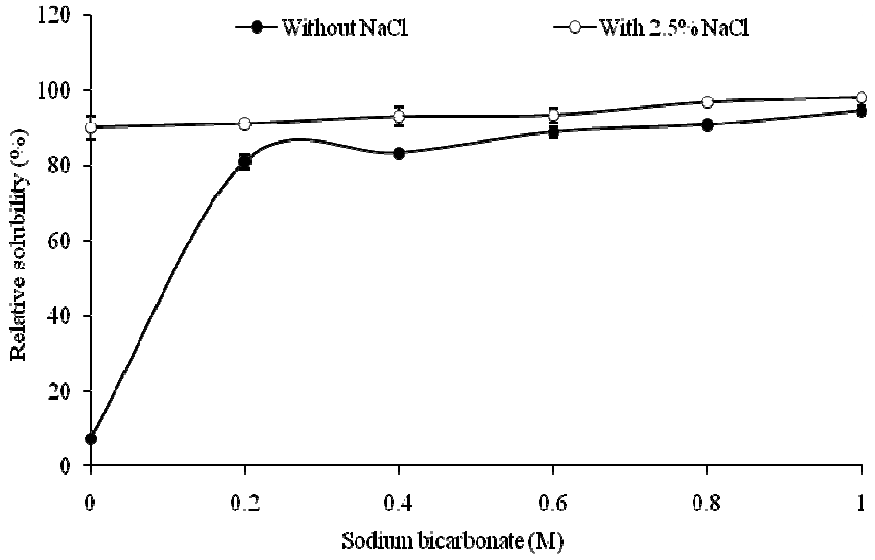


Figure. 1. Turbidity (A) and solubility (B) of natural actomyosin from Pacific white shrimp treated with sodium bicarbonate at different concentrations in the presence or absence of 2.5% NaCl. Bars represent standard deviation (n = 3).

Surface hydrophobicity, total sulfhydryl group and disulphide bond contents of NAM from Pacific white shrimp as affected by sodium bicarbonate at different concentrations

Changes in surface hydrophobicity (S_oANS) of NAM from Pacific white shrimp suspended in $NaHCO_3$ at different concentrations in the presence or absence of 2.5% NaCl are depicted in Figure. 2. S_oANS of NAM continuously increased when the concentrations of $NaHCO_3$ increased up to 0.8 M ($P < 0.05$). Thereafter, S_oANS was constant when $NaHCO_3$ concentration was above 0.8 M. The increase in S_oANS indicated the structural changes of NAM by increasing $NaHCO_3$ concentrations. Upon treatment with $NaHCO_3$, the aromatic hydrophobic amino acid residues, i.e. phenylalanine and tryptophan, might be exposed to a greater extent. ANS, an effective fluorescent probe, has been found to bind at non-polar regions of protein (Wicker et al., 1986). The increase in ANS binding of NAM was more likely due to the exposed hydrophobic domains. This was in agreement with the greater solubility of NAM in the presence of $NaHCO_3$ at the higher concentrations (Figure 1A). At the same concentration of $NaHCO_3$, the higher S_oANS was observed in NAM suspended in medium containing 2.5% NaCl, compared with that without 2.5% NaCl ($P<0.05$). The exposure of hydrophobic domains could be synergistically induced by NaCl. Increasing ionic strength decreases the sphere of each charge on the proteins, thereby weakening the structural integrity of myofibrils (Raymond and Zubay, 1984). As the protein moves away from the pI, the ionisable groups in proteins become increasingly charged up to a point where the charge repulsion causes the protein molecule to unfold. Along with NaCl, which is able to solubilise the myofibrillar proteins, the solubilised protein molecules with the modified charge caused by alkaline pH of $NaHCO_3$ were more likely unfolded as evidenced by the increased surface hydrophobicity.

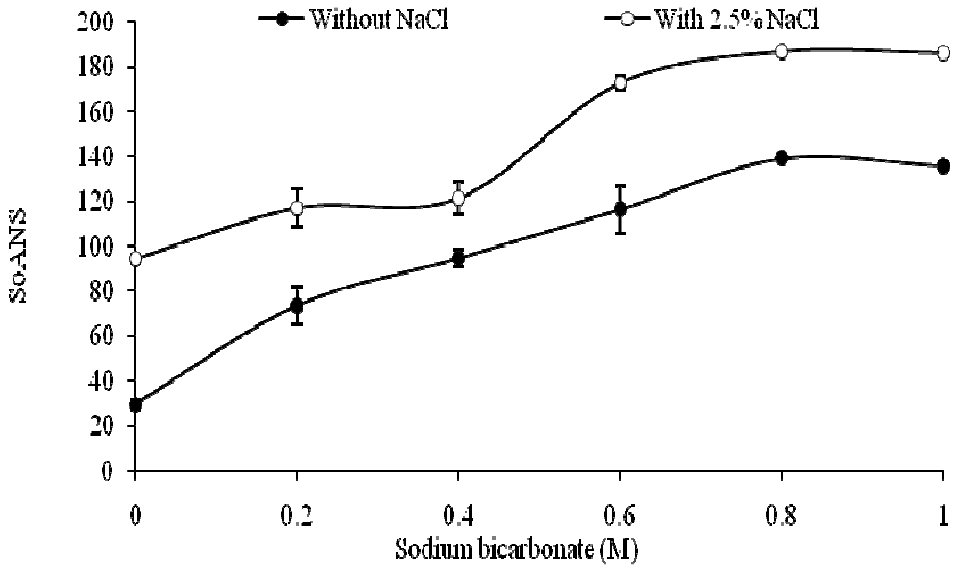


Figure. 2. Changes in surface hydryphobicity of natural actomyosin Pacific white shrimp treated with sodium bicarbonate at different concentrations in the presence or absence of 2.5% NaCl. Bars represent standard deviation (n = 3).

Total sulfhydryl (SH) group and disulphide bond contents of NAM from Pacific white shrimp treated with $NaHCO_3$ at various concentrations in combination with and without 2.5% NaCl are shown in Table 1. Slight increase in total SH group content of NAM was observed as $NaHCO_3$ increased up to 1.0 M ($P < 0.05$), regardless of NaCl incorporation. No changes in disulphide bond content of NAM samples were obtained when $NaHCO_3$ concentration increased up to 0.8 M ($P > 0.05$). However, a slight decrease in disulphide content bond was noticeable when $NaHCO_3$ at 1 M was used. Furthermore, NaCl had no impact on disulphide bond formation in NAM. At very high $NaHCO_3$ concentration, disulphide bond might be destroyed to some degree under alkaline condition. Chan et al. (1995) reported that myosin contained 42 SH groups. Two type of SH groups on the myosin head portion, named SH1 and SH2, have been reported to be involved in ATPase activity of myosin; another SH group (SHa) localised in the light meromyosin contributes to oxidation (Benjakul et al., 1997).

Apart from induction of the exposure of hydrophobic domains, $NaHCO_3$ at high concentration also enhanced the unfolding of NAM molecules, in which SH groups were more

exposed. The breakdown of disulphide bond might also cause the looser muscle structure, leading to the higher water holding capacity of muscle treated with $NaHCO_3$. Chantarasuwan et al. (2011) reported that Pacific white shrimp treated with 2.0% $NaHCO_3$ had the increase in water uptake.

Table 1. Changes in total sulphydryl group and disulphide bond contents of natural actomyosin Pacific white shrimp treated with sodium bicarbonate at different concentrations in the presence or absence of 2.5% NaCl

Sodium bicarbonate (M)	Total SH group content (mole/10^5 g protein)		Disulfide bond content (mole/10^5 g protein)	
	without NaCl	with 2.5% NaCl	without NaCl	with 2.5% NaCl
0	3.102 ± 0.013^{fB}	3.278 ± 0.015^{eA}	0.334 ± 0.004^{aA}	0.329 ± 0.003^{aA}
0.2	3.156 ± 0.009^{eB}	3.294 ± 0.001^{deA}	0.331 ± 0.007^{aA}	0.324 ± 0.002^{abA}
0.4	3.231 ± 0.012^{dB}	3.307 ± 0.005^{dA}	0.329 ± 0.003^{aA}	0.320 ± 0.005^{bB}
0.6	3.351 ± 0.033^{cA}	3.370 ± 0.005^{cA}	0.332 ± 0.003^{aA}	0.322 ± 0.005^{abB}
0.8	3.405 ± 0.004^{bB}	3.501 ± 0.017^{bA}	0.330 ± 0.006^{aA}	0.325 ± 0.004^{abA}
1.0	3.473 ± 0.012^{aB}	3.535 ± 0.017^{aA}	0.305 ± 0.006^{bA}	0.310 ± 0.006^{cA}

Means $\pm$ SD (n=3).

The different letters in the same column indicate significant differences ($p<0.05$). Different capital letters in the same row within the same parameter tested indicate the significant differences ($p<0.05$).

Ca^{2+}-ATPase and Mg^{2+}-ATPase activities of NAM from Pacific white shrimp as affected by sodium bicarbonate at different concentrations

Remaining Ca^{2+}-ATPase activity of NAM treated with $NaHCO_3$ at various concentrations in the presence or absence of 2.5% NaCl is shown in Figure. 3A. After incubation at 4 °C for 30 min, Ca^{2+}-ATPase activity of the NAM treated with $NaHCO_3$ decreased slightly as the concentration of $NaHCO_3$ increased ($P < 0.05$), suggesting the partial denaturation of myosin heavy chain, especially at the head portion. At the same $NaHCO_3$ concentration, no differences were found between samples treated without and with 2.5% NaCl ($P > 0.05$). Prevalent bicarbonate ion (HCO_3^-) might induce denaturation of Ca^{2+}-ATPase by unfolding of protein, the exposure of hydrophobic residues, etc. Induced dissociation of the actomyosin complex by $NaHCO_3$ at high concentration might release free myosin, which underwent denaturation easily. Xiong et al. (2000) reported that chicken myofibrillar proteins treated with 0.2 to 0.4 M NaCl had the higher contents of extractable actin and α-actinin. Tropomyosin and troponin-T were extracted at 0.3 M NaCl, whilst the noticeable extraction of myosin was detected at 0.4 M NaCl. Extraction of the both major (myosin and actin) proteins and other myofibrillar components gradually increased at higher salt levels (Wu and smith, 1987).

The effects of $NaHCO_3$ at different concentrations in combination with or without 2.5% NaCl on Mg^{2+}-ATPase activity of NAM from Pacific white shrimp muscle are shown in Figure. 3B. In the absence of 2.5% NaCl, Mg^{2+}-ATPase activity of NAM decreased as $NaHCO_3$ concentrations increased up to 0.8 M ($P < 0.05$). Thereafter, no further decreases were noticeable when $NaHCO_3$ concentrations increased up to 1.0 M ($P > 0.05$). The result indicated that $NaHCO_3$ at higher concentrations was effective in dissociating the actomyosin complex, as evidenced by the lower Mg^{2+}-ATPase activity retained. The decrease in Mg^{2+}-ATPase can be used as an indicator for the selective denaturation of actin, which is reported to be the activator for myosin Mg^{2+}-ATPase (Torigai and Konno, 1996).

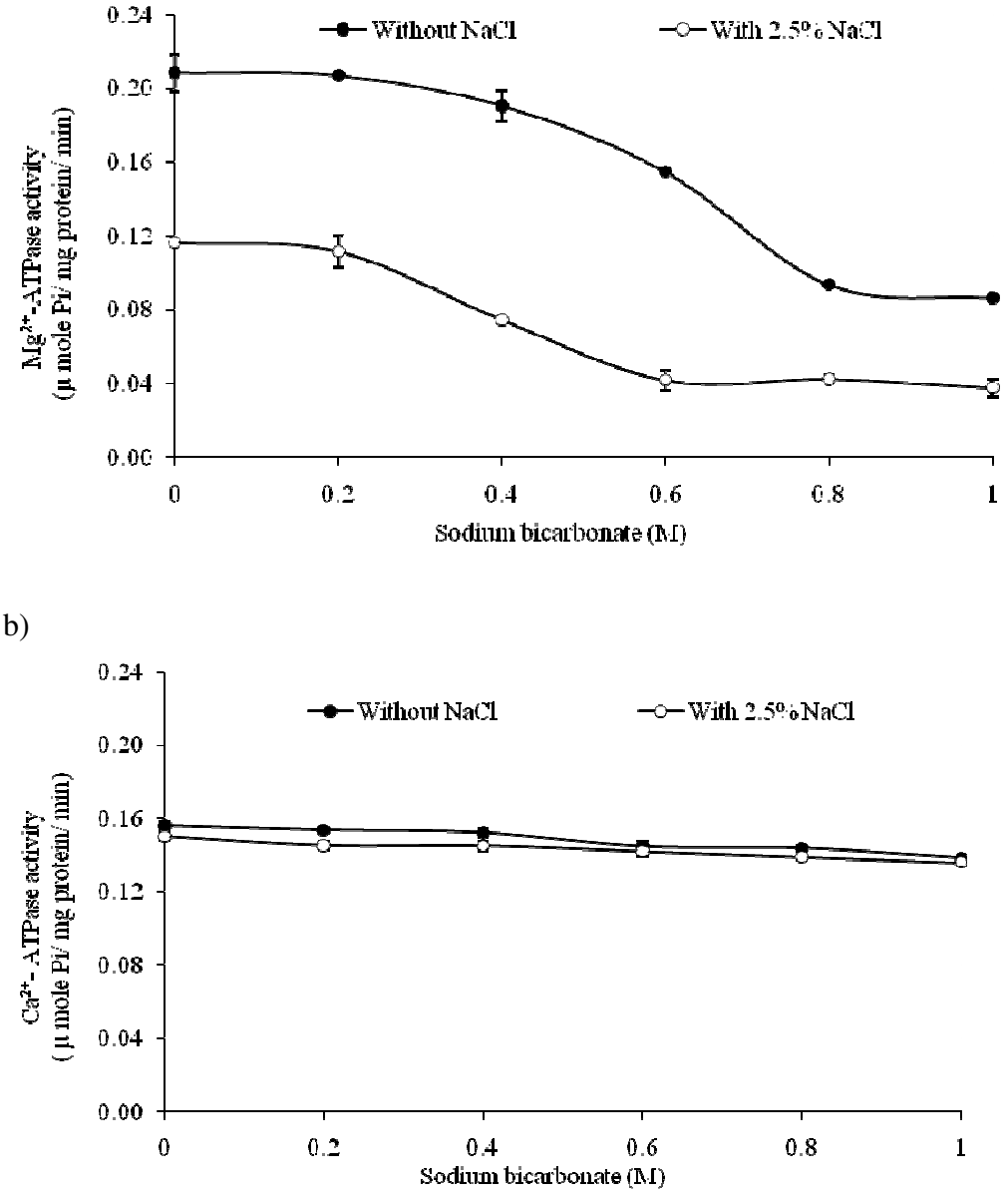


Figure. 3. Ca^{2+}-ATPase activity (A) and Mg^{2+}-ATPase activity (B) of natural actomyosin from Pacific white shrimp treated with sodium bicarbonate at different concentrations in the presence or absence of 2.5% NaCl. Bars represent standard deviation (n = 3).

For NAM treated with $NaHCO_3$, Mg^{2+}-ATPase activity was lower in the sample with 2.5% NaCl, compared with those without NaCl. In the presence of 2.5% NaCl, Mg^{2+}-ATPase activity decreased as $NaHCO_3$ concentrations increased up to 0.6 M ($P < 0.05$) and remained constant when the concentration was in the range of 0.8-1 M. The result suggested that $NaHCO_3$

could promote the release of actin and make actin more susceptible to denaturation, especially in the presence of 2.5% NaCl. In general, myosin plays a role in protection of actin from salt denaturation (Torigai et al., 1996). Therefore, the repulsive force mediated by $NaHCO_3$ at high concentration more likely contributed to the dissociation of actomyosin complex. This was confirmed by the increased solubility, decreased turbidity as well as the changes in SoANS and SH group content.

Zeta potential of NAM from Pacific white shrimp as affect by sodium bicarbonate at different concentrations

The zeta potential (ζ) representing the surface charge of NAM of Pacific white shrimp suspended in $NaHCO_3$ at various concentrations in combination with or without 2.5% NaCl is shown in Table 2. NAM solutions turned to become more negatively charged ranging from -12.12 to -26.98 as $NaHCO_3$ concentrations increased. In the presence of 2.5% NaCl, higher negative charge was obtained in all samples, in comparison with the absence of NaCl, when the same $NaHCO_3$ concentration was used. NaCl could facilitate the solubilisation or unfolding of protein molecules, where the $COOH^-$ group of side chains could be deprotronated with ease. Also, at the higher $NaHCO_3$ concentration, the ability of proteins to obtain the negative charge could be more pronounced. As a result, the higher negative charge was obtained in NAM treated with $NaHCO_3$ at high concentrations, particularly in the presence of NaCl. Benjakul et al. (2010) reported that a protein in an aqueous system has a zero net charge at its isoelectric point (pI), when the positive charges are balanced out by the negative charges and noted that the differences in net surface charge at different pHs were most likely governed by the different unfolding or exposure of charged amino acids, in which protonation or deprotonation could take place at different degrees. Thus, distinct negative net charge on surface of protein might enhance the dissociation of actomyosin complex, leading to the increased solubility of proteins. This might

be associated with the increased water holding capacity or yield of Pacific white shrimp treated with $NaHCO_3$ (Chantarasuwan et al., 2011).

Table 2 Zeta (ζ) potential of natural actomyosin from Pacific white shrimp treated with sodium bicarbonate at different concentrations in the presence or absence of 2.5% NaCl

Treatments	Sodium bicarbonate (M)	Zeta potential (mV)
$NaHCO_3$ without NaCl	0	-12.12 ± 0.07^{e}†
	0.2	-12.94 ± 0.43^{e}
	0.4	-16.41 ± 0.40^{d}
	1	-22.13 ± 0.22^{b}
$NaHCO_3$ with 2.5% NaCl	0	-17.19 ± 0.18^{d}
	0.2	-17.22 ± 1.20^{d}
	0.4	-20.86 ± 0.81^{c}
	1	-26.98 ± 1.02^{a}

†Mean ± SD (n = 3)

Different subscripts in the same column indicate the significant differences ($P < 0.05$).

Transmission electron micrograph of NAM from Pacific white shrimp as affected by sodium bicarbonate at different concentrations

Microstructures of NAM from Pacific white shrimp treated with $NaHCO_3$ at various concentrations in the presence or absence of 2.5% NaCl are illustrated in Figure. 4. The NAM suspended in water (without 2.5% NaCl) was found as the filamental aggregates. When 2.5% NaCl was incorporated, filaments were more dispersed and the aggregation was lowered. NaCl at a level of 3.0% has been shown to solubilise surimi and NAM from ling cod (*Ophiodon elongatus*) (Sultanbawa and Li-Chan, 2001). Transmission electron microscopy is used to obtain information on the changes in the shape of the actomyosin filaments caused by chemical treatment (Hsu et al., 2007). This filamental structure of actomyosin was still observed after

being treated with $NaHCO_3$ at low concentrations (0.2 M), but was more disrupted with increasing $NaHCO_3$ concentrations. In the presence of 1.0 M $NaHCO_3$ and 2.5% NaCl, filamental structure was intensively disrupted (Figure. 4H). The less disruption was found in the absence of 2.5% NaCl when 1 M $NaHCO_3$ was used. Actomyosin filaments were shortened by salt treatment, probably due to the dissociation of myosin subunits and depolymerisation of actin (Ko et al., 1990). The NAM of Pacific white shrimp treated with $NaHCO_3$ could undergo more dissociation or disruption, mainly caused by the increased negative charge (Table 2). Furthermore, under the high ionic strength condition, hydrophobic interactions stabilising the protein structure might be destroyed to some extent, leading to the dissociation of filament structure. Destructibility of protein was associated with the lost of intermolecular covalent and noncovalent interactions, including disulphide bonds and hydrophobic interactions (Lee and Lanier, 1995). The higher dissociation was in accordance with the increases in solubility (Fig. 1B) and lower turbidity (A660 nm) (Figure. 1A). The degree of unfolding or destruction of actomyosin varied depending on $NaHCO_3$ concentration used. Many proteins are partially unfolded into a compact state called 'molten globule', which retains most of the secondary structure whilst losing their tertiary structure (Mohan et al., 2007). In the presence of 2.5% NaCl, the attachment of the cross-bridges is further weakened as the Cl^- causes the increased electrostatic repulsive forces. If the lattice swells appreciably, the cross-bridges cannot remain attached (Ko et al., 1990). However, the excessive disruption of filamental structure contributed to mushy texture of shrimp or transparency in appearance as reported by Rattanasatheirn et al. (2008) and Chantarasuwan et al. (2011).

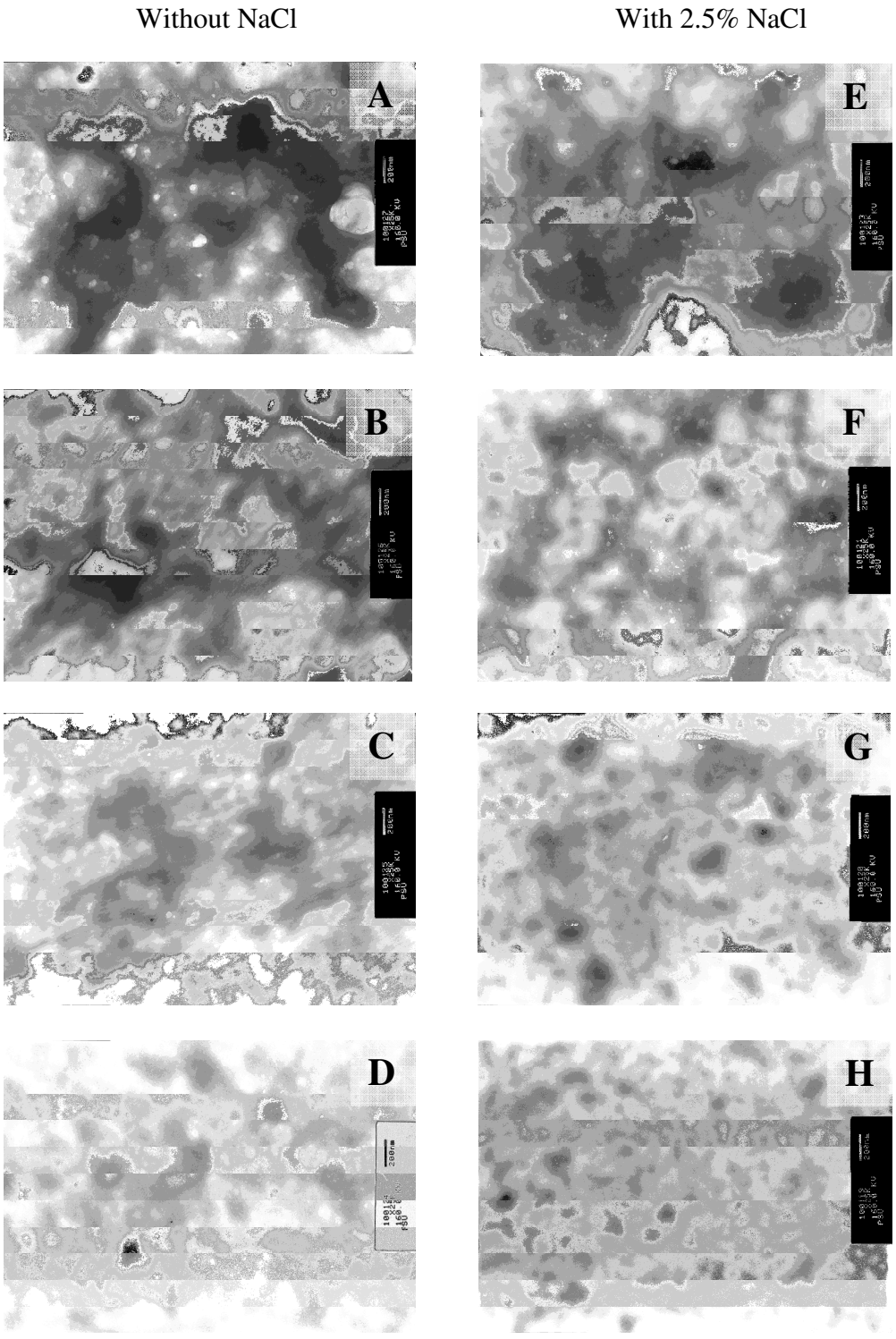


Figure. 4. Transmission electron micrograph of natural actomyosin from Pacific white shrimp treated with sodium bicarbonate at various concentrations in the presence or absence of 2.5% NaCl (A and E: 0 M $NaHCO_3$; B and F: 0.2 M $NaHCO_3$; C and G: 0.6 M $NaHCO_3$; D and H: 1 M $NaHCO_3$). Magnification: 25,000×.

CONCLUSIONS

Sodium bicarbonate can be used as non-phosphate compounds to increase the yield of white shrimp. It caused the dissociation of filamental structure of actomyosin complex associated with the increased solubility. The actions could be enhanced with the aid of 2.5% NaCl or increasing the concentration of sodium bicarbonate used. However, the high concentration could lead to the loss in yield due to the excessive solubilisation or disruption of protein filaments.

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