Improvement of Water Holding Capacity and Yield of Pacific White Shrimp by Phosphate and Bicarbonate Replacers

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ชื่อวิทยานิพนธ์ **การปรับปรุงความสามารถในการอุ้มน้ำและผลผลิตของกุ้งขาวโดยสารทดแทนฟอสเฟตและไบคาร์บอเนต**

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บทคัดย่อ

ฟอสเฟตและสารประกอบที่ไม่ใช่ฟอสเฟตถูกนำมาใช้อย่างแพร่หลายเพื่อปรับปรุงผลผลิตและลดการสูญเสียน้ำหนักของกุ้งภายหลังการให้ความร้อน อย่างไรก็ตามเมื่อเร็ว ๆ นี้ ทางประเทศคู่ค้าได้กำหนดข้อจำกัดในการใช้สารกลุ่มดังกล่าวจึงมีการเสาะหาสารอื่นที่มีศักยภาพใกล้เคียงเพื่อใช้สำหรับการแปรรูปกุ้งจากการศึกษาผลของสารละลายโซเดียมไฮดรอกไซด์ (NaOH) และโพแทสเซียมไฮดรอกไซด์ (KOH) ที่ระดับความเข้มข้นต่างกัน (ร้อยละ 0.125 0.25 0.5 และ 0.75, น้ำหนักต่อปริมาตร) ในสภาวะที่มีเกลือโซเดียมคลอไรด์ (NaCl) ร้อยละ 2.5 ต่อผลผลิตและคุณลักษณะของกุ้งขาวพบว่า น้ำหนักที่เพิ่มขึ้นภายหลังจากการแช่ในสารละลาย ผลผลิตหลังจากการให้ความร้อน และ พีเอช ของกุ้งขาวเพิ่มขึ้นเมื่อความเข้มข้นของสารละลายที่ใช้แช่กุ้งเพิ่มขึ้น ($P<0.05$) นอกจากนี้การแช่กุ้งในสารละลายโซเดียมไฮดรอกไซด์ ร้อยละ 0.75 ในสภาวะที่มีเกลือร้อยละ 2.5 (พีเอช 13.04) สามารถเพิ่มผลผลิตภายหลังจากการให้ความร้อนได้สูงสุด อย่างไรก็ตามการแช่กุ้งในสภาวะดังกล่าวทำให้ค่าความเป็นสีแดง (a^*-value) เพิ่มขึ้น ในขณะที่แรงเฉือนและคะแนนความชอบในทุกคุณลักษณะลดลง

จากการศึกษาผลของสารละลายโซเดียมไฮดรอกไซด์ ร้อยละ 0.75 ในสภาวะที่มีเกลือร้อยละ 2.5 ที่พีเอชต่างๆ (8.5, 10, 11.5 และ 13) สำหรับแช่กุ้งขาวพบว่า ขณะที่พีเอชของสารละลายเพิ่มขึ้นส่งผลให้น้ำหนักที่เพิ่มขึ้นภายหลังจากการแช่ในสารละลายและผลผลิตหลังจากการให้ความร้อนเพิ่มขึ้นซึ่งสอดคล้องกับค่าแรงเฉือนที่ลดลง ($P<0.05$) ภายหลังจากการแช่กุ้งในสารละลายโซเดียมไฮดรอกไซด์ ร้อยละ 0.75 ในสภาวะที่มีเกลือร้อยละ 2.5 (พีเอช 13) พบว่า ค่า a^*-value ของกุ้งดิบเพิ่มขึ้นในขณะที่ a^*-value ของกุ้งต้มลดลง ($P<0.05$) นอกจากนี้กุ้งต้มที่ผ่านการแช่ด้วยสารละลายโซเดียมไฮดรอกไซด์ ร้อยละ 0.75 ในสภาวะที่มีเกลือร้อยละ 2.5 (พีเอช 13) มีคะแนนความชอบลดลง ดังนั้นสารละลายโซเดียมไฮดรอกไซด์ ร้อยละ 0.75 ในสภาวะที่มีเกลือร้อยละ 2.5 (พีเอช 11.5) (ASS) สามารถใช้เป็นสารทดแทนฟอสเฟตและไบคาร์บอเนตสภาวะด่างสามารถ

เหนี่ยวนำให้แอกโตไมโอซินธรรมชาติ (NAM) เกิดการแตกตัว กิจกรรม Ca^{2+} และ Mg^{2+} ATPase ลดลงเมื่อพีเอชของสารละลายเพิ่มขึ้น นอกจากนี้สภาวะด่างทำให้เกิดการเปลี่ยนแปลงของไฮโดรโฟบิกซิตี้พื้นผิวของ NAM พีเอชของสารละลายที่เพิ่มขึ้นส่งผลให้พันธะไดซัลไฟล์ลดลงแต่หมู่ซัลฟ์ไฮดริลเพิ่มขึ้น ($P<0.05$) ดังนั้นสภาวะด่างส่งผลต่อคุณลักษณะทางฟิสิกส์-เคมีของโปรตีนกล้ามเนื้อ

เมื่อใช้ ASS (โซเดียมไฮดรอกไซด์ร้อยละ 0.75 และเกลือร้อยละ 2.5, พีเอช 11.5) ร่วมกับสารเติมแต่งชนิดต่างๆ พบว่าการใช้ ASS ร่วมกับน้ำตาล (กลูโคส) และน้ำตาลแอลกอฮอล์ (ซอร์บิทอลและกลีเซอรอล) ที่ระดับความเข้มข้นร้อยละ 0.25-1 ไม่มีผลต่อน้ำหนักที่เพิ่มขึ้นภายหลังการแช่ในสารละลายรวมถึงน้ำหนักที่สูญเสีย และผลผลิตหลังจากการให้ความร้อน ส่วนการแช่กุ้งด้วย ASS ร่วมกับกรดอะมิโน (ไกลซีน กรดกลูตามิก และ อาร์จินีน) ที่ระดับความเข้มข้นร้อยละ 1-3 พบว่า กุ้งที่ผ่านการแช่ในสารละลาย ASS ร่วมกับกรดกลูตามิกร้อยละ 3 (ASS+3% glutamic acid) มีน้ำหนักที่สูญเสียต่ำสุดและผลผลิตหลังจากการให้ความร้อนสูงสุด นอกจากนี้พบว่ากุ้งที่แช่ด้วย ASS+3% glutamic acid ที่พีเอช 7 มีน้ำหนักที่เพิ่มขึ้นภายหลังจากการแช่ในสารละลายและร้อยละผลผลิตหลังจากการให้ความร้อนต่ำกว่า รวมถึงน้ำหนักที่สูญเสียภายหลังจากการให้ความร้อนสูงกว่ากุ้งที่แช่ด้วย ASS+3% glutamic acid ที่พีเอช 11.5 ($P<0.05$) ส่วนการแช่กุ้งด้วย ASS ที่มีโมโนโซเดียมกลูตาเมตร้อยละ 3 พีเอช 11.5 (ASS+3%MSG) (โมลเทียบเท่า กรดกลูตามิก) สามารถให้น้ำหนักที่เพิ่มขึ้นภายหลังการแช่และผลผลิตของกุ้งหลังจากการให้ความร้อนสูงกว่ากุ้งที่แช่ด้วย ASS+3% glutamic acid ที่พีเอช 11.5 การใช้ ASS+3%MSG แช่กุ้งไม่ส่งผลกระทบต่อสี ค่าแรงเฉือนของกุ้งต้ม อีกทั้งยังให้คะแนนความชอบสูงสุด ดังนั้น ASS+3%MSG สามารถใช้เป็นสารทดแทนฟอสเฟตและไบคาร์บอเนตที่มีประสิทธิภาพในการแช่กุ้ง

จากการศึกษาการเปลี่ยนแปลงคุณภาพของกุ้งที่ผ่านการแช่ด้วยสารละลายต่างๆที่ประกอบด้วย 1) ASS 2) ASS+3%MSG และ 3) สารละลายเกลือร้อยละ 2.5 ที่ประกอบด้วยฟอสเฟตผสมภายหลังการแช่แข็งและทำละลายด้วยจำนวนรอบต่างๆ พบว่า ปริมาณของเหลวที่ปลดปล่อยจากตัวกุ้งลดลง อีกทั้งไม่พบกิจกรรมของเอนไซม์ α- glucosidase (AG) และ β-N-acetyl-glucosaminidase (NAG) ในกุ้งที่ผ่านการแช่ด้วย ASS+3%MSG การเพิ่มขึ้นของ a^*-value ในกุ้งดิบเพิ่มขึ้นเมื่อจำนวนรอบของการแช่แข็งและทำละลายเพิ่มมากขึ้น อย่างไรก็ตาม a^*-value ของกุ้งต้มที่ผ่านการแช่ใน ASS+3%MSG ลดลงเมื่อจำนวนรอบของการแช่แข็งและทำละลายเพิ่มมากขึ้น ($P<0.05$) ค่าแรงเฉือนของกุ้งดิบและกุ้งต้มที่ผ่านการแช่ในสารละลายต่างๆเพิ่มขึ้นเมื่อจำนวนรอบของการแช่แข็งและทำละลายเพิ่มขึ้นถึง 3 รอบ จากนั้นค่าแรงเฉือนลดลงอย่างมากใน

รอบที่ 5 ของการแช่แข็งและทำละลาย ($P<0.05$) ดังนั้นการแช่กุ้งใน ASS+3%MSG พีเอช 11.5 สามารถชะลอการเปลี่ยนแปลงของกล้ามเนื้อซึ่งเหนี่ยวนำโดยกระบวนการแช่แข็งและทำละลาย

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ABSTRACT

Phosphate and non-phosphate compounds have been used widely to improve the yield and lower the cooking loss of shrimp. However, the uses of those compounds have been recently restricted by the importers. Potential alternative has been therefore searched for shrimp processing. Effects of sodium hydroxide (NaOH) and potassium hydroxide (KOH) solutions with different concentrations (0.125, 0.25, 0.50 and 0.75%, w/v) in the presence of 2.5% NaCl on yield and characteristics of Pacific white shrimps *(Litopenaeus vannamei)* meat were studied. The increases in weight gain, cooking yield and pH of soaked shrimp were observed with increasing concentrations of both alkaline solutions ($P<0.05$). Treatment of shrimp with 0.75% NaOH + 2.5% NaCl (pH 13.04) rendered the highest cooking yield, however it resulted in the increased *a**-value, the decreased shear force and lower likeness score for all attributes.

When the selected soaking solution (0.75% NaOH solution containing 2.5% NaCl) with different pHs (8.5, 10, 11.5 and 13) were used for treatment of Pacific white shrimp, weight gain and cooking yield increased with the coincidental decrease in shear force as the pH of solution increased ($P<0.05$). When being treated at pH 13, raw shrimp showed the marked increase in *a**-value, whereas cooked shrimp had the decreased *a**-value ($P<0.05$). Cooked shrimp with prior treatment using the soaking solution (pH 13) exhibited the decreased likeness score. Thus, 0.75% NaOH containing 2.5% NaCl (pH 11.5) could be an alternative to phosphate and bicarbonate for shrimp treatment. Alkaline condition could induce the dissociation of natural actomyosin (NAM). Ca^{2+} and Mg^{2+} ATPase activity decreased as pH of solution increased. Surface hydrophobicity (SoANS) of NAM was also altered by alkaline condition. The increasing formation of disulfide bonds with concomitant decrease in

total sulfhydryl group content was observed as pHs increased ($P<0.05$). Therefore, physicochemical properties of muscle protein were affected by alkaline treatment.

As alkaline soaking solution (0.75% NaOH and 2.5% NaCl, pH 11.5; ASS) was incorporated with several additives, it was found that sugar (glucose) and sugar alcohols (sorbitol and glycerol) at various levels (0.25-1%) had no pronounced effect on weight gain, cooking loss and cooking yield. When different amino acids (glycine, glutamic acid and arginine) were added in ASS at various levels (1-3%), the lowest cooking loss but highest cooking yield were obtained for the sample treated with ASS containing 3% glutamic acid. When ASS containing 3% glutamic acid had pH of 7.0, higher cooking loss but lower weight gain and cooking yield were obtained, compared with pH 11 ($P<0.05$). For shrimp treated with ASS having 3% monosodium glutamate (glutamic acid mole equivalent), higher weight gain and cooking yield were achieved, in comparison with those treated with ASS containing 3% glutamic acid. For cooked shrimp, the treatment using ASS containing 3% MSG had no effect on color and shear force, but yielded the cooked shrimp with the highest overall likeness score. Therefore, ASS containing 3%MSG could be used as the potential phosphate and bicarbonate replacer for shrimp treatment.

Quality changes of shrimp subjected to different treatments including 1) ASS, 2) ASS+3%MSG and 3) 2.5% NaCl with mixed phosphates were monitored after freeze-thawing with various cycles. Drip loss of raw shrimp was reduced with treatment using ASS+3% MSG and no α- glucosidase (AG) as well as β-N-acetyl-glucosaminidase (NAG) activities were coincidentally detected. The increase in a^*-values of raw shrimp increased as freeze-thaw cycles increased. On the other hand, a^*-value of cooked shrimp treated with ASS containing 3% MSG decreased with increasing freeze-thaw cycles ($P<0.05$). Shear force of both raw and cooked shrimp with all treatments increased when freeze-thaw cycles increases up to 3 cycles, however, it was drastically decreased after 5 freeze-thaw cycles ($P<0.05$). Therefore, treatment of shrimp with 0.75% NaOH containing 2.5% NaCl and 3% MSG (pH 11.5) could retard the deteriorative changes of muscle induced by freeze-thawing process.

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CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

1.1 Introduction

Seafood industry of Thailand is well known for its long-standing excellent reputation worldwide, owing to its prime quality, freshness, variety, and taste (Benjakul *et al.*, 2003a). Thailand has exported seafood and seafood products to different countries such as America, Europe and Asia. Shrimp is one of the seafood products, which have become economically important for Thailand. In 2012, Thailand exported 17 million tons of shrimp and shrimp products with a value of 4,520 Million baths. Among the products, frozen shrimp and shrimp products accounted for 57.21% and the remainders were the processed seafood products (The Customs Department, 2012).

For frozen cooked shrimp processing, cooking and freezing are implemented. Those processes cause protein denaturation, resulting in the decrease in cooking yields and increased cooking loss. To minimize those negative effects, some additives, particularly phosphate compounds, have been widely used in shrimp and shrimp products (Ratanasatein *et al.*, 2008). Appropriate amount of NaCl in combination with phosphate or bicarbonate has been introduced to enhance water holding of shrimp (Hamm and Cormack, 1972, Rattanasatheirn, 2008). Due to the strict regulation of using phosphates in seafood, especially shrimp, other additives termed non-phosphate compounds with the similar properties in increasing the yield or lowering the cooking loss have been paid increasing attention (Chantarasuwan *et al.*, 2011).

Sodium bicarbonate has 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). Shrimp soaked in 2.5% NaCl with 2.0% sodium bicarbonate had the improved water retention and lower cooking loss (Chantarasuwan *et al.*, 2011). This was mediated by the repulsive force between muscle fiber under the alkaline condition (Chantarasuwan *et al.*, 2011b). Charge of protein molecules can be modified by alkaline solution, and charged domain in muscle bind water effectively.

However, EU Standing Committee meeting on May 17th 2013 announced the new regulation, where carbonate/bicarbonate are not allowed to be used in marine products. Due to the strict regulation in use of phosphate and carbonate as the processing aid in shrimp and shrimp products, other alternative, especially alkaline compounds, can be considered as the agent with the equivalent property in improving quality of shrimp. Additionally, the substances with water binding capacity such as amino acid, sugar or sugar alcohol, etc. can be used in conjunction with alkaline compounds. As a sequence, those compounds can exhibit the synergistic effect in holding water in shrimp muscle. Furthermore, some hydrocolloids with high water holding capacity can be used as the water binding agent after an appropriate hydrolysis to yield the oligosaccharides. Oligosaccharide with the proper size distribution could penetrate in the shrimp muscle under the alkaline condition with ease. The use of those compounds in combination with the selected alkali can be therefore potential means to replace phosphate or bicarbonate, which have been recently banned in many countries. Most importantly, the yield and quality of cooked and uncooked shrimp can be improved.

The information gained can be of benefit for shrimp processing industry. Those phosphate and carbonate replacers can be used with equivalent efficacy. As a whole, shrimp products of Thailand can be competitive in the world market and both farmer and processor can gain increasing revenue. Also, shrimp farming can be sustainable.

1.2 Review of literature

1.2.1 Pacific white shrimp (*Litopenaeus vannamei*)

Pacific white shrimp is distributed naturally throughout the Eastern Pacific coast from the Gulf of California, Mexico to Tumbes in Northern Peru. It is the major species cultivated in the east hemisphere and contributes 30% of farmed production of penaeid shrimp in the world (Afero, 2005). This shrimp is non-indigenous species, which has been aquacultured in some parts of the southern United States, including Florida, Texas, Georgia, South Carolina, Alabama, Arizona, Indiana, and Illinois (Samocha *et al*., 2002), and Hawaii (McGovern-Hopkins and Tamaru, 2001).

Recently, this species has also widely been cultivated in Thailand and Indonesia (Miranda, 2010).

1.2.1.1 Taxonomy and biological characteristics

Taxonomic status of Pacific white shrimp can be placed as follows (Holthuis, 1980):

Phylum : Arthropoda
Class : Crustacea
Subclass : Malacostraca
Series : Eumalacostraca
Superorder : Eucarida
Order : Decapoda
Suborder : Natantia
Section : Penaeidea
Family : Penaeidae
Subfamily : Penaeinae
Genus : *Penaeus* Boone, 1931
Subgenus : *Litopenaeus* (Perez Farfante, 1969)
Species : *Litopenaeus vannamei* (Boone, 1931)

The bodies of Pacific white shrimp are translucent but often have a bluish-green hue due to the presence of pigmented chromatophores (molecules evolved to collect/reflect light). Shrimp can reach 230 mm (9 inches) in length (Farfante and Kensley, 1997). Pleopods are often marked with dark red, while the margin of the uropods of the tail are green along their margins. The preferred habitat ranges from muddy bottoms near the shoreline down to depths of 72 m (235 feet) (Dore and Frimodt, 1987). The growth and survival of Pacific white shrimp postlarvae are strongly dependent on temperature and salinity. The highest survival and growth are generally found at temperature around 28-30°C and salinity of 33-40 ppt. Survival of juveniles is usually compromised at low salinities and high temperatures (Ponce-Palafox *et al.*, 1997).

1.2.2 Import-export

Thailand is now the world's leading exporter of shrimp products, supplying over 20 percent of the world trade in shrimps and prawns. Thailand exports shrimp in various forms, including fresh chilled and frozen, dried, boiled, canned and other preparation. Fresh chilled and frozen are the largest exports, accounting more than 70 percent of all shrimp exports each year (Sriboonchitta, 2000). However, in the last 6 years, fresh chilled and frozen shrimps tend to be declined, while other prepared shrimps tend to be increased (Sriboonchitta, 2000). The major markets of Thai frozen shrimp are USA and Japan. EU, China and Singapore are also important markets. The export statistic of shrimp and shrimp product from 2010-2012 is shown in **Table 1.**

Table 1 Export statistic of shrimp and shrimp product of Thailand

Month	2010		2011		2012	
	Quantity (Kg)	Value (Baht)	Quantity (Kg)	Value (Baht)	Quantity (Kg)	Value (Baht)
January	15,604,243	3,199,687,547	12,842,459	3,082,495,433	10,452,463	2,945,640,320
February	15,649,279	3,187,371,842	11,712,083	2,871,253,418	11,348,838	3,133,036,757
March	18,252,714	3,883,029,296	15,880,824	3,927,721,763	12,865,069	3,349,354,276
April	15,124,515	3,096,002,607	13,090,627	3,284,320,564	12,801,795	3,114,409,548
May	20,145,673	3,992,997,282	14,877,318	3,846,371,154	19,202,966	4,486,494,229
June	27,315,072	5,591,014,914	17,284,248	4,451,958,876	17,456,701	4,343,124,882
July	23,007,960	5,219,274,331	18,973,084	5,074,092,071	18,850,928	4,553,131,033
August	21,977,070	5,139,851,094	21,207,010	5,767,117,381	15,364,077	3,769,233,267
September	23,259,690	5,423,980,961	20,194,567	5,189,234,343	14,679,489	4,054,924,960
October	23,188,611	5,378,009,836	19,876,689	5,201,776,067	15,808,935	3,949,337,720
November	19,668,048	4,505,618,851	18,686,065	5,013,835,472	15,733,803	4,189,613,268
December	18,547,506	4,356,477,346	15,761,438	4,338,087,347	13,338,241	3,320,526,670
Total	241,740,381	52,973,315,907.00	200,386,412	52,048,263,889.00	177,903,305	45,208,826,930.00

Source: The Customs Department (2012).

1.2.3 Chemical composition

Fish and shellfish are rich in nutrients, especially proteins and amino acids. The main constituents of fresh fish are water (65-80%), crude protein (40-53.5% dry basis), fat (9.9-18.8%, dry weight basis), total carbohydrate (23.7-37.3%, dry weight basis) and inorganic substances (6.4-11.4%, dry weight basis) (Jabeen and Chaudhry, 2011). Compositions and quality can be varied, depending upon muscle type, feeding period, time of harvesting and spawning stage, etc. (Chaijan *et al.*, 2011; Kong *et al.*, 2006; Kong *et al.*, 2007). Chemical compositions of shrimp vary, depending on species **(Table 2)**. However, Pacific white shrimp is rich in protein (18-25%) (Sriket *et al.*, 2007a).

Table 2 Chemical composition of edible portion of different shrimps

Shrimp	Composition (%) wet wt. basis				References
	Moisture	Protein	Ash	Fat	
Pacific white shrimp	77.2±0.2	18.8±0.2	1.5±0.1	1.3±0.1	Sriket *et al.* (2007)
Black tiger shrimp	80.5±0.3	17.1±0.6	0.9±0.0	1.2±0.4	Sriket *et al.* (2007)
Red shrimp	74.5±0.7	21.4±0.2	2.0±0.1	0.1±0.1	Rosa and Nones (2004)
Pink shrimp	74.6±0.7	20.8±0.3	1.9±0.1	0.2±0.0	Rosa and Nones (2004)
Norway Lobster	75.2±0.9	20.4±0.4	2.0±0.1	0.1±0.0	Rosa and Nones (2004)

1.2.4 Fish and shellfish muscle

1.2.4.1 Proteinaceous components

There are different proteins in fish and shellfish muscles. These proteins perform different tasks and have varying properties (Joo *et al.*, 1999; Sikorski *et al.*, 1990). Muscle proteins can be classified into three groups based on solubility as follows:

1.2.4.1.1 Sarcoplasmic proteins

Sarcoplasmic proteins are located in sarcolemma and are soluble at low salt concentrations (<0.1 M KCl) (Sriket, 2012). Sarcoplasmic proteins were found as the second predominant proteins in fish and shrimp meat (black tiger and white shrimp), accounting about 30-35% of total muscle protein (Jafarpour and Gorczyca, 2012; Sriket *et al.*, 2007a). Despite their diversity, sarcoplasmic proteins share several common physicochemical properties. Most are of relatively low molecular weight, with high isoelectric pH, and have globular or rod-shaped structures (Sikorski *et al.*, 1990b). Sarcoplasmic proteins can be extracted by homogenizing the muscle tissue with water or solutions of neutral salts with ionic strength below 0.15 (Sriket, 2012). Enzymes influencing the quality of fish also belong to sarcoplasmic proteins. Those include the enzymes of the glycolytic pathway and the hydrolytic enzymes of the lysosomes (Ladrat *et al.*, 2003).

1.2.4.1.2 Myofibrillar proteins

Myofibrillar proteins are the major proteins in fish muscle. Normally, these proteins constituted as the major protein (56.8-64.3% of total proteins) in black tiger and white shrimps (Sriket *et al.*, 2007). The myofibrillar proteins are also mainly responsible for the water holding capacity of fish, for the textural development of fish product, as well as for the functional properties of fish minces and homogenates (Sikorski *et al.*, 1990a). Myofibrillar proteins undergo changes during the rigor mortis and extended frozen storage (Shahidi, 1994). Myofibrillar proteins are soluble in solution of neutral salts with ionic strength less than 0.5 and are often called the “salt-soluble proteins” (Rattanasatheirn, 2008).

Myofibrillar proteins in fish or shellfish vary in content, depending upon species. Sriket (2007) reported that Pacific white shrimp had the higher myofibrillar protein than black tiger shrimp **(Table 3).**

Table 3 Nitrogenous constituents of black tiger shrimp and Pacific white shrimp meat

Composition (mg N/g muscle)	Black tiger shrimp	Pacific white shrimp
Non-protein nitrogen	4.68±0.31	1.44±0.23
Sarcoplasmic protein	6.16±0.02	7.81±0.62
Myofibrillar protein	12.62±0.35	14.25±0.99
Alkaline-soluble protein	0.65±0.05	0.36±0.03
Stromal protein	0.21±0.01	2.66±0.11

Source: Sriket (2007)

Moreover, myofibrillar proteins can be further divided into the subgroups as follows:

1.2.4.1.2.1 Contractile proteins

Contractile proteins, which are different in size and location in the muscle, are listed in **Table 4** (Ashie and Simpson, 1997).

- Myosin

Myosin is the most abundant myofibrillar component, constituting approximately 40-60% of total protein content (Bechtel, 1986). It is a large fibrous protein with a molecular weight of about 500 kDa (Ogawa *et al.*, 1994). Myosin consists of six polypeptide subunits, two large heavy chains and four light chains arranged into an asymmetrical molecule (Watabe, 2002; Xiong, 1997a) **(Figure 1).** The molecular structure of myosin comprises two globular heads (S-1s) and a double-stranded α -helical rod. The S-1 globular heads in myosin have ATPase activity and actin binding ability. The α -helical rod forms a filament (Ogawa *et al.*, 1994). The globular head regions of myosin bind and hydrolyze ATP to ADP. The activity reaches its maximum with 3-5 mM Ca^{2+}. This activity is due to myosin alone and thus is not essentially affected by the presence of actin (Ochiai and Chow, 2000). Ca^{2+}-ATPase activity is a good parameter to estimate the quality or the extent of deterioration of protein in fish muscle (Watabe, 2002). Myosin ATPase is also largely affected by chemical modification of reactive SH residue (SH_1, SH_2). Modification of SH_2 results in the inactivation of Ca^{2+}-ATPase (Ochiai and Chow, 2000).

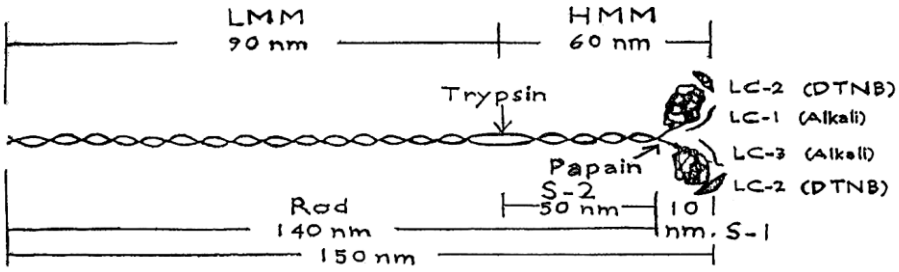

Figure 1. Schematic representation of the myosin molecule. Light meromyosin (LMM), heavy meromyosin (HMM), rod, S-1 and S-2 subfragments of HMM, the light chains, and the hinge regions susceptible to trypsin and papain are indicated.

Source: Xiong (1997)

Myosin heavy chain (MHC) is not stable and susceptible to degradation, especially improper handling and storage. The decrease in band intensity of MHC and actin was noticeable in whole white shrimp kept in ice for 12 days (Sriket, 2007). Various proteinases play a role in autolytic process of ice-stored fish, depending on several factors e.g. tissue compartmentization, presence of activators or inhibitors and the susceptibility of the protein toward cleavage by the respective enzymes (Ladrat *et al.*, 2003) The decrease in the relative amount of MHC with a concomitant increase in the number and intensity of bands with molecular size about 100 kDa cross-reacting with anti-MHC antiserum were found in *Penaeus borealis*. The disappearance of band of about 67 and 50 kDa after 24 h and the appearance of a band of slightly less than 50 kDa after 5 h of iced storage were noticeable (Martinez *et al.*, 2001)

- Actin

Actin is the second most abundant myofibrillar protein, constituting about 22% of myofibrillar proteins. This protein has a molecular weight of 42 kDa (Jafarpour and Gorczyca, 2012). In muscle tissue, actin is naturally associated with tropomyosin and the troponin complex. It also contains a myosin binding site, which allows myosin to form temporary complex with it during muscle contraction or the permanent myosin-actin complex during rigor mortis development in postmortem

(Xiong, 1997a). Actin is a quite resistant to autolysis during post-mortem storage (Rawdkuen *et al*., 2007).

- Actomyosin

When actin and myosin are mixed *in vitro*, a complex, called actomyosin, is formed. This complex can be dissociated by addition of ATP. Actomyosin is the main state of actin and myosin in postmortem muscle because ATP is depleted by postmortem metabolism (Ochiai and Chow, 2000). Myosin and actomyosin are generally found in extracts of postmortem muscle (Foegeding *et al*., 1996). The dissociation constant for actin and myosin is 10^{-8} to 10^{-7} M (Ochiai and Chow, 2000). The actomyosin specifically dissociates with ATP, pyrophosphate, and other polyanions (Ochiai and Chow, 2000). The higher the ionic strength, the less ATP is required. Mg^{2+} is also required for the dissociation of actomyosin complex (Ochiai and Chow, 2000). However, unlike prerigor muscle, simple addition of ATP and other solubilizing compounds such as Mg^{2+} to meat does not dissociate all the myosin from actin, and extraction of myosin from postrigor meat is therefore difficult (Foegeding *et al*., 1996).

1.2.4.1.2.2 Structural and regulatory proteins

Myofibrils also contain structural and regulatory proteins at lower quantities. They are present in the myofibril filament structure, e.g., A-band, I-band, Z-disc, namely, α-,β-, -actinin, C-, M-, H-, and X-protein paratropomyosin, and others (Kijowski, 2001; Xiong, 1997a). Those are involved in contraction-relaxation of muscle **(Table 4).**

- Tropomyosin

Tropomyosin, representing 5% of myofibrillar proteins, is composed of two α-helical polypeptides wound together into a two-stranded, coiled-coil supersecondary structure. In skeletal muscle, two polypeptides, α- and β-tropomyosin, can combine to form a tropomyosin dimer. The α - and β -tropomyosin polypeptides have molecular weights of 37 and 33 kDa, respectively (Xiong, 1997). Tropomyosin and troponin are combined in a complex that regulates interaction of myosin with the thin filament (Foegeding *et al.*, 1996). In physiological conditions, it binds to F-actin at 1:7

stoichiometric ratio (G-actin), binds to troponin at a 1:1 ratio, and regulates the activity of myosin ATPase (McCormick, 1994). Each tropomyosin molecule is about 385 A° long and associates in head-to-tail fashion to form a filament that follows and associates with the coil of the F-actin filament (McCormick, 1994). The shift of tropomyosin in the actin folds due to binding or releasing calcium ions by troponin results in masking or exposing the actin active center involving in myosin binding. Formation of actomyosin complex is not feasible under masking of the center. Tropomyosin demonstrates a tendency toward head-to-tail polymerization of the molecules, and it shows a high resistance to denaturation (Kijowski, 2001).

Table 4. Contractile proteins in food myosystems

Protein	Relative Abundance (%)	Size (kDa)	Location
Myosin	50-60	470	Thick filaments
Actin	15-30	43-48	Thin filaments
Tropomyosin	5	65-70	Thin filaments
Troponins	5		Thin filaments
Troponin-C			
Troponin-I		17-18	
Troponin-T		20-24	
		37-40	
C-Protein	-	140	Thick filaments
α-Actin	-	180-206	Z-disc
Z-nin	-	300-400	Z-disc
Connective/Titin	5	700-1,000	Gap filaments
Nebulin	5	~600	N_2-line

Source: Adapted from Jafarpour and Gorczyca (2012).

- Troponin

Troponin has a molecular weight of 76 kDa. Troponin, accounting for 5% of myofibrillar proteins, consists of three subunits designated troponin C (for calcium binding), troponin I (for inhibition), and troponin T (for binding with tropomyosin)

(Xiong, 1997a). Each subunit of troponin has distinct functions. Troponin C is a calcium binding protein and confers calcium regulation to the contractile process via the thin filament. It has four sites to bind calcium ions and shows an ability to bind to other subunits in the presence of Ca^{2+}. The binding of calcium ions is associated with conformative changes in troponin C followed by regulatory action of troponin-tropomyosin complex in muscle contraction (Kijowski, 2001). Troponin I, when tested without the other subunits, strongly inhibits ATPase activity of actomyosin. Troponin T functions to provide a strong association site for binding of troponin to tropomyosin (Foegeding *et al.*, 1996).

1.2.4.1.3 Stromal protein

Stroma protein is connective tissue protein of fish flesh, representing approximately 3% of the total protein content of muscles. This is a reflection of the different structural arrangements of muscle cells in fish, compared to mammals. Connective tissue proteins are mainly collagens and elastin (Mackie, 1994; Kijowski, 2001; Sriket 2012). These proteins are neither soluble in neutral salt solution of ionic strength nor in weak solution of NaOH and HCl (0.05 M) (Kijowski, 2001). Content of connective tissue or stromal proteins varies with species. Sriket (2007) reported that white shrimp meat comprised higher stromal protein with greater pepsin-soluble collagen than black tiger shrimp meat. Changes in stromal protein or connective tissue in fish and shellfish, mainly via degradation, contribute to the softening of meat. Several proteases are able to hydrolyze collagen and other connective tissue. Ezquerra *et al.*, (1997) reported that a collagenolytic enzyme in the muscle of white shrimp *(Penaeus vannamei)* resulted in softening of muscle during iced storage. It was associated with the weakening of endomysium and the collapse of collagen fibrils (Shigemura *et al.*, 2004). Sriket *et al.* (2010) reported that the muscle softening of fresh water prawn during iced storage resulted from the degradation of collagen caused by trypsin released from cephalothorax during the extended iced storage.

1.2.5 Water-holding capacity of muscle proteins

Water holding capacity is defined as the ability of a food matrix to prevent water release from the three-dimensional structure (Chantrapornchai and McClement, 2002). This property is affected by several factors, e. g. pore and capillary size, the charges of the protein matrix, force (hydrophobic interaction, hydrogen bonds, S-S bonds, Van Der Waals forces) (Chou and Morr, 1979; Chantrapornchai and McClements, 2002), ionic strength, ion species, pH, temperature (Choi *et al.*, 2000) and the presence of low molecular weight substances (Correia and Mittal, 2000; Sawyer *et al.*, 2008). Binding of water to the surface of protein through hydrogen bonds between water molecules and charges and dipolar amino acid residues seems to be insignificant for water retention in meat (Xiong, 1997). The surroundings of myofibrils causing the increased protein charges or dipoles (high concentrations of salt and pH away from the protein isoelectric point) would bring about the increase water retention in meat (Xiong, 1997).

The water holding capacity of shrimp meat depends on many intrinsic factors and process used. After death, the muscle has gone into rigor-mortis stage and the drip losses tend to increase (Jolley *et al.*, 1980). During rigor mortis, the contraction of muscle reduces the amount of space available for water to reside in the myofibril (Huff-Lonergan. 2005). The continuous decreases in water holding capacity of black tiger shrimp and Pacific white shrimp were observed throughout the storage of 12 days at 4°C (Sriket *et al.*, 2007)

During storage, water-holding capacity of shrimp meat can be altered (Huff-Lonergan. 2005). Freezing method and frozen storage condition play a crucial role in water holding capacity of shrimp meat. Frozen black tiger shrimp with cryogenic freezing had the lower freezing loss than those subjected to air blast freezing (Boonsumrej *et al.*, 2007). Thawing process is another factor influencing the drip loss. Repeated freeze-thawing generally results in the poorer water holding capacity of shrimp meat. Higher amounts of exudate were observed in Pacific white shrimp and black tiger shrimp when the freeze-thaw cycles increased (Sriket *et al.*, 2007). Therefore, multiple freeze-thawing cycles showed the detrimental effects on the shrimp muscle. Repeated melting and reformation of ice crystals caused the damage

to cell membranes and organelles (Sriket *et al.*, 2007). Freeze-thawing was also shown to increase the cooking loss of shrimp muscle (Srinivasan *et al.*, 1997).

Freezing and thawing increase the toughness of seafood during frozen storage, mainly owing to the enhanced myosin denaturation, as well as aggregation of myofibrillar proteins (Sikorski *et al.*, 1976). Increase in shear force of Pacific white shrimp was noticeable when shrimp was frozen-stored for an extended time (Sriket, 2007). This was coincidental with the increased drip loss associated with the loss in protein solubility (Sriket, 2007).

1.2.6 Thermal stability of fish muscle protein

Heating and thermal process show the pronounced impact on quality, particularly textural property of muscle foods. The texture and taste of muscle become rapidly undesirable with excessive firmness and a lack of juiciness when the heating temperature exceeds 70°C (Mizuta *et al.*, 1999). Those changes are associated with the thermal denaturation of proteins. Protein stability under thermal stress could be described by means of the transition temperature and heat capacity. DSC has been used to study the thermal properties and stability of muscle proteins and to measure the extent of their denaturation under various processing conditions (Wright and Wilding, 1984). The transition represents point where the conformational changes occur in protein structure due to denaturation and is generally expressed as peak maximum temperature (T_{max}). When protein denatures, both inner- and intramolecular bonds are disrupted, often in a coorperative manner, or the protein is thought to change its conformation from a highly ordered state to a less ordered counterpart (Kilara and Harwarwakar, 1996). T_{max} of the first peak of muscle proteins is assumed to correspond to myosin denaturation. Peak 2 is assumed to correspond to actin denaturation. Ratthanasatheirn *et al.* (2008) reported that DSC thermogram of Pacific white shrimp meat revealed 2 major endothermic peaks with T_{max} of 50.1 and 71.3 ◦C, corresponding to myosin and actin peaks. Sriket *et al.* (2007) reported that myosin from black tiger shrimp (T_{max} = 51.28 °C) and from white shrimp (T_{max} = 50.13 °C) had the similar temperature required for denaturation. T_{max} of actin of black tiger shrimp and white shrimp were 66.20 and 71.17 °C, respectively.

Thermal denaturation of the myosin was dependent on pH and ionic strength (Wright and Wilding, 1984). Raising and lowering pH from neutral pH reduced T_{max} values and ΔH. After being treated with mixed phosphate, *T*max of both peaks (myosin and actin) of fresh Pacific white shrimp shifted to the lower temperature (Ratthanasatheirn *et al.*, 2008). Salt and pyrophosphate decreased the heat stability of Pacific white shrimp muscle proteins, leading to the denaturation at lower temperature with less energy input. The destabilizing effect of salt and pyrophosphate on the shrimp proteins affects the properties of shrimp proteins after heating or cooking. At higher salt concentration, muscle proteins may denature, resulting in stronger protein-protein bonds, shrinkage of the muscle and dehydration. This is attributed to the enhanced hydrophobic interactions and the modification of water structure in the muscle (Smyth *et al*., 1998).

1.2.7 Changes of muscle protein as affected by frozen storage and freeze-thawing process

During frozen storage, quality changes generally take place in fish and shellfish. There are three accepted theories to explain denaturation of muscle proteins during freezing and frozen storage, namely, (1) an increase in solute concentration, (2) dehydration of the cell, and (3) auto-oxidative changes that alter the balance of protein-protein and protein-water interactions (Morrison, 1993; Haard, 1992; Zotos *et al*., 1985). Water holding capacity is defined as the ability of a food matrix to prevent water release from the three-dimensional structure (Chantrapornchai and McClement, 2002). This property is affected by pore and capillary size, the charges of the protein matrix, bonding (hydrophobic interaction, hydrogen bonds, S-S bonds and Van Der Waals forces) (Chou and Morr, 1979; Chantrapornchai and McClements, 2002) ionic strength, ion species, pH, temperature, equilibrium between protein and water (Choi *et al.*, 2000) and the presence of low molecular weight substances (Correia and Mittal, 2000; Sawyer *et al.*, 2008)

As freezing proceeds, proteins are exposed to the increased ionic strength in the non-frozen aqueous phase. This leads to extensive modification of the native structure of proteins (Connell, 1995; Franks, 1995 and Lin and Park, 1998). In a dehydrated state, protein-water interactions in the tissue are disrupted, and protein

molecules are exposed to an environment that is less polar than water. These changes result in an increased exposure of hydrophobic side chains and therefore the changes in protein conformation occur (Jittinandana *et al*., 2003). Ice crystals and the increase in ionic strength of the system during frozen storage caused myosin denaturation and disruption of the actin-myosin complex, as indicated by the decrease in Ca^{2+}-ATPase activities (Benjakul and Bauer, 2000). During denaturation, which is commonly induced by freezing or frozen storage, hydrophobic and hydrogen bonds buried inside the protein molecules become exposed and broken from their native arrangement. As a consequence, conformational changes in coiled or helical section of the peptide chain occur and reform in a manner different from those in the native structure. This results in rearrangement of hydrophobic and hydrogen bonded regions on an intra- and inter-molecular basis (Morawetz, 1972). Disulfide bridges are the important covalent bonds, which relate to aggregation of protein (Sikorski *et. al.*, 1990b). The formation of disulfide bonds via oxidation of SH groups or disulfide interchanges was coincidental with the decrease in total and surface SH contents (Hayakawa and Nakai, 1985). Benjakul *et al.* (2003b) reported that ATPase activities of natural actomyosin from croaker, lizardfish, threadfin bream, and bigeye snapper decreased continuously during storage at -18 °C for 24 weeks and the degree of changes varied with species. In general, lizardfish was the most susceptible to quality changes. Total solubility of muscle protein from croaker, lizardfish, threadfin bream, and bigeye snapper in 0.6 M KCl decreased continuously during storage at -18 °C for 24 week (Benjakul *et al*., 2005). The loss in ATPase activity was due to tertiary structural changes caused by ice crystals and an increase in the ionic strength of the system (Benjakul and Bauer, 2000). The decreased solubility indicated aggregation as well as denaturation of proteins caused by the formation of disulfide bond and hydrophobic interaction during freezing and frozen storage. (Benjakul *et al*., 2005). Benjukul and Sutthipan (2009) reported chemical and physicochemical changes of muscles from hard and soft shell mud crabs *(Scylla serrata)* during 12 weeks of storage at -20 °C. Ca^{2+}-ATPase activity of natural actomyosin (NAM) from both crabs decreased continuously during storage, regardless of muscle types. After 8 weeks of storage, Ca^{2+}-ATPase activity of NAM from lump muscle of soft shell crab decreased to a greater extent than that of hard shell crab.

Temperature abuse during frozen storage or freeze-thawing generally induces the denaturation of muscle protein. After five cycles of freeze–thawing, loss of Ca^{2+}-ATPase activity, sulfhydryl group content and protein solubility with concomitant increases in disulfide bond formation and surface hydrophobicity were more pronounced in white shrimp muscle, than in black tiger shrimp muscle (Sriket *et al.*, 2007a). Sriket (2007) found that SH group of black tiger shrimp and white shrimp NAM decreased to 2.88 and 2.46 mole/10^5g protein, respectively after 5 freeze-thaw cycles.

Freezing and thawing also affect the membrane structures of muscle tissues. Normally enzymes in fresh tissue are retained in intracellular organelles. The leaked enzymes are regarded as markers of membrane damage and the activity of lysosomal enzymes in the centrifuged tissue fluid has been used to differentiate frozen from fresh fish (Rehbein, 1988). Membrane integrity was estimated as the volume of centrifuged tissue fluid (CTF) and by lysosomal β-N-acetyl-glucosaminidase activity in CTF (Nilsson and Ekstrand, 1995). When the number of freeze-thaw cycles of cod and catfish increased, the activities of α-glucosidase and β-N-acetyl-glucosaminidase increased, suggesting the greater disintegration of membrane structure (Benjakul and Bauer, 2001). Sriket *et al*. (2007b) found that white shrimp had the greater exudate loss, higher α-glucosidase (AG) as well as β-N-acetyl-glucosaminidase (NAG) activities than did black tiger shrimp, especially when the number of freeze-thaw cycles increased.

In general, the deterioration in texture, flavor, and color of muscles frequently occurs during frozen storage (Benjakul and sutthipan, 2009). After 5 freeze-thaw cycle, shear force of black tiger shrimp and white tiger shrimp were decreased (Sriket, 2007). The decrease in shear force suggested the loss in integrity of muscle fiber, leading to the weakening of muscle. Repeated melting and reformation of ice crystals caused the damages of cell membranes, organelles as well as muscle structure (Srinivasan *et al.*, 1997). Moreover, the destruction of Z-disks was more pronounced in white shrimp after freeze-thawing. MHC was also crosslinked through disulfide and nondisulfide covalent bonds (Sriket, 2007).

1.2.8 Quality index and acceptability of shrimp

The appearance is one of major quality attributes, which plays a significant role in consumer acceptance (Teerawut and Patumchart, 2014). Various chemical and physical changes taken place after harvesting and during storage through the processing are related with color changes. For shrimp quality, color is foremost importance (Ahmed and Shivhare, 2001). Changes in color occur at the time of harvest, prior to cooking, and after cooking (Teerawut and Patumchart, 2014).

For raw shrimp, translucence is a desired characteristic but shrimp turns to be darker with extended storage. The change in color is dependent on sample composition and storage temperature (Imran *et al.*, 2013). Unfavorable color change associated with melanosis on the surface of shrimp products has been of great concern to shrimp processors (Nirmal and Benjakul, 2011). Melanosis or blackspot is a natural post-mortem process originated by the polymerization of phenols into insoluble black pigments, the melanins, by polyphenoloxidase (also called phenoloxidase) followed by non-enzymatic polymerization of the quinones, giving rise to dark pigments of high molecular weight (Nirmal *et al.*, 2009). When the storage time increased, the melanosis score of Pacific white shrimp increased continuously (Nirmal *et al.*, 2010). To retard or prevent melanosis, pretreatment of shrimp with catechin or feluric acid (Nirmal and Benjakul, 2009) or green tea extract (Nirmal and Benjakul, 2011a) or lead seed extract (Nirmal and Benjakul, 2011b) before ice storage were reported.

The red-orange color of cooked shrimp is the most attractive component of the color product (Lucien-Brun and Vidal, 2006). Astaxanthin is a red-orange carotenoid found in fish and shellfish. It is mainly associated with the color of invertebrate animals such as shrimp, crabs and lobster. Generally, it presents in crustaceans as a protein-pigment complex (Armenta-Lopez *et al.*, 2002). This complex can be green, purple or blue in the living animal. However a red color is formed when being subjected to heat treatment (Britton, 1996). The color depends upon the carotenoid content (Okada *et al.*, 1994).. Shrimp without sufficient pre-cooking have faced melanosis, particularly during the extended storage (Manheem *et al.*, 2013). Manheem *el al.* (2013) found that shrimps pre-cooked with longer time showed a lower development of black spots as evidenced by a lower melanosis score throughout storage of 7 days at 4 °C. Furthermore, some additive used in cooked for cooking

shrimp can have the impact on color. Lopkulkiaert *et al.* (2009) reported that the sample treated with sodium chloride and sodium bicarbonate containing traces of citric acid caused a brighter but less reddish and yellowish color, compared to the precooked sample. Additionally, heating consition also determines the color of shrimp. Tepaneyasin *et al.* (2004) found that use of a constant inlet air temperature of 100°C yielded the dried shrimp of the best quality, especially high value of redness, compared to shrimp dried using other conditions

Texture is another important attribute affecting shrimp acceptability (Monaco *et al.*, 2007) and is defined as an expression of structural, mechanical and surface attributes detected through human senses (Szczesniak, 2002). Shrimp meat should be firm and meets the customer expectation. Unwanted textural changes such as muscle softening often occur in shrimp, particularly which shrimp stored after capture for a long time. Susceptibility toward texture softening is dependent on species and level of proteolytic enzyme. Calpain system and the cathepsins, in synergy, are suggested to be responsible for post mortem muscle protein degradation (Delbarre-Ladrat *et al.*, 2006). Sriket *et al.* (2010) reported that the muscle softening of fresh water prawn during iced storage resulted from the degradation of collagen situated in the non-helical region rather than myofibrillar and sarcoplasmic proteins. During the storage, autolysis of cephalothorax, where hepatopancreas and other internal organs are located, could take place, thereby releasing the active proteases into the muscle. Hepatopancreas extracts from crustacean contain proteinases such as trypsin, chymotrypsin and collagenases, which are capable of degrading the native collagen under physiological conditions (Garcia-Carreo *et al.*, 1994). This leads to mushy texture and undesirable characteristic.

Shrimp has been known to posess savory taste and flavor. This makes shrimp very popular for customers. Nevertheless, some cultured shrimps have faced off-flavor, particularly muddy flavor, grassy or soil-like taste and odor called ‘corn smell’ (or ‘olor a choclo’in Ecuador). This type of flavor causes customers to react immediately (Lucien-Brun and Vidal. 2006). This ‘off flavor’ is the result of an excess of certain types of blue green algae or cyanobacteria in the pond. Those microorganisms produce the compound causing off- flavor, named geosmin and MIB

(Yarnpakdee *et al.*, 2014). Those compounds can be accumulated in shrimp muscle (Yarnpakdee *et al.*, 2014). Off-odor associated with the spoilage or deterioration is one of major cause for consumer rejection. Sriket (2007) reported that Pacific white shrimp and black tiger shrimp had the decreasing sensory score when storage in ice for 8 days. This was in accordance with the increase in microbial load, especially spoilage bacteria e.g. *Pseudomonas* sp. (Sriket, 2007). Those off-flavor or off-odor become a frequent reason for major quality claims (Lucien-Brun and Vidal. 2006) as well as rejection by market.

1.2.9 Food additives for yield improvement and cryo-stability

Yield has been considered as the prime factor since it is closely related with increasing revenue or profit. Uptake of water into the fish/shellfish has been a strategy commonly implemented in fish/shellfish processing industry. Some additives with capacity of water binding or holding have been widely used. In addition to increase the weight or yield, some additives have been known to retard the denaturation of protein associated with quality changes of muscle during the extended frozen storage. With the uses of those additives, the quality and shelf life of product can be increased.

1.2.9.1 Phosphates

Phosphate compounds have been widely used in seafood products to improve the qualities such as increased bound water and cooking yields (Chantarasataporn *et al*., 2013). Certain chemical properties of phosphates enable them to produce a wide variety of effects in food products such as the adjustment of pH, buffer properties, sequestration of selected cations, changing the ionic charge distributions, changing the ionic strength of environment and/or bacteriostatic effect (Dziezak, 1990). Several commercial phosphates are used in the meat and fish industry and they differ from one another in their properties **(Table 5).**

Table 5. Classes, formulas, pH, solubility, and functions of several phosphates

Class of phosphate and basic structure	Phosphate name	Generally accepted formula	pH (1% solution)	Solubility at 25% (g/100g water)	Function
Orthophosphate	Monosodium phosphate	NaH_2PO_4	4.6	87	Emulsifier, buffer
	Disodium phosphate	Na_2HPO_4	9.2	12	Emulsifier, buffer
	Disodium phosphate dehydrate	$Na_2HPO_4.2H_20$	9.1	15	Emulsifier, buffer
	Trisodium phosphate	Na_3PO_4	11.8	14	Emulsifier, buffer
	Monosodium phosphate	NaH_2PO_4	4.6	25	Water binding in meats
	Dipotassium phosphate	K_2HPO_4	9.3	168	Emulsifier, buffer
	Trisodium phosphate	Na_3PO_4	11.9	107	Emulsifier, buffer
	Monocalcium phosphate	$Ca(H_2PO_4).H_2O$	3.8	-	Acidulant, leaving acid, dough condition, yeast food, nutrient
Condensed phosphate					
pyrophosphate	Sodium acid pyrophosaphate	$Na_2H_2P_2O_7$	4.3	15	Emulsifier, buffer, sequestrant, water binding in meat
	Tetrasodium pyrophosphate	$Na_4P_2O_7$	10.3	8	Dispersant, coagulant, crysrallization inhibitor in canned tuna
	Tetapotassium pyrophosphate	$K_4P_2O_7$	10.5	187	Emulsifier, water binding in meat
tripolyphosphate	Sodium tripolyphosphate	$Na_5P_3O_{10}$	9.9	15	Emulsifier, water binding in meat
	Potassium tripolyphosphate	$K_5P_3O_{10}$	9.6	193	Emulsifier, water binding in meat
Long chain polyphosphate	Sodium polyphosphates, glassy or Graham's Salt;	$(NaPO_3)_6.Na_2O$	7.7	40	Sequestrant, emulsifier, water binding in meat, suspending agent
	Three chain lengths;	$(NaPO_3)_{13}.Na_2O$	6.9	40	Sequestrant, emulsifier, water binding in meat, suspending agent
	Sodium hexametaphosphate has an average chain length 0f 13	$(NaPO_3)_{21}.Na_2O$	6.3	40	Sequestrant, emulsifier, water binding in meat, suspending agent
Metaphosphate	Sodium trimetaphosphate	$(NaPO_3)_5$	6.7	23	
	Sodium tetrametaphosphate	$(NaPO_3)_4.4H_2O$	6.2	18	

Source: Dziezak (1990).

Phosphate act as polyelectrolytic to increase ionic strength, resulting in increased water holding capacity by direct binding of water to the phosphate and inducing the repulsion of proteins by introducing negative charges on the protein group. This repulsing effect opens up protein structure, and increase the number of binding sites available for water, which allows for more water to be retained in the meat (Xiong, 2005). Phosphate solutions have been used to increase cooking yield and lower cooking loss of Pacific white shrimp. However, water holding capacity depends on types of phosphate (Rattanasatheirn *et al.*, 2008). The enhancing effect of phosphate compound was in the descending order: tetra-sodium pyrophosphate (TSPP)> sodium tripolyphosphate (STPP)> sodium hexametaphosphate (SHMP)> monopotassium phosphate (MKP)>control (non phosphate) (Madcharoen, 2003).

In general, phosphates have been used in combination with salt, especially NaCl to enhance the efficacy in water uptake or quality improvement. Such preferential binding for chloride ions by protein molecule at pHs above the isoelectric point increases its net negative charge and results in repulsive forces, thus permitting additional water absorption within the protein network (Albarracin *et al.*, 2011). In contrast, at pHs below the isoelectric point, the positive charge of protein is neutralised by chloride ions, thereby reducing net positive charge and water-holding capacity (Albarracin *et al.*, 2011). Madcharoen (2003) reported that TSPP (2.5%) in combination with 2.5% NaCl showed a synergistic effect on water holding capacity of black tiger shrimp, especially as soaking time increased up to 2 h. Faithong *et al.* (2013) found that soaking Pacific white shrimp with 2% tetrasodium pyrophosphate for 8 h or with 2% sodium tripolyphosphate for 10 h in the presence of 2% NaCl was effective in increasing the weight gain and lowering cooking loss, compared with those without phosphate treatment. Rattanasatheirn *et al.* (2008) reported that Pacific white shrimp *(Litopeneous vannamei)* soaked in 2.5% NaCl containing 0.875% sodium acid pyrophosphate (SAPP) and 2.625% tetrasodium pyrophosphate (TSPP) were generally less translucent and had high weight gain and cooking yield along with low cooking loss. Manheem (2013) reported that soaking of Pacific white shrimp with 3% mixed phosphates (TSPP/STPP: 2:1) solution in the presence of 2.5% NaCl before cooking was able to increase water retention, thereby increasing cooking yield.

Phosphates have been abused in the industry for years, and excessive use leads to water losses and adverse effects on flavor. Additionally, they might bring about translucent and crispy products with a slimy texture (Rattanasatheirn *et al.*, 2008; Chantaraporn *et al.*, 2013). Rattanasatheirn *et al.* (2008) reported that shrimp, especially, ice stored shrimp, was more translucent after being soaked in phosphate solution. This was concomitant with disappearance of M-line. Nowadays, there are strict regulations on the use of phosphates in many countries and the consumers have highly negative feelings with respect to their use in seafoods.

1.2.9.2 Sodium bicarbonate

Sodium bicarbonate or sodium hydrogen carbonate is the chemical with the formula of $NaHCO_3$. Sodium bicarbonate is a white solid that is crystalline but often appears as a fine powder. It has slightly salty and alkaline taste, resembling that of washing soda (sodium carbonate) (Dyni and Jones, 1998). It is among the food additives encoded by European Union, identified by the initials E 500. It has been used as non-phosphate additives for fish and meat industry.

$NaHCO_3$ at high concentration also enhanced the unfolding of NAM molecule, in which SH groups were more exposed. The breakdown of disulfide 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). Xiong and Delles (2009) explained that bicarbonate effectiveness is due to the ability to partially solubilize myofibrillar proteins and increase their electrostatic repulsion by raising the pH. This function causes a transverse swelling of myofibrils, permitting higher water absorption and retention. Sodium bicarbonate has 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 flavour in sow meat (Sindelar *et al.*, 2003). Soaking Pacific white shrimp in 2.0% $NaHCO_3$ in the presence of 2.5% NaCl for 4 h at 4 °C (pH 8.5) increased the yield and lowered cooking loss without negative effect on sensory properties (Chantarasuwan *et al.*, 2011). Sen *et al.* (2014) reported that treatment of pre- and post-chill breast meat with 3% sodium bicarbonate containing

2% NaCl could enhance water holding capacity, cooking yield and sensory attribute, compared with control after storage at 4 °C for 24 h. Wynveen *et al.* (2001) found that injection of mixed solution (0.23% sodium bicarbonate and 0.07% NaCl) was effective in preventing post mortem pH decline and appeared to improve the quality of pork. Sheard and Thali (2004) demonstrated that the marinating pork loin with 3% sodium bicarbonate containing 5% NaCl gave the higher yield (84%) than the control (70%).

1.2.9.3 Sodium carbonate

Sodium carbonate (also known as washing soda, soda ash and soda crystals), Na_2CO_3, is a sodium salt of carbonic acid (soluble in water). It most commonly occurs as a crystalline heptahydrate, which readily effloresces to form a white powder, the monohydrate. Pure sodium carbonate is a white odorless powder that absorbs moisture from the air. It has an alkaline taste and forms a strongly alkaline water solution. Sodium carbonate is domestically well known for its everyday use as a water softener. It became known as "soda ash " because of the sodium content (Dyni and Jones, 1998).

Sodium carbonate lowers the H+ ion concentration. When the pH of the intramuscular aqueous phase shifts away from the isoelectric point of myosin, electrostatic repulsion increased, leading to the expansion of myofilament lattices. This expansion would increase protein surface to promote hydrogen bonding and electrostatic interactions between water and muscle proteins (Xiong and Delles, 2009). Sodium carbonate is widely used as a marinade in Chinese cookery (Skurray *at al.*, 1986). Chantarasuwan *et al.* (2011) reported that shrimp treated with 2.5% NaCl containing 2.0% sodium carbonate for 4 h at 4 °C (pH 8.5) had the reduced cooking loss and increased cooking yield. However, the effectiveness was lower, when compared with 2.0% sodium bicarbonate under the same condition used. CO_3^{2-}, a divalent ion, could provide the net negative charge for 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, leading to higher solubilization associated with higher loss.

1.2.9.4 Hydrocolloids and polysaccharides

Hydrocolloids or gums are a diverse group of long chain polymers characterized by their property of forming viscous dispersions and/or gels when dispersed in water. These materials were firstly found in exudates from trees or bushes, extracts from plants or seaweeds, flours from seeds or grains, gummy slimes from fermentation processes, and many other natural products (Milani and Maleki, 2012). In addition, they produce a dispersion, which is intermediate between a true solution and a suspension, and exhibit the properties of a colloid. Considering these two properties, they are appropriately termed as 'hydrophilic colloids' or 'hydrocolloids' (Milani and Maleki. 2012.) Hydrocolloids have been widely used in food products to modify texture, improve moisture retention, control water mobility, and maintain overall product quality during storage (Rodge *et al*., 2012). They are readily available and they are not expensive. Most of them are compatible with muscle proteins and can improve the yield without negative effect on texture. Generally, they do not affect significantly the color of products. Hydrocolloid or gums have been used to improve water holding capacity of products (Ramírez *et al.*, 2011). Occurrence of a large number of hydroxyl groups noticeably increases their affinity for binding water molecules.

The effects of carrageenan on the water holding capacity of different kinds of gelled meat products (especially based beef meat) have been extensively studied. Addition of increasing levels of carrageenan to low fat meat balls was more effective than guar gum for the textural properties after cooking (Ulu., 2006). Hsu and Chung (2001) observed an increase in cooking yield, hardness, and other textural profile analysis parameters by adding up to 2% carrageenan to low-fat emulsified meatballs. Ayadi *et al.* (2009) reported that 0.5%-1.5% carrageenan caused a decrease in emulsion stability, and an increase in water holding capacity, hardness and cohesiveness of the formulated sausage samples.

Alginate is of particular interest for a broad range of applications because it has many functional properties; I. physical properties (Solubility, selective ion binding and gel formation), II. material properties (stability and ion cross linked gel) and III. biological properties (Draget *et al.*, 2002). Its applications span from traditional technical utilization to foods and biomedicine as well as other industries

(Prompaphagorn, 2008). Hong and Chin (2010) reported that 5% sodium alginate contributed to the improved water-binding ability and cold-set porcine myofribrillar protein gel formation. Alginate was added into Alaska pollock surimi to increase the water retention ability (Kim, 2003). However, shear stress and shear strain values were reduced. Cong- Gui *et al.* (2006) showed that pork muscle had the increased enhanced water-holding capacity (WHC) as sodium alginate increased from 0.25% to 1.0%. Juemanee *et al.* (2009) reported that frozen battered shrimp burger with optimized formulation (0.3% modified tapioca starch + 0.7% sodium alginate) had a higher moisture content and juiciness scores, compared with the control.

1.2.9.4.1 Alginate oligosaccharide

Oligosaccharides are linear or branched carbohydrates consisting primarily of 2-20 sugar units. Most commonly seen oligosaccharides include fructooligosaccharides, galactooligosaccharides, isomaltooligosaccharides, soybeanoligosaccharides, and xylooligosaccharides (Chou *et al.*, 2010). Oligosaccharides have similar mouth feel to sugar but are only 20-70% as sweet as table sugar, and have been reported to possess unique functional characteristics, including promoting growth of intestinal probiotics, increasing bowel movement, and lowering serum cholesterol and triglycerides (Chou *et al.*, 2010). Alginate oligosaccharide and their derivatives have been widely used as releasing agents in pharmacy, and additives in food industry. Recently, alginate oligosaccharide and their derivatives have been attracting considerable attention due to their bioactivity of antitumor and promoting plant growth (Li *et al.*, 2010).

Alginate oligosaccharide has gained increasing interest as the additive for increasing water holding capacity of food systems. Due to hydroxyl group, it can bind with water via H-bond. Zhang *et al.* (2013) studied the effect of alginate oligosaccharides on water-holding, textural and flavor properties of fresh, frozen and boiled shirimp *(Litopenaeus vannamei)* meat. Alginate oligosaccharides with average degree polymerization of 3~6 were used. After treatment, weight gain of fresh shrimp was significantly improved. The drip loss of frozen shrimp after thawing was significantly decreased, and the product yield of boiled shrimp meat was also increased significantly. Furthermore, Li *et al.* (2010) reported that soaking shrimp

with 1% (w/w) solution of alginate oligosaccharides could increase yield up to 11.7%, which was significantly higher than 3% phosphate treated sample with the yield of 6.2%. After being frozen storage of 10 days, drip loss was lower than shrimps treated with phosphate.

1.2.9.5 Protein and protein hydrolysate

The addition of functional proteins as water- and fat-binding agents to muscle food has been practiced and is well established in the meat industry. Commercially available proteins are obtained both from animal and plant sources. The functionality of protein ingredients is dependent on their origin, molecular structure, method of isolation, various modifications of the isolated proteins, and their interactions with other ingredients in the food products (Thorarinsdottir *et al.*, 2001). Proteins are added to foods for various reasons to improve water- and fat-binding properties, nutritional value, viscosity, gelation, emulsification, and foaming properties. Soy protein isolates may be used in emulsified fish products to improve water- and fat-binding properties of products (Thorarinsdottir *et al.*, 2001). When soy protein was combined with salt and phosphates, the performance was better than salt and phosphate alone. However the appearance was adversely affected in frozen cod fillets (Thorarinsdottir *et al.*, 2004). Pietrasik (2003) reported that 2% egg albumin had a synergistic effect with carrageenan to improve water binging capacity and textural properties of cooked beef homogenates. Jafarpour *et al.* (2012) demonstrated that 3% egg white power significantly improved texture properties of common carp *(Cyprinus carpio)* surimi gel. Tabilo-Munizaga and Barbosa-Canovas, (2005) found that surimi gels from Alaska pollock and Pacific whiting added with 1% egg white and induced by high pressure had higher water holding capacity compared with the control.

Additionally, protein hydrolysates have gained increasing interest due to their functional properties**.** The main functional properties of protein hydrolysates include solubility, water holding, emulsifying and foaming properties (Kristinsson and Rasco, 2000). Protein hydrolysates are highly hygroscopic. The presence of polar groups such as COO^- and NH_3^+ generated during enzymatic hydrolysis has a substantial effect on the amount of adsorbed water and moisture sorption isotherm for these materials (Kristinsson and Rasco, 2000b). Roselle seed protein hydrolysates were also

found to be effective in enhancing water-holding capacity and cooking yield in a meat model system (Tounkara *et al.,* 2013). Shrimp soaked in 4% NaCl containing 7% protein hydrolysate from salted egg white and 2.5% mixed phosphates (0.625% sodium acid pyrophosphate [SAPP] and 1.875% tetrasodium pyrophosphate [TSPP]) had the highest cooking yield with the lowest cooking loss (Keawmanee *et al.,* 2009). Canola protein hydrolysate (1%) was found to improve water holding capacity and cooking yields of meat product (Cumby *et al.* 2008). Kittiphattanabawon *et al.* (2012) reported that gelatin hydrolysate with 10%DH was able to prevent the denaturation of surimi protein comparably to commercial cryoprotectant. The highest Ca^{2+}-ATPase activity with the coincidental lowest surface hydrophobicity was observed after repeated freezing-thawing, compared with the control. Nikoo *et al.* (2014) indicated that peptide Pro-Ala-Gly-Tyr (PAGT) at a level of 25 ppm showed the preventive effect against water loss in Japanese sea bass mince induced by freeze/thaw process.

1.2.9.6 Amino acid

Amino acids are biologically important organic compounds, composed of amino ($-NH_2$) and carboxylic (-COOH) functional groups, along with a side-chain specific to each amino acid (Damodaran. 1996). In addition to differences in size, these side groups carry different charges at physiological pH (e.g., nonpolar, uncharged but polar, negatively charged, positively charged); some groups are hydrophobic (e.g., branched chain and aromatic amino acids) and some hydrophilic (most others) (Damodaran. 1996). These groups exhibit different effectiveness in binding with water molecule (Damodaran. 1996). Some amino acids have been used as cryoprotectant (Fungfuang *et al.,* 2009; Shima *et al.,* 2003). The dicarboxylic amino acids, glutamic acid and aspartic acid, showed good cryoprotective properties, while their acid amides, glutamine and asparagine, did not show any appreciable effects. Among the basic amino acids, lysine, ornithine and histidine showed some cryoprotective effect, whereas arginine showed only a little effect (Matsumoto and Noguchi, 1992). Mohammed *et al.* (2007) reported that amino acids (arginine, lysine and histidine) as potential cryoprotectants for the stabilisation of liposomes. Amino acids not only offer hydrogen bonds, but also provide electrostatic interactions for effective lyophilisation (Mohammed *et al.,* 2007). In sperm cryopreservation, the

addition of L-glutamine into the cryopreservation solution prevents the sperm from freezing and thawing stress and enhances the post-thaw motility of frozen/thawed sperm (Takeo and Nakagata, 2010).

1.2.9.7 Sugar and Sugar alcohol

Sugars or sugar alcohols of low molecular weight are common ingredients in a variety of processed foods (Zhou *et al.*, 2006: Baek *et al.*, 2004). They alter the thermal and physical properties of the food system (Baek *et al.*, 2004). Sugars can behave differently, possibly depending on the food system and storage conditions. Sugars may be used either as plasticizers, like water, affecting the thermal transitions of food system, or as water immobilizers that reduce the mobility of water in the food system (Baek *et al.*, 2004). The role of sugars and sugar alcohols as cryoprotectants in frozen food systems is widely accepted (Roos, 1993; Slade and Levine, 1991). Cryoprotectants are known to lower the denaturation and/or aggregation of myofibrillar protein during frozen storage, thereby maintaining functional properties, such as gel-forming ability, water holding capacity and solubility of proteins. (Kittiphattanabawon *et al.*, 2012). Hydroxy groups of sugars and sugar alcohols interact with water molecules, thus contributing to retardation of ice crystallization. Uedaira *et al.* (1990) proposed that the sugar molecule having a larger number of -OH groups had a stronger stabilizing effect on the structure of water surrounding the sugar molecule. The average number of -OH groups is smallest in ribose and increase in the order fructose, mannose, xylose, glucose, sucrose, maltose and trehalose (Uedaira and Ishimura, 1989). Sugar and sugar alcohol have been used as cryoprotectant in surimi (Kittiphattanabawon *et al.* 2012; Jin *et al.*, 2011; Zhou *et al.*, 2006;) starch (Baek *et al.*, 2004) and meat simulation (Chou *et al.*, 2010). Wu *et al.* (2014) reported that addition of trehalose (2.5-10 g dry weight) to myofibrillar protein from *Lateolabrax japonicas* (100 g) showed an effectiveness to prevent the loss of Ca^{2+}-adenylpyrophosphatase (ATPase) activity, solubility, sulfhydryl content, and to maintain unfrozen water content of myofibrils during storage at -18°C for 90 days. Imelda *et al.* (2009) reported that Pacific cod surimi containing cryoprotectant (sucrose: sorbitol blend, ratio 1:1) had the reduced cooking loss after 6 freeze/thaw cycles, compared with the control (without cryoprotectant).

1.2.10 Objectives

1. To study the effect of various phosphate and bicarbonate replacers and some factors on water holding capacity and characteristics of raw and cooked Pacific white shrimp.
2. To elucidate the mechanism of the selected phosphate and bicarbonate replacer in improvement of water holding capacity of Pacific white shrimp muscle.
3. To study the impact of some additives in combination with the selected phosphate and bicarbonate replacer on quality of raw and cooked Pacific white shrimp.
4. To investigate the effect of freeze-thawing on quality of frozen raw and cooked shrimp treated with the developed phosphate and bicarbonate replacer.

1.2.11 References

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CHAPTER 2

EFFECT OF STRONG ALKALINE SOLUTIONS ON YIELD AND CHARACTERISTICS OF PACIFIC WHITE SHRIMP *(LITOPENAEUS VANNAMEI)*

2.1 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 soaking solution (12.24-13.02) and soaked shrimp muscle (6.84-9.44) increased as the concentrations of 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. Thus, further improvement for the use of strong alkali as shrimp soaking solution is still required.

Keywords: Strong alkaline solution, yield, Pacific white shrimp, characteristics.

2.2 Introduction

Shrimp is the fishery product with the most representative international economy, 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 lead to the changes in quality attributes, affecting textural and physicochemical properties and causing loss of weight. 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 widely used. Phosphate and bicarbonate have been widely used in conjunction with NaCl to exploit their synergistic action (Murphy and Zerby, 2004; Chantarasuwan *et al*., 2011b). Those additives are used in fish and fishery products to improve yield as well as quality, 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; Chantarasuwan *et al*., 2011b; Manheem *et al*., 2012; Carneiro *et al*,.2013 and Rattanasatheirn *et al*., 2008). The mechanism for quality improvement of muscle foods by phosphates is based on the ionization of protein molecules. 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, bicarbonate effectiveness 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 a transverse swelling of myofibrils, permitting higher water absorption and retention.

Due to the strict regulation in the uses of phosphate and bicarbonate as the processing aid in shrimp and shrimp products, other alternatives, especially alkaline compounds, can be considered as the agent with the equivalent property in improving quality of shrimp. However, the conditions for treatment and some factors could determine their efficacy 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.

2.3 Materials and methods

2.3.1 Collection and preparation of shrimp

Pacific white shrimp (*Litopeneaeus vannamei)* (55-60 shrimp/kg) were purchased from a local market in Hat Yai, Songkhla province, Thailand. Shrimp with storage time less than 6 h after capture were stored in the insulated box containing 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.

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

2.3.2.1 Preparation of shrimp treated with alkaline solutions

Shrimp (peeled and deveined) were mixed with KOH and NaOH solutions with various concentrations (0.125, 0.25, 0.5 and 0.75%, w/v) in the presence of 2.5% NaCl 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) (Manheem, 2012) and in 2.5% NaCl containing 2% $NaHCO_3$ (Chantarasuwan *et al.*, 2011b) were used as the positive controls. Sample without soaking was also used as the control. Prior to soaking, all solutions were measured for pH.

After treatments, 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 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 analyzed as follows:

2.3.3 Analyses

2.3.3.1 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:

Weight gain (%) = [(B-A)/A] × 100

where: A = initial weight (before soaking)

B = weight after soaking, followed by draining

2.3.3.2 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 equation:

Cooking loss (%) = [(B-C)/B] × 100

Cooking yield (%) = (C/A) × 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

2.3.3.3 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 (Sartorious North America, Edgewood, NY, USA).

2.3.3.4 Determination of NaCl content

NaCl content of both raw and cooked shrimp was determined as per the method of AOAC (2000). Sample (1 g) was added with 10 ml of 0.1 M $AgNO_3$ and 10 ml of concentrated 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. Thereafter, 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, UK) and adjusted to 100 ml with distilled water. The mixture was titrated with standardized 0.1 M KSCN until the solution became permanent brownish red. The salt content was then calculated as follows:

$$NaCl\ (\%) = 5.8 \times [(V_1 \times N_1)-(V_2 \times N_2)]/W$$

where, V_1 =volume of $AgNO_3$ (ml); N_1 =concentration of $AgNO_3$ (M); V_2=volume of KSCN (ml); N_2=concentration of KSCN (M); W= weight of sample (g)

2.3.3.5 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 for measurement using a Hunterlab colorimeter (Hunter Associates Laboratory, Inc., Reston, VA, USA), using a CIE Lab scale (Young and Whittle, 1985).

2.3.3.6 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.

2.3.3.7 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 into solutions.

SDS-PAGE was performed using 10% running 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 *xg* 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 protein was estimated.

2.3.3.8 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, odor, taste, texture and overall likeness. Samples were presented in the plates coded with three-digit random numbers.

2.3.3.9 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 (4x4x4 mm) with a razor blade. The prepared samples were fixed in 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 20-100% 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.

2.3.4 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).

2.4 Results and discussion

2.4.1 pH

The pHs of various solutions and pH of soaked shrimp are shown in **Figure 2a** and **2b,** respectively. In general, pHs of solutions increased when the concentration increased ($P<0.05$). pHs of both alkaline solutions were higher than those of the mixed phosphate and bicarbonate solutions. NaOH and KOH are strong alkali and can completely ionized in water. 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). For pH of mixed phosphates, slightly alkaline pH (9.4-9.5) was obtained, reflecting alkaline nature of those phosphates. In general, pH of shrimp meat increased when pH of solution increased ($P<0.05$). However, the pHs of shrimp meat was much lower than those of solutions. 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 soltion ($P<0.05$). 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 ($P\geq0.05$). The pH changes of shrimp meat more likely determined the changes in muscle, particularly the modification of charge as well as conformation of protein (Chantarasuwan *et al.*, 2011b).

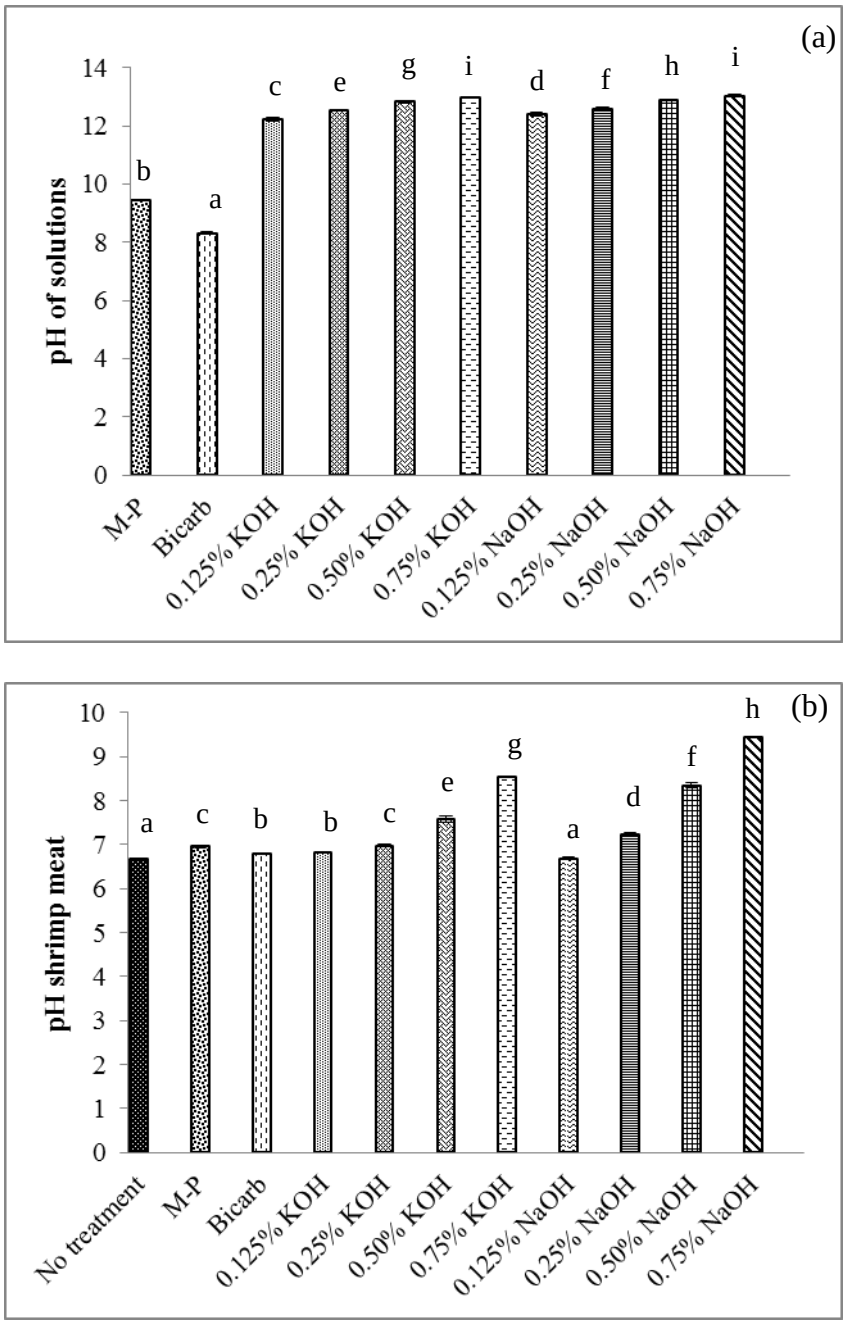

Figure 2. pH of initial solution (a) and pH of meat (b) of Pacific white shrimp after soaking in KOH and NaOH solutions at different concentrations in the presence of 2.5% NaCl. Note: M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1 (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).

2.4.2 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 2.5% NaCl are shown in **Figure 3.** Weight gain **(Figure 3a)** and cooking yield **(Figure 3b)** 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, proteins had negative charge, in which protein molecules repulsed each other. This resulted in the swollen muscle fiber, 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 3c.** 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 increase 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.

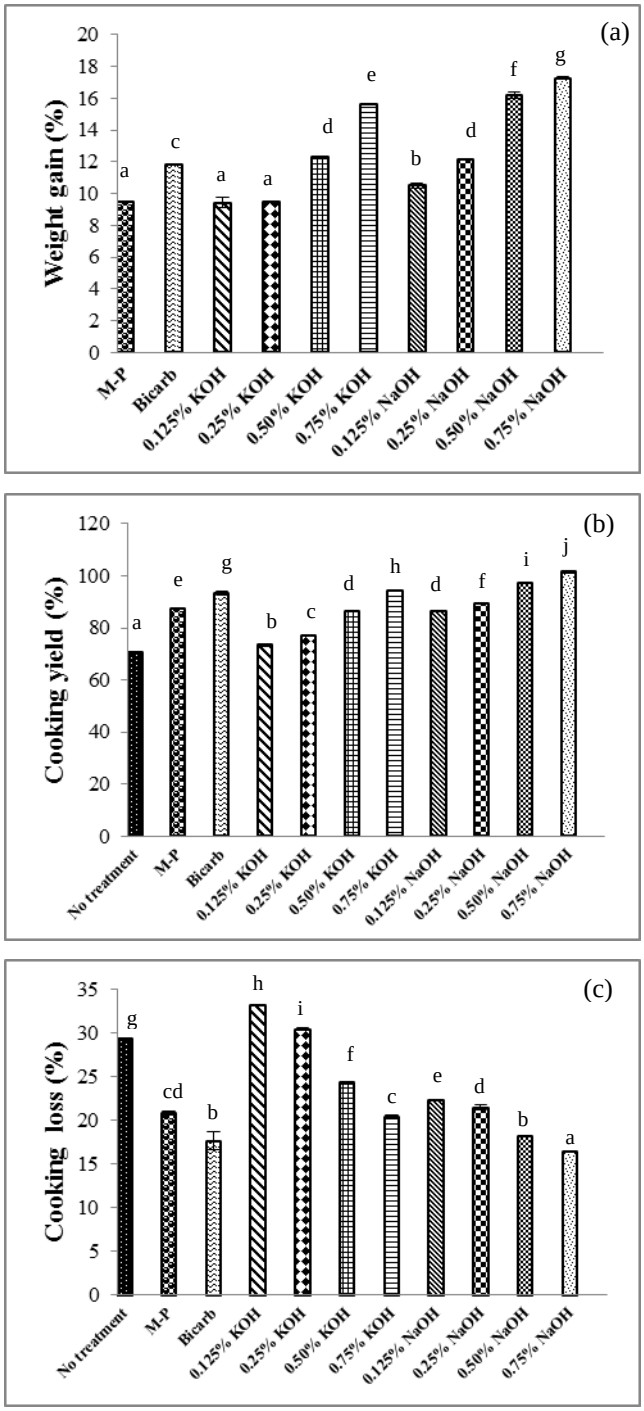

Figure 3. Weight gain (a), cooking yield (b) and cooking loss (c) of Pacific white shrimp after soaking in KOH and NaOH solutions at different concentrations in the presence of 2.5%. Note: M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 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).

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 negative charge of muscle proteins might bind with water, thereby enhancing water binding capacity (Chatarasuwan *et al.*, 2011b).

2.4.3 Salt content

Salt contents (dry weight basis) of raw and cooked shrimp treated with KOH and NaOH solutions at different concentrations in combination with 2.5% NaCl are shown in **Figure 4.** Similar NaCl content was found in all samples, except for the control, which had the lowest NaCl content ($P<0.05$). It was noteworthy that all solutions used contained 2.5% NaCl. During soaking, NaCl could be penetrated into the meat, regardless of alkaline compound used. 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). The increase in salt content was noticeable in Pacific white shrimp as the pH of solution (2.0% sodium carbonate and 2% sodium bicarbonate) increased. (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, 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. Therefore, NaCl could penetrate into muscle as indicated by the increase in NaCl content in the treated shrimp.

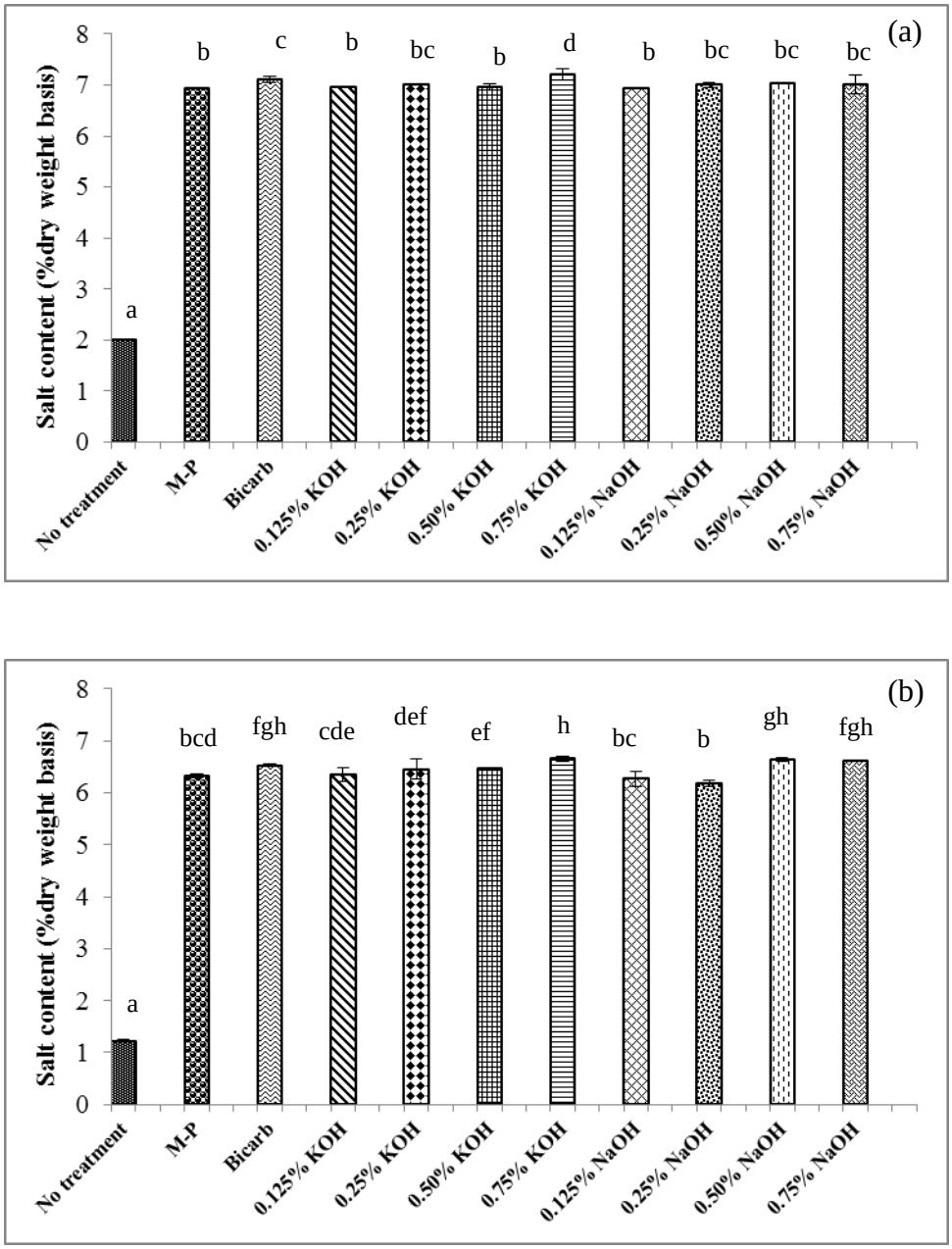

Figure 4. Salt content of raw (a) and cooked (b) of Pacific white shrimp after soaking in KOH and NaOH solutions at various concentrations in the presence of 2.5% NaCl. Note: M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1 (w/w)).; 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).

2.4.4 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 6**. Color is one of the quality parameters of shrimp *(Litopenaeus vannamei)* (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 associated with the swollen structure, in which the light could pass through and was associated with increased transparency. Shrimp treatment 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 but the translucence depended on pH of solution.

Shrimp turned to be red after being treated with both alkaline solutions **(Figure 5).** This was coincidental with the increase in *a** value **(Table 6)**. At alkaline pH, carotenoproteins could be partially solubilized in soaking solution. Also, alkaline pH could induce the denaturation of carotenoid proteins, leading to the appearance of red color caused by free carotenoids, especially astaxanthin (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 induced by both alkaline solution 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.

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 **(Figure 6).** This was in accordance with the decreases in both *a** and *b** values **(Table 6).** 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 carotenoid proteins could possess the highest content of pigments retained. Dense structure caused by the intense aggregations of muscle protein under the environment with less water was also contributed to the turbidity and redder in color **(Figure 6).**

Table 6. 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 †,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).

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 superscripts in the same column under the same state of sample indicate the significant differences ($P<0.05$).

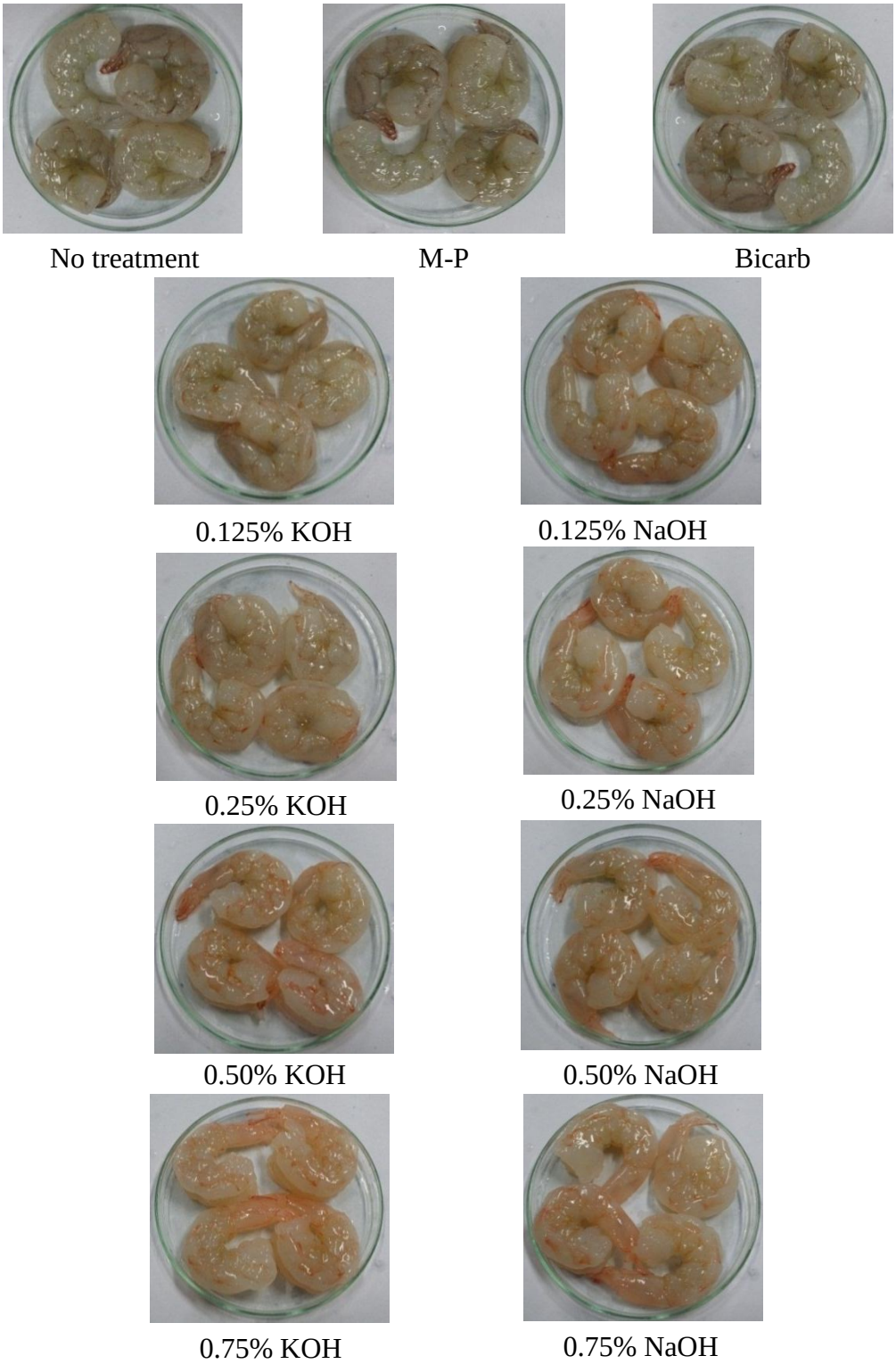

Figure 5. Raw shrimp treated with KOH and NaOH solutions at different concentrations in the presence of 2.5% NaCl. Note: M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1 (w/w)).; Bicarb: solution containing 2.5% NaCl and 2.5% sodium bicarbonate.

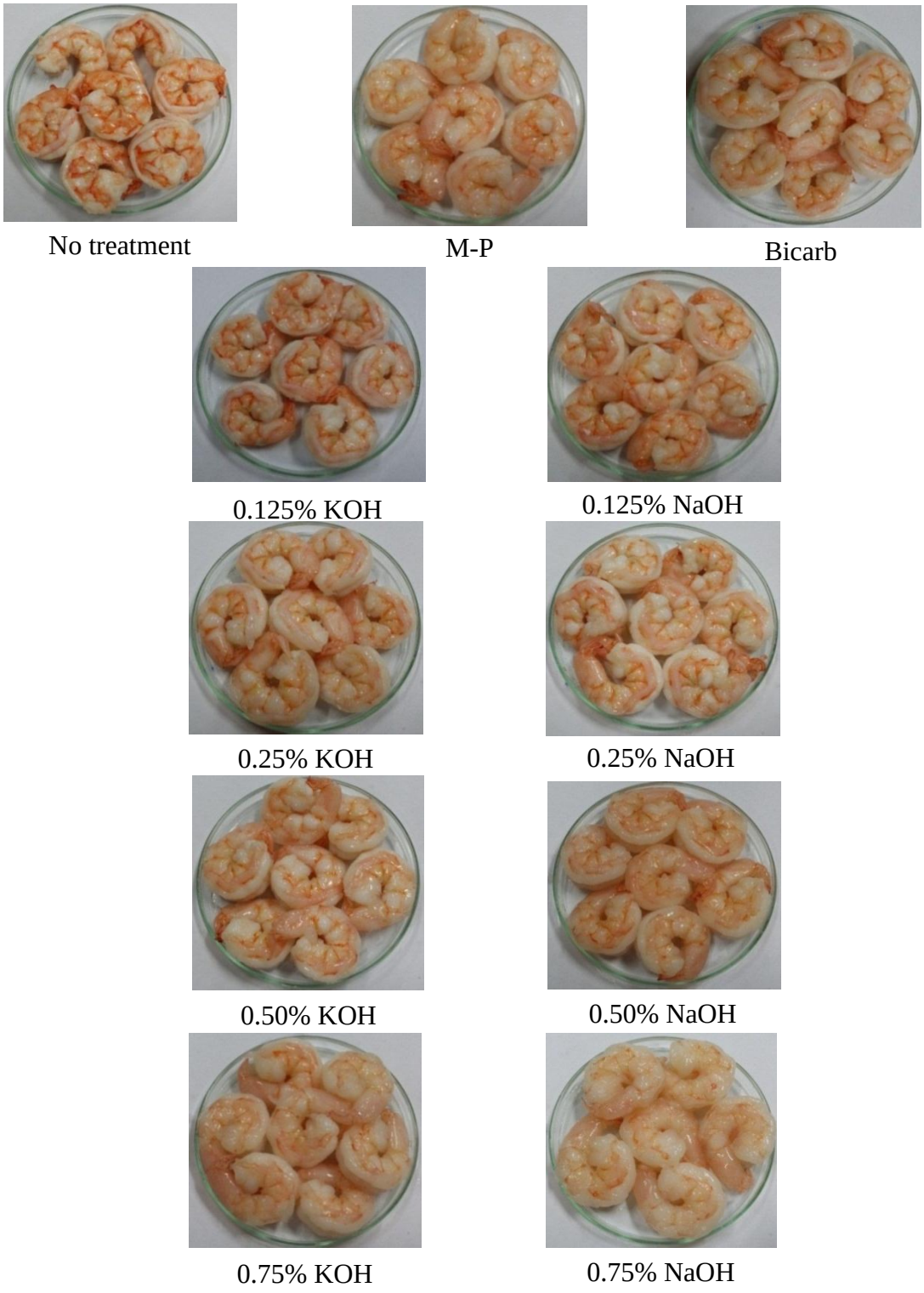

Figure 6. Cooked shrimp treated with KOH and NaOH solutions at different concentrations in the presence of 2.5% NaCl. M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1 (w/w)).; Bicarb: solution containing 2.5% NaCl and 2.5% sodium bicarbonate.

2.4.5 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 6.** 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 note that similar shear force was observed between shrimp treated with bicarbonate and those treated with the mixed phosphate ($P\geq0.05$). For cooked shrimp treated with alkaline solutions or mixed phosphates or bicarbonate, shear force decreased, in comparison with the control ($P<0.05$). In general, there was no difference in shear force among all samples subjected to difficult treatments ($P\geq0.05$) 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 is enhanced in 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. Additionally, the cleavage of peptides induced by alkaline condition during heating could lower the firmness of shrimp meat. Therefore, the treatment of shrimp using alkaline solutions lowered the shear force of cooked shrimp.

2.4.6 Protein pattern of soaking solution

Protein patterns of different alkaline solutions containing 2.5% NaCl after soaking with shrimp are shown in **Figure 7.** Myosin heavy chains (MHC) and actin were noticeable in solutions of mixed phosphate, bicarbonate and NaOH or KOH solutions at a concentration of 0.125%. 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 proteins become more polar and could bind water more effectively. This was coincidental with the increased weight gain **(Figure 3a).** 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. As a result, the less compact structure was formed and the water absorption could be enhanced.

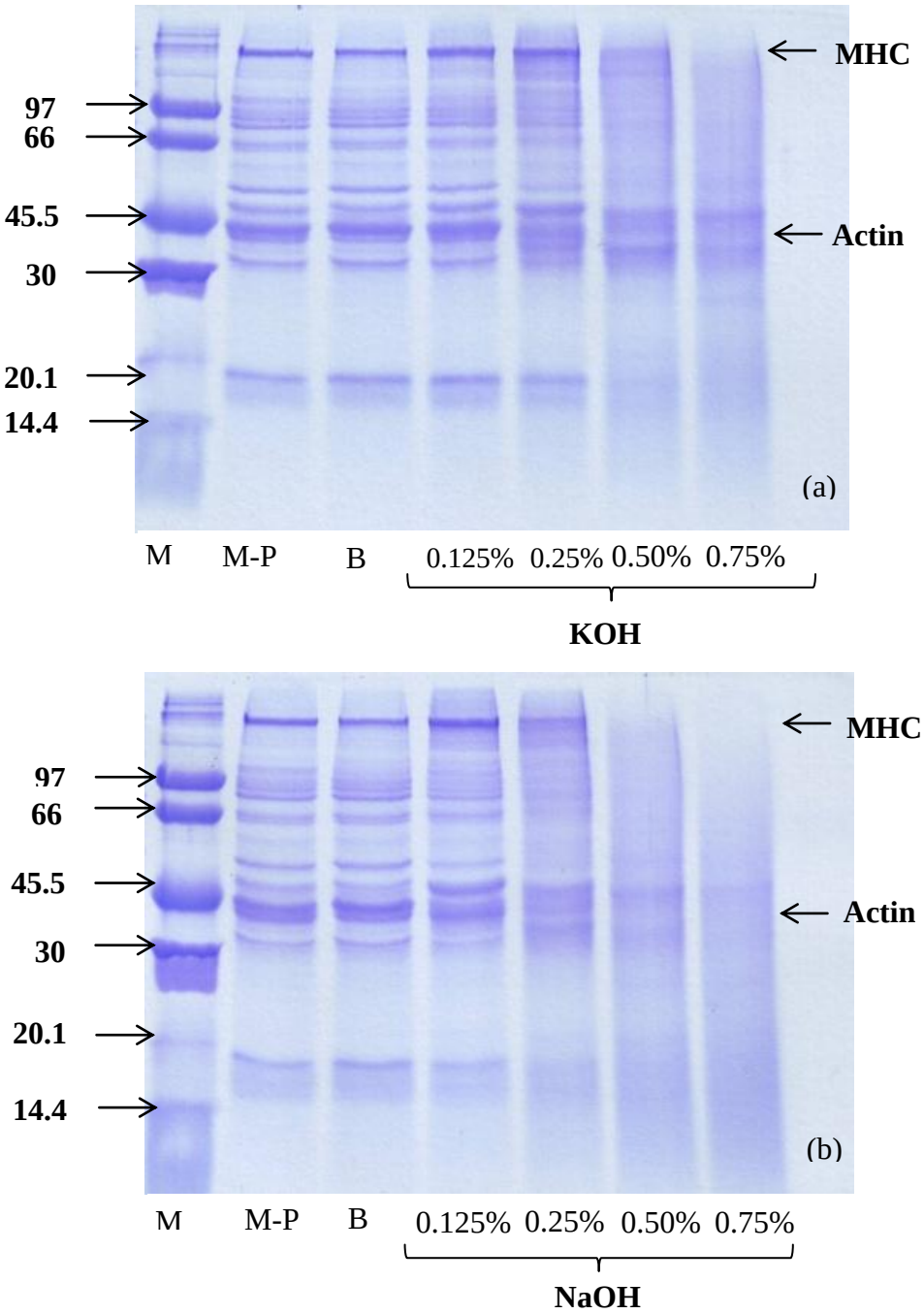

Figure 7. Protein pattern of KOH solution (a) and NaOH solution (b) containing 2.5% NaCl after soaking with shrimp.

Note: 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.

2.4.7 Characteristics of Pacific white shrimp treated with the selected alkaline solution

2.4.7.1 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 7.** Lower score in all attribute were 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 6).** 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 with 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 and the development is still required.

Table 7. 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).

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 superscripts in the same row indicate the significant differences ($P<0.05$).

2.4.7.2 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 8 and 9.** 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 the control. However, myofibrils were less attached as indicated by gaping occurred. 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 transparency of treated samples, especially under alkaline conditions.

For the transverse sections **(Figure 9)**, the dense structure was noticeable in the control shrimps. 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 myofibrils to align closely, leading to the more compact structure. Treated shrimps with 2.5% NaCl containing mixed phosphate, bicarbonate and 0.75% NaOH in the presence of 2.5% NaCl had 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, even though after cooking. This would lead to the higher cooking yield with lower cooking loss in shrimp treated with alkaline solution.

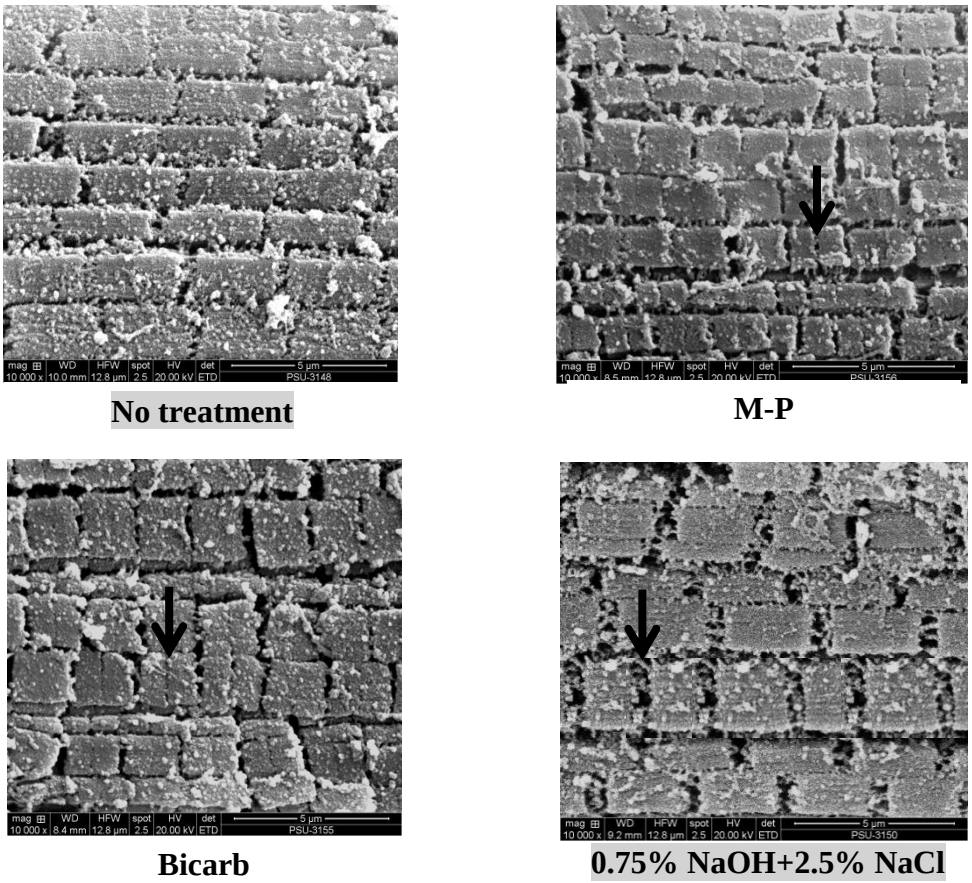

Figure 8. SEM micrographs of longitudinal section of cooked shrimp muscle treated with different treatments. Magnification= 10,000x

∗Arrow indicates M-line

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.

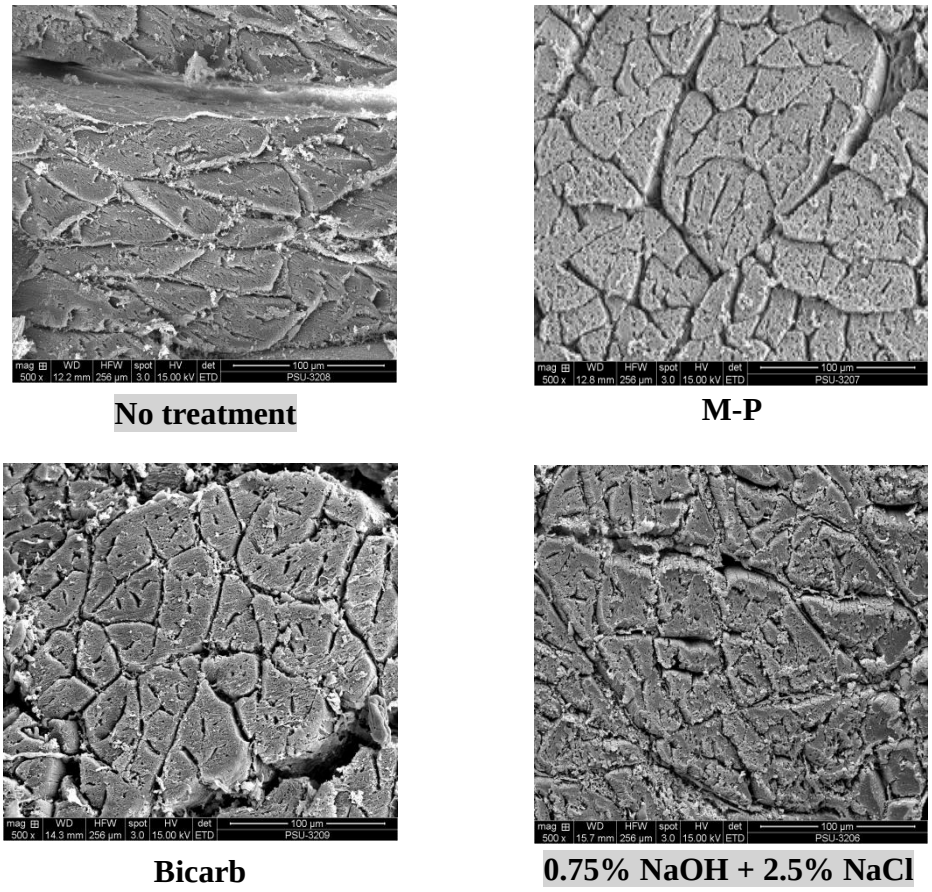

Figure 9. SEM micrographs of transverse sections of cooked shrimp muscle treated with different treatments. Magnification= 5,000x

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.

2.5. 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 cause partial solubilization and disruption of the muscle structure. 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.

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CHAPTER 3

EFFECT OF ALKALINE SOLUTION AT VARIOUS PHS ON WATER HOLDING CAPACITY AND PHYSICOCHEMICAL PROPERTIES OF MUSCLE PROTEINS FROM PACIFIC WHITE SHRIMP *(LITOPENAEUS VANNAMEI)*

3.1 Abstract

Impacts of 0.75% NaOH solution containing 2.5% NaCl with different pHs (8.5, 10, 11.5 and 13) on the water holding capacity of Pacific white shrimp were investigated. Weight gain and cooking yield increased with the coincidental decrease in shear force as the pH of solution used increased ($P<0.05$). When being treated at pH 13, raw shrimp showed the marked increase in redness (a^*-value), whereas pre-cooked shrimp had the decreased redness ($P<0.05$). Based on sensory analysis using 9-point hedonic scale, pre-cooked shrimp with prior treatment using NaOH solution, pH 13 exhibited the lowest score for all attributes including appearance, color, flavor, texture, taste and overall likeness, compared with untreated shrimp, TSPP+STPP treated shrimp and those treated with NaOH solution at other pHs ($P<0.05$). When physicochemical properties of natural actomyosin (NAM) from shrimp meat subjected to different pHs were determined, Ca^{2+} ATPase activity decreased as pH of solution increased and the complete loss in activity was observed at pH 13. Surface hydrophobicity (SoANS) of NAM was gradually increased when pH increased up to pH 11.5, however the decrease in SoANS was found at pH 13. The increase in disulfide bonds with concomitant decrease in total sulfhydryl group content was observed with increasing pHs ($P<0.05$). Extremely high pH thus more likely induced the drastic change of muscle protein. Therefore, 0.75% NaOH containing 2.5% NaCl (pH 11.5) could be an alternative, but the efficacy was still lower than phosphates.

Keywords: Water holding capacity, physicochemical properties, Pacific white shrimp

3.2 Introduction

Shrimp is one of the seafood products, which have become economically important for Thailand (Benjakul *et al.,* 2003). A variety of shrimp products have been produced and exported worldwide. Frozen cooked shrimp is one of popular products due to its attractive color and ready-to-cook perspective. During cooking, protein denaturation takes place, resulting in the decrease in cooking yield and increased cooking loss. To minimize those negative effects, some additives, particularly phosphate and bicarbonate, have been widely used in shrimp prior to cooking (Rattanasatherin *et al.*, 2008). Phosphates have been used in combination with NaCl for increasing water uptake in shrimp (Rattanasatherin *et al.*, 2008). Mixed phosphates (tetrasodium pyrophosphate+sodium tripolyphosphate) were reported to show the higher efficacy in increasing the yield of shrimp in comparison with single phosphate (Manheem *et al.*, 2012). Bicarbonate is another chemical termed "non-phosphate", which has been used widely for improving the yield and lowering cooking loss of shrimp and other seafoods (Chatarasuwan *et al.*, 2011). Bicarbonate played a role in raising the pH, in which repulsion was enhanced and led to dissociation of muscle fibers. This resulted in the increase in water uptake and water holding capacity of muscle. Pacific white shrimp treated with 2% bicarbonate (pH 8.5) had the increased yield with no negative effect on sensory property for both raw and cooked sample (Chatarasuwan *et al.,* 2011). Treatment of Na_2CO_3 or $NaHCO_3$ resulted in the increased in negative charge of natural actomyosin (NAM) of Pacific white shrimp (Chantarasuwan *et al.,* 2011)**.** Additionally**,** Chantarasuwan *et al.,* (2011) found that surface hydrophobicity of NAM underwent alternation when treated with Na_2CO_3 or $NaHCO_3$, especially at high pHs.

Due to the strict regulation in the uses of phosphate and bicarbonate as the processing aids in shrimp and shrimp products, other alternatives, especially alkaline compounds, can be considered as the agent with the equivalent property in improving quality of shrimp. Alkaline solution such as NaOH under the appropriate condition could serve as the replacer of both phosphate and bicarbonate for shrimp processing. For better understanding and effective application, the factors affecting NaOH treatment as well as its mode of action in increasing water holding capacity of muscle

proteins should be elucidated. Nevertheless, no information regarding the use of NaOH for shrimp treatment has been reported. Thus, the objective of this study was to investigate the effect of NaOH as phosphate and bicarbonate replacer, on the yield and characteristics of Pacific white shrimp. The impact of NaOH on physicochemical properties of Pacific white shrimp muscle protein was also investigated.

3.3 Materials and methods

3.3.1 Study on alkaline treatment at various pHs on yield and characteristics of Pacific white shrimp

3.3.1.1 Preparation of alkaline solutions

Solution containing 0.75% NaOH (w/v) and 2.5% NaCl (w/v) was prepared. Thereafter, the solution was adjusted to different pHs (8.5 10, 11.5 and 13) using 1 N or 6 N HCl. Prior to soaking, all solutions were measure for pH and NaCl content. Solutions obtained were cooled down to 4 °C in a walk-in cold room overnight before use.

3.3.1.1.1 Determination of pH

The pH of soaking solution was determined using a pH-meter (Sartorious North America, Edgewood, NY, USA).

3.3.1.1.2 Determination of NaCl content

NaCl content of soaking solution was determined as per the method of AOAC (2000). Soaking solution (20 ml) was added with 10 ml of 0.1 M $AgNO_3$ and 10 ml of concentrated HNO_3. The mixture was boiled gently for 10 min. The mixture was then cooled using running water. Thereafter, 50 ml of distilled water and 5 ml of 50 g/l Ferric alum ($FeNH_4(SO_4)_2 .12\ H_2O$) indicator were added. The mixture was filtered with Whatman No.1 filter paper (Whatman International Ltd, Maidstone, UK) and adjusted to 100 ml with distilled water. The mixture was titrated with standardized 0.1 M KSCN until the solution became permanent brownish red. The salt content was then calculated as follows:

$$NaCl\ (\%) = 5.8 \times [(V_1 \times N_1)-(V_2 \times N_2)]/W$$

where, V_1 =volume of $AgNO_3$ (ml); N_1 =concentration of $AgNO_3$ (M); V_2=volume of KSCN (ml); N_2=concentration of KSCN (M); W= weight of sample (g).

3.3.1.2 Treatment of shrimp using alkaline solutions

Prepared shrimps (peeled and deveined) were mixed with the prepared alkaline solutions at a ratio of shrimp/solution 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 samples were placed on 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) (Manheem *et al.*, 2012) was used as the positive control. Sample without any treatment was also used as the control. Shrimps of different treatments were divided into two portions, raw and cooked shrimp. To prepare the cooked shrimp, the prepared sample were heated by steaming until core temperature of the second segment of abdomen reached 85°C and the shrimp were held at that temperature for 1 min. Thereafter, shrimps were placed in iced water suddenly. The samples were placed on the plastic screen at 4°C for 5 min. Both raw and cooked shrimps were subjected to analyses.

3.3.1.3 Analyses

3.3.1.3.1 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

3.3.1.3.2 Determination of cooking loss and cooking yield

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

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

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

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.

3.3.1.3.3 Determination of color

Color expressed as *L** (lightness), *a** (greenness/ redness) and *b** (yellowness/ blueness) was determined at the second segment of shrimp abdomen using a Hunterlab colorimeter (Hunter Associates Laboratory, Inc., Reston, Virginia, USA). (Young, 1985)

3.3.1.3.4 Determination of shear force

Shear force of raw and cooked shrimp were 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 consist 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. The peak of the shear force profile is regarded as the shear force value.

3.3.1.3.5 Determination of pH

pH of shrimp meat was determined as per the method of Manheem (2012). using a pH-meter (Sartorious North America, Edgewood, NY, USA). 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 (Sartorious North America, Edgewood, NY, USA).

3.3.1.3.6 Sensory evaluation

The cooked samples treated with alkaline solutions at different pHs were subjected to sensory analysis in comparison with the control (without treatment) and positive control. 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. (Manheem *et al.*, 2012). 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.

3.3.2 Study on physicochemical changes of natural actomyosin from Pacific white shrimp as affected by alkaline solutions at various pHs

3.3.2.1 Extraction of natural actomyosin (NAM)

Shrimp mince (50 g) was homogenized 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 homogenizer (IKA, Labortechnik, Selangor, Malaysia). To avoid overheating, the sample was placed in ice and homogenized for 20 s, followed by a 20 s rest interval for a total extraction time of 4 min. The extract was centrifuged at 5000*xg* for 30 min at 4 ºC using a refrigerated centrifuge (Avanti® J-E, Beckman Coulter, Inc., Palo Alto, CA, USA). Three volumes of chilled deionized water was added to precipitate NAM. The NAM was collected by centrifuging at 5000 *xg* for 20 min at 4 ºC. The NAM pellet was stored in ice until use.

3.3.2.2 Preparation of NAM in various alkaline solutions

NAM was mixed with alkaline solutions at various pHs (8.5, 10, 11.5, 13) in the presence of 2.5% NaCl. The NAM dissolved in 2.5% NaCl was used as the control. The final concentration of NAM solution was adjusted to 4.5 mg protein/ml using the corresponding solutions. The mixture was stirred gently for 30 min in ice at 4 ºC. The resulting NAM samples were analyzed.

3.3.2.3 Analyses

3.3.2.3.1 Determination of surface hydrophobicity

Surface hydrophobicity was measured according to the method of Benjakul and Bauer (2001) using 8-anilo-1-naphthalenesulfonic acid (ANS) as a probe. NAM dissolved in 10 mM phosphate buffer, pH 6.0 containing 0.6 M NaCl was diluted to 0.1, 0.2, 0.3, and 0.5% (w/v) protein using the same buffer. The diluted protein solution (2 ml) was added with 10 µl of 8 mM ANS in 0.1 M phosphate buffer, pH 7.0. The fluorescence intensity of ANS-conjugates was measured using a FP-750 spectrofluorometer (Shimadzu, Kyoto, Japan) at an excitation wavelength of 374 nm and an emission wavelength of 485 nm. Surface hydrophobicity was calculated from the initial slope of the plot of fluorescence intensity against protein concentrations and referred to as ‘SoANS’.

3.3.2.3.2 Determination of total sulfhydryl content

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 solution (0.4%), 9 ml of 0.2 M Tris–HCl buffer, pH 6.8, containing 8 M urea, 2% SDS and 10 mM EDTA, were added. To 4 ml of the mixture, 0.4 ml of 0.1% DTNB in 0.2 M Tris–HCl (pH 8.0) was added and incubated at 40 °C for 25 min. The absorbance at 412 nm was measured using a UV-16001 spectrophotometer (Shimadzu, Kyoto, Japan). A blank was conducted by replacing the sample with 0.6 M KCl.The total SH content was calculated using a molar extinction coefficient of 13,600 $M^{-1}cm^{-1}$.

3.3.2.3.3 Determination of disulfide bond content

Disulfide bond content in samples was determined using the 2-nitro-5-thiosulphobenzoate (NTSB) assay as described by Thannhauser *et al.* (1987). To 0.5 ml of NAM sample (1 mg/ml), 3.0 ml of freshly prepared NTSB assay solution, pH. 9.5 were added. The mixture was incubated in dark at room temperature (25–27 °C) for 25 min. Absorbance at 412 nm was measured. The disulfide bond content was calculated using a molar extinction coefficient of 13,900 M^{-1} cm^{-1}.

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

ATPase activities were determined as described by Benjakul *et al.* (1997). An aliquot (1 ml) of NAM solution (2.5-4 mg/mL) in 0.6 M KCl, pH 7.0, was added to 0.6 ml of 0.5 M Tris-maleate, pH 7.0. To the mixture, one of the following solutions was then added for each. ATPase activity assay to a total volume of 9.5 ml: 10 mM $CaC1_2$, for Ca^{2+}-ATPase and 2mM $MgCl_2$, for Mg^{2+}-ATPase. To each assay solution, 0.5 ml of 20 mM ATP was added to initiate the reaction. The reaction was conducted for exactly 8 min at 25 °C and terminated by adding 5 ml of chilled 15 % (w/v) trichloroacetic acid (TCA). The reaction mixture was centrifuged at 3500xg for 5 min and the inorganic phosphate liberated in the supernatant was measured by the method of Fiske and Subbarow (1925). Specific activity was expressed as μmol inorganic phosphate released/mg protein/min.

3.3.3 Statistical analyses

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

3.4 Results and discussion

3.4.1 Characterization of soaking solution at various pHs

3.4.1.1 pH

Soaking solution (0.75% NaOH containing 2.5% NaCl) adjusted to various pHs were used for shrimp treatment, pH varied from 8.5 to 13. The neutral pH (6.93) was observed in 2.5% NaCl solution. pH of 2.5% NaCl solution containing mixed phosphate (tetrasodiumpyrophosphate+sodiumtripolyphosphate: TSPP+STPP, 2:1 (w/w)) was 9.38. It is well known that pH plays an important role in water holding capacity of meat. Chatarasuwan *et al.* (2011) reported that Pacific white shrimp soaked in 2.5% NaCl containing sodium carbonate and sodiumbicarboante at different levels of pH (5.5, 7, 8.5, 10 and 11.5) showed an increase in the weight gain and cooking yield and reduced cooking loss as pH of solution increased. Therefore, alkaline pH of solution containing mixed phosphate might partially be involved in changes in muscle proteins, apart from phosphate themselves. For solution comprising 0.75% NaOH and 2.5%NaCl, pH could be another prime factor affecting shrimp treatment.

3.4.1.2 NaCl content

Salt contents of soaking solution (0.75% NaOH containing 2.5% NaCl) adjusted to various pHs are shown in **Figure 10.** Soaking solution with pH 8.5 showed the highest NaCl content, compared with those with other pHs ($P<0.05$). Salt content of 0.75% NaOH containing 2.5% NaCl (pH 13) was similar to that of 2.5% NaCl solution. It was noted that as alkaline solution (0.75% NaOH) was adjusted to the lower pHs using HCl solution, NaCl was formed due to the neutralization. With the lower pH of solution after adjustment, the higher NaCl was generated. The result

suggested that NaCl could be further formed in soaking solution as the pH adjustment was carried out. However, Asena (2003) found that cooking yield of Pacific white shrimp treated in TSPP was not affected by the levels of NaCl (1.5-3.5%). Thus, additional NaCl formed in alkaline soaking solution during pH adjustment might not affect weight gain and cooking yield of treated shrimp in the present study.

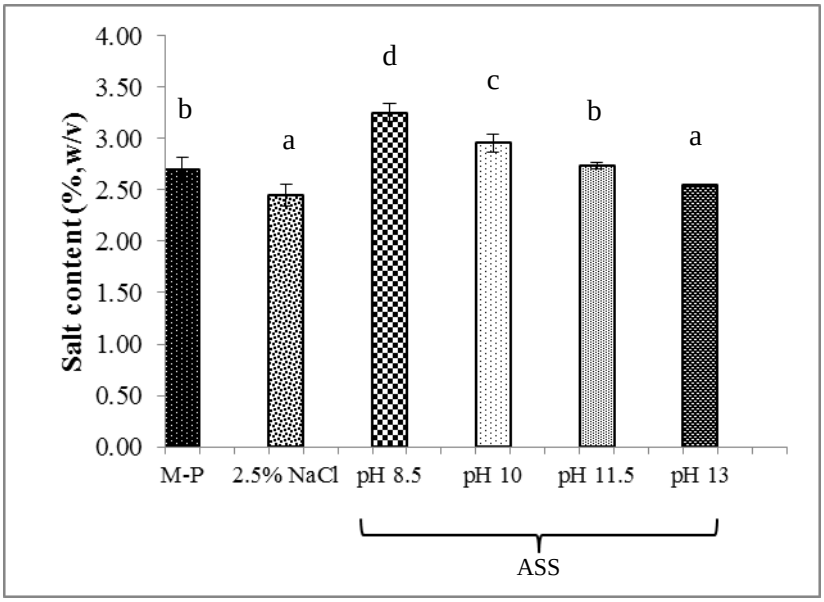

Figure 10. Salt content of soaking solution containing 0.75% NaOH and 2.5% NaCl adjusted to at various pHs. Note: ASS: 0.75% NaOH containing 2.5% NaCl.; M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1 (w/w)). Different lowercase letters on the bars indicate the significant differences ($P<0.05$). Bars represent the standard deviation (n=3).

3.4.2 Effect of alkaline treatment at various pHs on yield and characteristics of Pacific white shrimp

3.4.2.1 Weight gain, cooking loss and cooking yield of Pacific white shrimp

Weight gain, cooking loss and cooking yield of Pacific white shrimp treated with alkaline solution (0.75% NaOH) containing 2.5% NaCl with different pHs are shown in **Figure 11.** Weight gain and cooking yield increased when pH of solutions increased ($P<0.05$). Nevertheless, cooking loss decreased with increasing pH ($P<0.05$). Among all samples, that treated with the solution at pH 13 had the highest weight gain and cooking yield with the concomitantly lowest cooking loss ($P<0.05$). At a pH above pI or very alkaline pH, proteins have the negative charge, in which protein molecules repulse each other, resulting in the swollen and looser muscle structure (Chantarasuwan *et al.*, 2011). As a consequence, water could be more uptaken. In the presence of 2.5% NaCl, the chloride ion could penetrate into the muscle. As a result, water molecules might be bound with those ion or proteins via ionic interaction. Thus, the additional water absorption within the protein network could be achieved. When heat was applied, proteins in the muscle more likely underwent coagulation with coincidental loss in water holding capacity. This was evidenced by the highest cooking loss and the lowest cooking yield in the control (without treatment) ($P<0.05$). However, after being treated with alkaline solution, the negatively charged domains were coagulated at lower extent during cooking. Furthermore, the water was still bound with charged proteins via ionic interaction, especially at the very alkaline pH. Those led to the lower cooking loss of shrimp treated with alkaline solution, particularly at high pHs. Weight gain and cooking yield of shrimp treated with 2.0% sodium carbonate or bicarbonate were increased as pH of solution increased ($P<0.05$) (Chantarasuwan *et al.*, 2011). Therefore, pH of solution had the influence on weight gain, cooking yield and cooking loss of Pacific white shrimp.

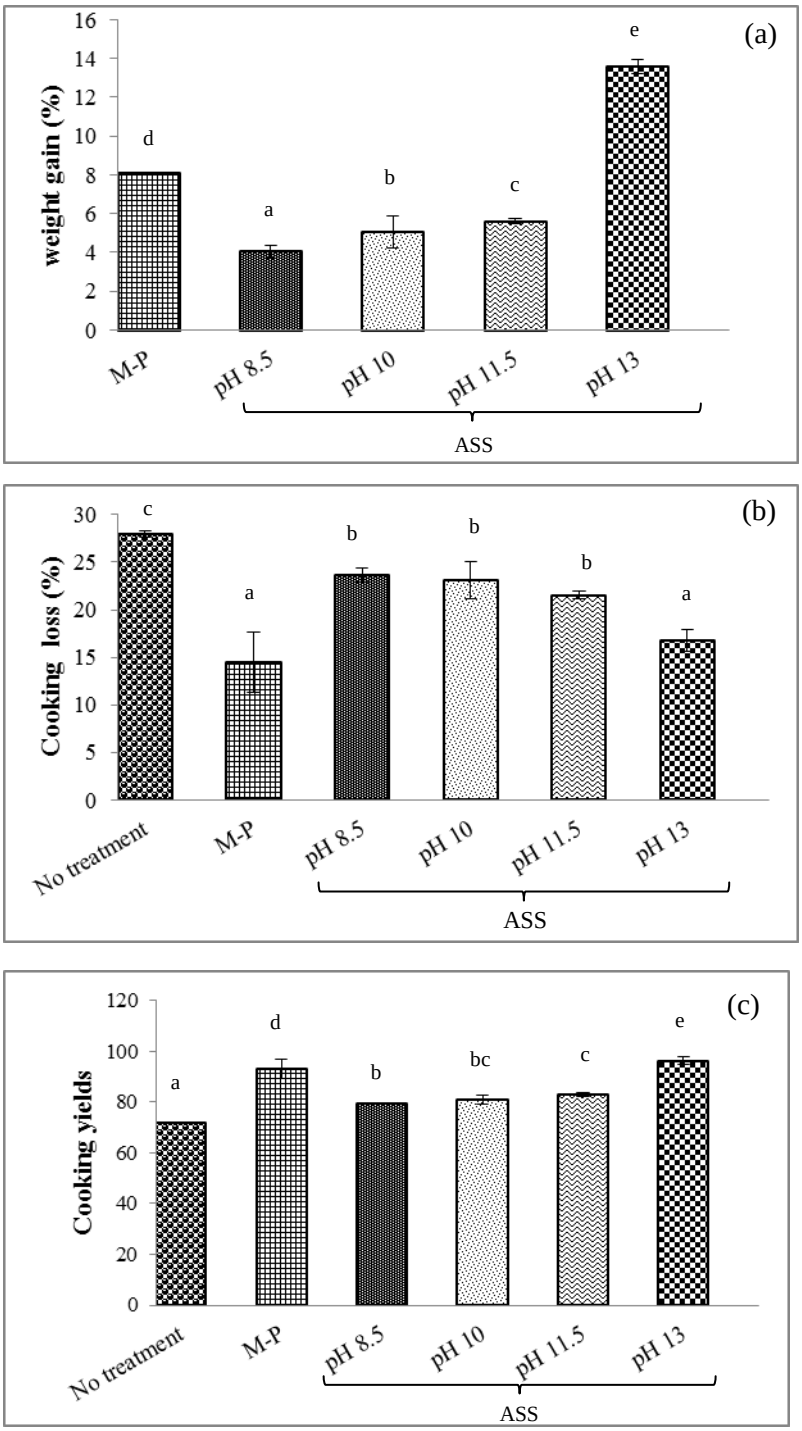

Figure 11. Weight gain (a), cooking loss (b) and cooking yield (c) of Pacific white shrimp treated with 0.75% NaOH solution at various pH in the presence of 2.5% NaCl. Note: ASS: 0.75% NaOH containing 2.5% NaCl.; M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1 (w/w)). Different lowercase letters on the bars indicate significant different ($P<0.05$) Bars represent the standard deviation (n=3).

3.4.2.2 Shear force and pH

Shear force of raw and cooked shrimp treated with alkaline solutions at various pHs in the presence of 2.5% NaCl is depicted in **Table 8.** Shear force of raw shrimp remained unchanged up to pH 11.5 ($P \geq 0.05$). Shear force of shrimp treated with alkaline solution was not different from that of the control (without treatment) and that treated with mixed phosphates ($P \geq 0.05$). However, shrimp treated with alkaline solution at pH 13 showed the lower shear force than that treated with solution having pH of 8.5 ($P < 0.05$). pH of solution more likely had the impact on muscle proteins. 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 (Chantarasuwan *et al.*, 2011). The result was in accordance with Chantarasuwan et *al*, (2011) who reported that shear force of raw and cooked shrimp decreased when pH of Na_2CO_3 or $NaHCO_3$ solutions used for shrimp treatment increased. For cooked shrimp treated with alkaline solutions, shear force decreased when pH of solution used increased ($P < 0.05$). During cooking, protein underwent denaturation and some weak bonds might be disrupted, particularly after treated with very alkaline solution. For shrimp soaked in solution with extremely alkaline pH, some partial hydrolysis might take place, particularly during cooking. The cleavage of peptides could lower the firmness of shrimp meat (Chantarasuwan *et al.*, 2011). Moller *et al.* (2010) reported that a decreasing shear force of pork meat was obtained as the pH increased. This was evidenced by the marked decrease in shear force of sample treated with alkaline solution at pH 13. For control shrimp, proteins were more likely aggregated as induced by heat. Those aggregated proteins were more resistant to the force applied. On the other hand, those treated with mixed phosphates had slightly lower shear force after cooking. The bound water in shrimp muscle treated with phosphates might play a role in preventing the coagulation of protein during heating.

Table 8. Shear force and pH of raw and cooked shrimp treated with 0.75% NaOH at various pHs in the presence of 2.5% NaCl

Samples	Treatments	Shear force (g)	pH
Raw	No treatment	2202 ± 106 †,ab	6.43±0.02 a
	M-P	2193 ± 87 ab	6.77±0.02 c
	ASS (pH 8.5)	2264 ± 164 b	6.42±0.02 a
	ASS (pH 10)	2208 ± 26 ab	6.54±0.04 b
	ASS (pH 11.5)	2150 ± 16 ab	6.81±0.02 c
	ASS (pH 13)	2051 ± 98 a	8.22±0.03 d
Cooked	No treatment	2568 ± 508 d	6.74±0.05 ab
	M-P	1899 ± 362 ab	6.94±0.04 c
	ASS (pH 8.5)	2459 ± 301 cd	6.71±0.03 a
	ASS (pH 10)	2055 ± 132 bc	6.77±0.02 ab
	ASS (pH 11.5)	2216 ± 142 bcd	6.80±0.01 b
	ASS (pH 13)	1630 ± 78 a	7.60±0.06 d

†Mean ± SD (n=3).

Note: ASS: 0.75% NaOH containing 2.5% NaCl.; M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1 (w/w)). Different lowercase superscripts in the same column under the same state of sample indicate the significant differences ($P<0.05$).

For pH, both raw and cooked samples had the increases in pH when pH of solutions increased. Raw shrimp treated with mixed phosphates showed slightly higher pH than that without treatment, but had similar pH to that with alkaline treatment at pH 11.5 ($P<0.05$). Alkaline solution could penetrate into the shrimp meat, affecting pH of muscle proteins. Changes in pH could induce the modification of charge as well as conformation of protein. Chantarasuwan *et al.* (2011) found that pH of Pacific white shrimp meat increased as pH of soaking solution increased.

3.4.2.3 Color

Color parameters (*L**, *a** and *b**) of raw and cooked shrimp treated with alkaline solution at various pHs in the presence of 2.5% NaCl are shown in **Table 9.** For raw shrimp, no marked difference in *L**-value was obtained when shrimp were treated with alkaline solution with different pHs. Nevertheless, *L**-value of sample treated at pH 13 was lower than that shrimp treated at pH 11.5. Raw shrimp turned to be reddish when soaked in the solution with pH 13 as indicated by the drastic increase in *a**-value ($P<0.05$). An extremely alkaline pH could induce the denaturation of carotenoproteins, leading to the appearance of red color caused by free carotenoids, especially astaxanthin (Chantarasuwan *et al.*, 2011). Shrimp soaked in 2.5% NaCl containing 2.0% sodium carbonate or 2.0% sodium bicarbonate at pH 10 and 11.5 showed the increased *a** value (Chantarasuwan *et al.*, 2011). The increased *b** value with conincidental decreased *L** value was observed in shrimp treated with alkaline solution at pH 13 ($P<0.05$). When shrimps were subjected to cooking, the pronounced increases in both *a** and *b**-values were obtained, in comparison with the raw counterparts. It was noted that lower *a** and *b**-values with low *L**-value were found in shrimp treated with alkaline solution at pH 13 ($P<0.05$). During soaking in very alkaline pH, the solubilization of carotenoproteins and swelling of muscle protein took place at high degree. As a result, carotenoproteins were leached out into the solution. The lower carotenoproteins were retained in shrimp, resulting in pale color, especially for shrimp treated with alkaline solution at pH 13.

Table 9. Color of raw and cooked shrimp treated with 0.75% NaOH at various pHs in the presence of 2.5% NaCl

Samples	Treatments	*L**	*a**	*b**
Raw	No treatment	42.51±2.27 †,ab	-2.48±0.56 a	-1.21±0.65 d
	M-P	41.03±3.06 a	-2.07±0.68 a	-3.26±1.26 a
	ASS (pH 8.5)	42.26±2.13 ab	-2.33±0.33 a	-3.14±0.82 ab
	ASS (pH 10)	43.55±2.59 ab	-2.61±0.76 a	-2.78±1.55 bc
	ASS (pH 11.5)	44.52±2.04 b	-2.18±0.45 a	-2.44±1.87 c
	ASS (pH 13)	40.96±1.58 a	0.99±0.45 b	-1.87±1.21 cd
Cooked	No treatment	75.55±2.79 c	7.46±3.12 b	15.48±3.18 bc
	M-P	68.05±3.06 a	6.99±2.72 b	12.77±2.58 ab
	ASS (pH 8.5)	74.70±2.80 bc	7.00±1.29 b	14.18±2.38 bc
	ASS (pH 10)	73.51±1.33 bc	5.66±2.71 b	14.87±1.48 bc
	ASS (pH 11.5)	72.17±2.60 b	6.98±1.62 b	16.35±2.27 c
	ASS (pH 13)	67.60±2.31 a	3.20±1.83 a	11.30±3.41 a

†Mean ± SD (n=3).

Note: ASS: 0.75% NaOH containing 2.5% NaCl.; M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1 (w/w)). Different lowercase superscripts in the same column under the same state of sample indicate the significant differences ($P<0.05$).

3.4.2.4 Sensory properties

Sensory score of cooked shrimp treated with alkaline solutions at various pHs in the presence of 2.5% NaCl is shown in **Table 10.** No differences in all attributes were found between the control (no treatment and treated with mixed phosphate) and those soaked in alkaline solution possessing pHs up to 11.5 ($P\geq0.05$). Nevertheless, the lower scores in all attribute were found in the sample soaked in alkaline solution at pH 13 ($P<0.05$). This was due to the slimy surface associated with the excessive solubilization of protein at extremely alkaline pH and strong alkaline odor. The lower color likeness score was in agreement with pale color (low *a** value) of shrimp treated with solution at pH 13. Additionally, shrimp treated in alkaline solution at pH 13 showed alkali flavor, leading to unacceptability. Strong alkali flavor of ground buffalo meat patties became obvious when treated with 1.0% and 2.0% ammonium hydroxide (Naveena *et al.*, 2011).

Table 10. Sensory score of raw and cooked Pacific white shrimp soaked in 0.75% NaOH at various pHs in the presence of 2.5% NaCl

Attributes	No treatment	Treatments				
		M-P	ASS (pH 8.5)	ASS (pH 10)	ASS (pH 11.5)	ASS (pH 13)
Appearance	6.44±1.23 †,b	6.60±1.35 b	6.36±1.04 b	6.92±1.00 b	6.84±0.94 b	5.52±1.45 a
Color	6.52±1.16 b	6.84±1.34 b	6.60±1.12 b	6.88±1.05 b	6.80±1.19 b	5.52±1.16 a
Flavor	6.28±1.46 b	6.60±1.47 b	6.44±1.08 b	6.36±1.29 b	6.52±1.16 b	5.16±1.84 a
Texture	7.00±1.22 b	7.24±0.97 b	7.36±0.86 b	7.24±1.05 b	7.24±0.88 b	5.76±1.59 a
Taste	7.24±0.93 b	7.12±1.24 b	7.00±1.15 b	7.28±0.94 b	7.20±1.00 b	5.36±1.93 a
Overall	7.12±0.78 b	7.28±0.94 b	7.08±0.95 b	7.20±0.91 b	7.12±0.93 b	5.64±1.35 a

†Mean ± SD (n=3). Sensory score: 1= dislike extremely; 5 = neither like nor dislike and 9 = like extremely.

Note: ASS: 0.75% NaOH containing 2.5% NaCl.; M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1 (w/w)). Different lowercase superscripts in the same row indicate the significant differences ($P<0.05$).

3.4.3 Physicochemical changes of natural actomyosin from Pacific white shrimp as affected by alkaline solution with various pHs

3.4.3.1 Total sulfhydryl group and disulfide bond content

Total sulfhydryl group content of NAM decreased, while disulfide bond content increased when pH of solution increased ($P<0.05$) as shown in **Figure 12.** The decrease in sulfhydryl groups generally resulted from the formation of disulfide bonds through oxidation of sulfhydryl (SH) groups or disulfide interchanges (Wicker *et al.*, 1986).

Sulfhydryl groups on the myosin head portion, named SH_1 and SH_2, were reported to be involved in ATPase activity; another SH group (SHa) localized in the light meromyosin contributes to oxidation (Benjakul *et al.*, 1997). As pH of solution increased, protein underwent unfolding via repulsion, thereby releasing the reactive SH groups to a higher extent. Those SH group could be oxidized under alkaline condition, as shown by the decrease in SH group with the concomitant increase in disulfide bond. The accelerated denaturation of myosin molecules, especially the

conformational changes, in which the reactive sulfhydryl groups were exposed to oxidation, might result in increased disulfide bond formation (Sriket *et al.*, 2007).

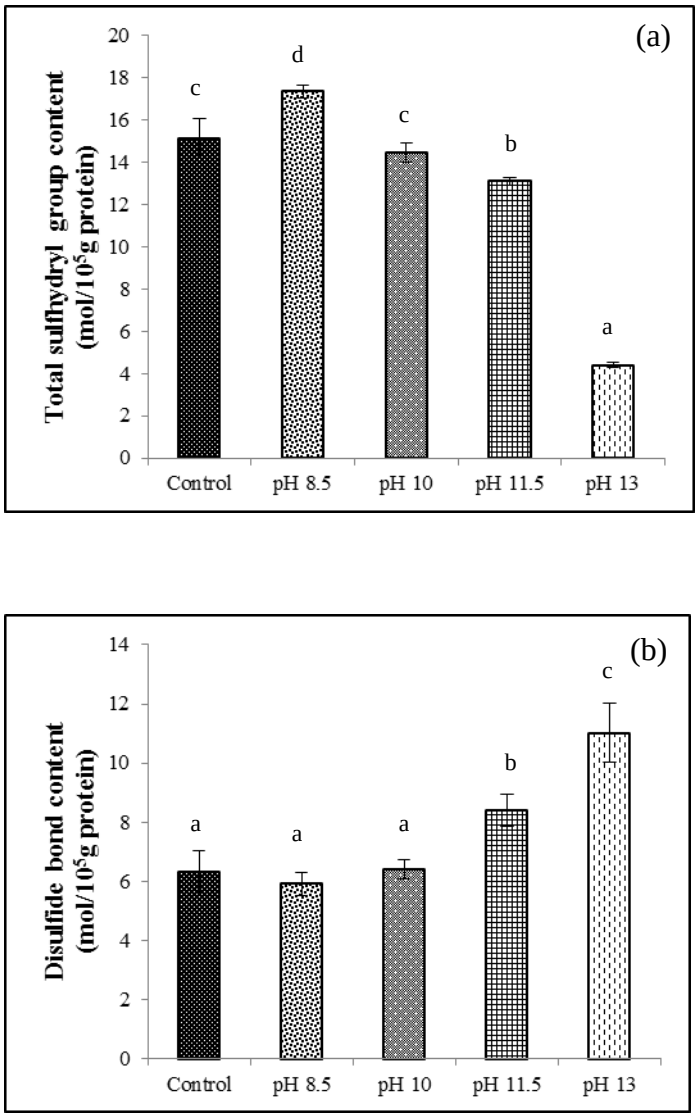

Figure 12. Total sulfhydryl (a) and disulfide bond (b) of natural actomyosin from Pacific white shrimp in 0.75% NaOH containing 2.5% NaCl with different pHs. Note: Control (NAM without treatment). Different lowercase letters on the bars indicate significant differences ($P<0.05$). Bars represent standard deviation ($n = 3$).

Cysteine is perhaps the most susceptible amino acid residue and it is usually one of the first to be oxidised (Thanonkaew *et al.*, 2006). The amino acids with reactive side chains (sulfhydryl, thioether, amino group, imidazole ring and indole ring) are

particularly susceptible to oxidation initiated by oxidizing lipids and their products (Gardner, 1979). Oxidised myofibrils showed substantial changes in sulfhydryls and disulfide bonds (Xiong, 2000).

3.4.3.2 Surface hydrophobicity (SoANS)

SoANS of NAM from Pacific white shrimp suspended in 0.75% NaOH containing 2.5% NaCl at various pHs is depicted in **Figure 13.** SoANS of NAM continuously increased when pH of solution increased up to 11.5 ($P<0.05$). Thereafter, SoANS sharply decreased when pH was above 11.5. The increase in SoANS indicated the structural changes of NAM, in which the hydrophobic domains were exposed, particularly aromatic hydrophobic amino acid residues, i.e. phenylalanine and tryptophan. ANS, an effective fluorescent probe, has been found to bind at non-polar regions of protein (Hayagawa *et al.*, 1985). Generally, myofibrillar proteins are soluble in salt solution. It was noted that the control (NAM in 2.5% NaCl) showed lower SoANS to NAM suspend in alkaline solution with pH of 8.5, 10 and 11.5 ($P<0.05$). The exposure of hydrophobic domains could be synergistically induced by NaCl (Chantarasuwan *et al.*, 2011). Myofibrillar proteins, solubilized by

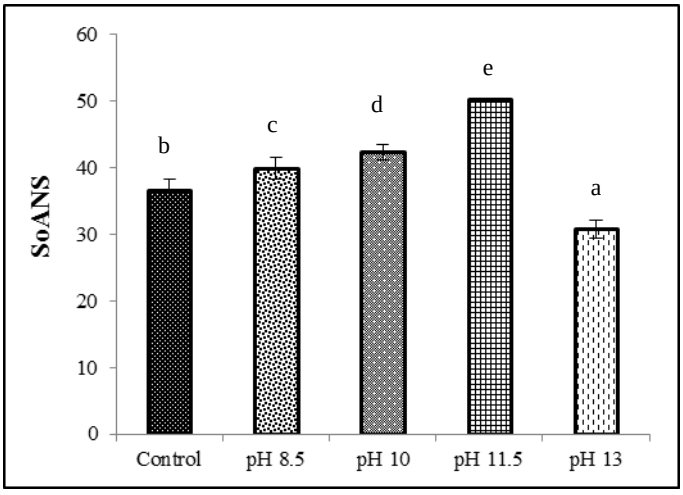

Figure 13. Surface hydrophobicity of natural actomyosin from Pacific white shrimp in 0.75% NaOH containing 2.5% NaCl with different pHs. Note: Control (NAM without treatment). Different lowercase letters on the bars indicate significant differences ($P<0.05$). Bars represent standard deviation (n = 3).

NaCl could have the modified charge caused by alkaline pH (Chantarasuwan *et al.*, 2011). Increasing ionic strength decreases the sphere of each charge on the proteins, thereby weakening the structural integrity of myofibrils (Raymond and Zubay, 1983). As the protein moves away from the pI, the ionizable groups in proteins become increasingly charge up to a point where the charge repulsion causes the protein molecule to unfold (Chantarasuwan *et al.*, 2011). Such an unfolding more likely favored the exposures of hydrophobic domains, as evidenced by increased surface hydrophobicity. The increase in SoANS of samples was generally in agreement with the decrease in Ca^{2+}-ATPase **(Figure. 14a).**

When pH of alkaline solution was 13, the decrease in SoANS was obtained ($P<0.05$). The exposed or unfolded domains of NAM at pH 13 might undergo aggregation via hydrophobic interaction, as indicated by the lower SoANS. Therefore, the conformational change of NAM was influenced by pH of alkaline solutions.

3.4.3.3 Ca^{2+}-ATPase and Mg^{2+}-ATPase activities

Ca^{2+}-ATPase activity of NAM treated with 0.75% NaOH at various pHs containing 2.5% NaCl is shown in **Figure 14a.** Ca^{2+}-ATPase activity decreased as pH of solution increased ($P<0.05$), suggesting the partial denaturation of myosin heavy chain, especially at the head portion. At very alkaline pH (13), no activity was retained. At alkaline pH, the dissociation of actomyosin complex was augmented (Benjakul *et al.*, 1997). The complete unfolding of head domain led to the total loss in activity of Ca^{2+}-ATPase. The decrease in Ca^{2+}-ATPase activity was possibly due to the conformational changes of the myosin globular head as well as the aggregation of this portion (Okada *et al.*, 1986). When muscle proteins were adjusted to pH above pI, the protein molecule became negatively charged. As a consequence, the repulsion became dominated, leading to the dissociation of protein as well as unfolding of myosin head with ATPase activity (Chanarat and Benjakul, 2013). Chantarasuwan *et al.* (2011). reported that 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 ($P<0.05$). Ca^{2+}-ATPase activity can be used as an indicator for the integrity of myosin molecules. Chaijan *et al.*, (2006) reported that myosin of sardine

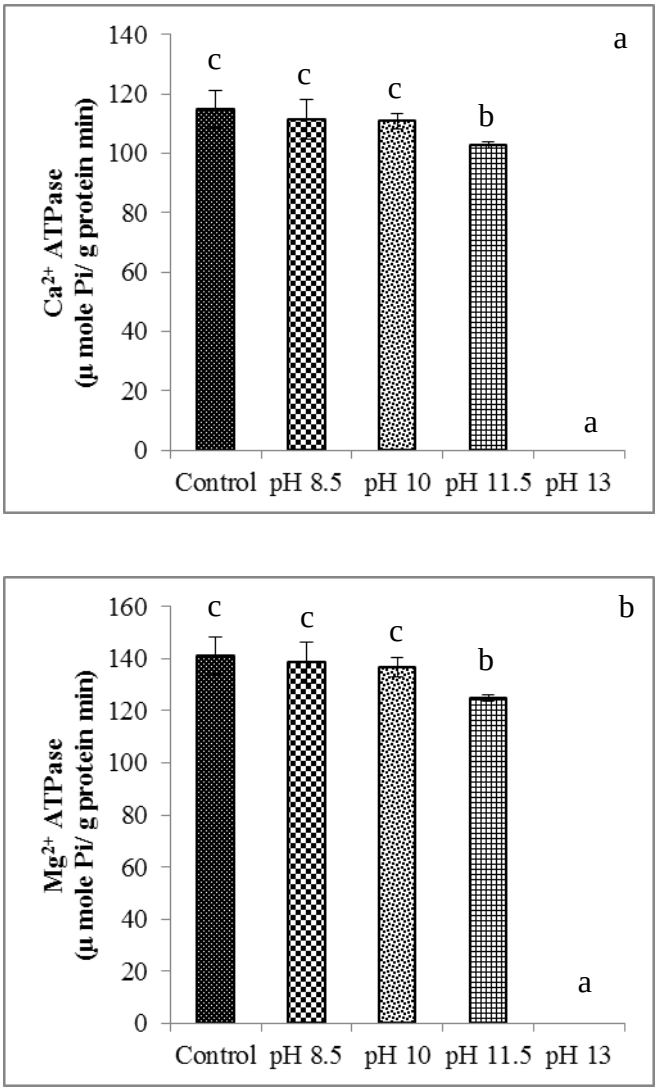

Figure 14. Ca^{2+} ATPase (a) and Mg^{2+} ATPase (b) of natural actomyosin from Pacific white shrimp treated in 0.75% NaOH containing with 2.5% NaCl with different pHs. Note: control (NAM without treatment). Different lowercase letters on the bars indicate significant differences ($P<0.05$). Bars represent standard deviation (n = 3).

(Sardinella gibbosa) and mackerel *(R. kanagurta)* underwent denaturation by alkaline solubilization. The effects of 0.75% NaOH at various pHs in the presence of 2.5% NaCl on Mg^{2+}-ATPase activity of NAM from Pacific white shrimp muscle are shown in **Figure 14b.** Mg^{2+}-ATPase activity of NAM slightly decreased as pH of solution increased up to 11.5 and drastically decreased at pH 13 ($P < 0.05$). The result indicated that at higher pH, the actomyosin complex underwent dissociation. Apart from myosin, free actin was liberated to the strong alkaline environment. As a result,

actin was denatured almost completely. The decrease in Mg^{2+}-ATPase can be used as an indicator for the denaturation of actin, which is reported to be the activator for myosin Mg^{2+}-ATPase (Torigai and Konno. 1996).

3.5 Conclusion

The pH of soaking solution was the prime factor governing the efficacy of shrimp treatment. Soaking the shrimp in 0.75% NaOH containing 2.5% NaCl (pH 11.5) could be an effective means to increase weight gain and cooking yield and had no negative effect on sensory properties. Alkaline treatment resulted in the dissociation of filamental structure of actomyosin complex along with the conformational changes. However, weight gain and cooking yield were lower than those of shrimp treated with mixed phosphates. For further improvement, some additives can be used in conjunction with alkaline soaking solution, in which its efficacy can be equivalent to phosphate or bicarbonate**.**

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CHAPTER 4

EFFECT OF TREATMENTS USING SOME ADDITIVES ON YIELD AND CHARACTERISTICS OF PACIFIC WHITE SHRIMP *(LITOPENAEUS VANNAMEI)*

4.1 Abstract

Effects of soaking solution, 0.75% NaOH containing 2.5% NaCl (pH 11.5), in the presence of different sugar and sugar alcohols (glucose, glycerol and sorbitol) at different concentrations on yield and characteristics of Pacific white shrimps *(Litopenaeus vannamei)* were studied. All sugar and sugar alcohols had no pronounced effect on weight gain, cooking loss and cooking yield. When different amino acids (glycine, glutamic acid and arginine) were incorporated in soaking solution at various levels, the lowest cooking loss but highest cooking yield were obtained for the sample treated with glutamic acid, especially at high level (3%). Soaking solution containing 3% glutamic acid (pH 11.5) reduced cooking loss and increase weight gain and cooking yield more effectively than that with pH 7 ($P<0.05$). When shrimp were treated with soaking solution having 3% monosodium glutamate (mole equivalent glutamic acid) higher weight gain and cooking yield were obtained, in comparison with those treated with mixed phosphates. For cooked shrimp, treatment using soaking solution containing MSG had no effect on color and shear force, but yielded the cooked shrimp with the highest overall likeness score. Treatment of shrimp using soaking solution involving MSG resulted in the shift of T_{max} for both MHC and actin to lower temperatures with lower enthalpy. It also caused the solubilization of some proteins. Such a treatment led to the swelling of muscle fibril as visualized by scanning electron microscope. Therefore, alkaline solution containing MSG could be used as the potential phosphate and bicarbonate replacers for shrimp treatment.

Keywords: Additives, Characteristic of shrimp, Pacific white shrimp

4.2 Introduction

Nowadays, Pacific white shrimp *(Litopenaeus vannamei)* is one of important species having a high market demand worldwide due to its appealed appearance, taste, flavor and texture (Manheem, 2013). Thailand is well known as the world largest shrimp producer, manufacturer and exporter. In 2013, shrimp exported from Thailand are accounted at 28,617 million baths (The Customs Department, 2013). Shrimp and their products of Thailand have been recognized as prime quality with high acceptability (Manheem, 2013). Shrimp processing, especially freezing, can lead to the denaturation or aggregation of proteins (Carnetro *et al.*, 2013). These changes can result in drip loss and sensorial changes in the product (Gonçalves and Ribeiro, 2009). Furthermore, cooking also leads to lower quality attributes, mainly by affecting textural and physicochemical properties and causing loss of weight (Carnetro *et al.*, 2013). The addition of water binding agent is required in order to retain the quality of shrimp during transportation and storage. Phosphate and bicarbonate have been widely used as water binding agent, which can increase water uptake and lower cooking loss (Rattanasatherin *et al.*, 2008; Carnetro *et al.*, 2013 and Chatarasuwan *et al.*, 2011). Due to the strict regulation of uses of phosphate and bicarbonate in shrimp and shrimp product, alternative additives with the property equivalent to both phosphate and bicarbonate are needed for quality improvement and maintenance.

Sugar or sugar alcohol have been commonly used in surimi industry as cryoprotectants, to retard the protein denaturation during freezing and frozen storage (Beak and Lim. 2004; Jin *et al.*, 2010). Apart from low molecular weight carbohydrate, amino acids had been reported to exhibit cryoprotective effects, thus retarding protein denaturation and retaining protein functionality of frozen fish muscle (Campo-Deano *et al.*, 2009; Zhou *et al.*, 2006). Owing to the hydrophilic nature of those compounds, their uses along with alkaline treatment could show the synergism in water uptake or water binding in shrimp muscle. Therefore, those compounds can serve as the potential additive for shrimp treatment and could replace phosphate or bicarbonate. The objective of this study was to investigate the impact of some sugar, sugar alcohol and amino acids in conjunction with alkaline treatment on

characteristics of shrimp and to elucidate the impact of those agents on physicochemical properties and acceptance of resulting shrimp.

4.3 Materials and methods

4.3.1 Collection and preparation of shrimp

Pacific white shrimp (*Litopenaeus vannamei)* (55-60 shrimp/kg) were purchased from a local market in Hat Yai, Songkhla province, Thailand. Shrimp with storage time less than 6 h after capture were stored in the insulated box containing 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. Prepared shrimp were placed in polyethylene bag and stored in ice until used.

4.3.2 Effects of sugar, sugar alcohols and amino acids at various concentrations in alkaline soaking solution on weight gain, cooking loss and cooking yield of shrimp.

4.3.2.1 Preparation of treated shrimp

Shrimp (peeled and deveined) were mixed with alkaline soaking solution (ASS; 0.75% NaOH containing 2.5% NaCl, pH 11.5) in the presence of sorbitol, glucose or glycerol at levels of 0.25, 0.5 and 1% (w/v) using the shrimp/ solution 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 treatment, the shrimp were placed on the plastic screen for 5 min (4 °C) to drain off solution. Samples soaked in 2.5% NaCl containing 3% mixed phosphates (sodium tripolyphosphate+tetrasodium pyrophosphate; 1:2, w/w) (Manheem, 2012) and in 2.5% NaCl containing 3% NaOH (pH 11.5) were used as the positive controls. Sample without soaking was also used as the control.

To study the effect of some amino acids on shrimp treatment, glycine, glutamic acid and arginine were added into ASS (pH 11.5) to obtain the final concentrations of 1, 2, and 3% (w/v). Shrimp were treated with the prepared solutions as previously described.

After treatments, those shrimp were divided to two portions. The first portion was used as raw shrimp and another portion was subjected to cooking to obtain the

cooked samples. To prepare 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 analyzed for weight gain, cooking yield and cooking loss.

4.3.2.2 Analyses

4.3.2.2.1 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:

Weight gain (%) = [(B-A)/A] × 100

where: A = initial weight (before soaking)

B = weight after soaking, followed by draining

4.3.2.2.2 Determination of cooking loss and cooking yield

Cooking loss and cooking yieldwere 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 equation:

Cooking loss (%) = [(B-C)/B] × 100

Cooking yield (%) = (C/A) × 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

4.3.3 Effect of glutamic acid at various concentrations and pHs in alkaline soaking solution on weight gain, cooking loss and cooking yield of shrimp

Shrimp (peeled and deveined) were mixed with ASS (0.75% NaOH containing 2.5% NaCl) in the presence of glutamic acid solutions with various concentrations (1, 2, and 3%) having pH 7 and 11.5. Shrimp were treated with solutions and analyzed as described in section 4.3.2.2.1 and 4.3.2.2.2, respectively.

4.3.4 Comparative study of glutamic acid and monosodium glutamate in soaking solution for shrimp treatment

4.3.4.1 Preparation of treated shrimp

Shrimp (peeled and deveined) were mixed with ASS (pH 11.5) in the presence of glutamic acid or monosodium glutamate (MSG) at various concentrations (1, 2, and 3%) with pH of 11.5. MSG was used at the same mole equivalent to glutamic acid. Samples were treated as mention above in section 4.3.2.1 Both raw and cooked shrimp were analyzed as described in section 4.3.2.2. Shrimp were subjected to the additional characterization.

4.3.4.2 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 for measurement using a Hunterlab colorimeter (Hunter Associates Laboratory, Inc., Reston, Virginia, USA) using a CIE Lab scale (Young and Whittle, 1985).

4.3.4.3 Determination of shear force

Shear force of raw and cooked shrimp were 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.

4.3.4.4 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 into solutions. SDS-PAGE was performed using 10% running 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 *xg* for 15 min to remove undissolved debris using a microcentrifuge (MIK-RO20), Hettich Zentrifugan, Tuttlingen, Germany). 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, 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 protein was estimated.

4.3.4.5 Characterization shrimp treated with the selected solutions

Shrimp treated with ASS (pH 11.5) containing MSG at a level rendering the highest cooking yield (ASS+3% MSG) were subjected to characterization, in comparison with those without treatment, those treated with 2.5% NaCl containing mixed phosphate (M-P) and those treated with ASS without any additional chemical (ASS).

4.3.4.5.1 Determination of pH

pH of raw and cooked 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 (Sartorious North America, Edgewood, NY, USA).

4.3.4.5.2 Determination of moisture content

Cooked shrimp were finely chopped prior to analyses. Moisture content was determined using an oven according to the method of AOAC (2000).

4.3.4.5.3 Determination of NaCl content

NaCl content of cooked shrimp was determined as per the method of AOAC (2000). Sample (1 g) was added with 10 ml of 0.1 M $AgNO_3$ and 10 ml of concentrated 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.

Thereafter, 50 ml of distilled water and 5 ml of 50 g/l ferric alum ($FeNH_4(SO_4)_2 .12 H_2O$) indicator were added. The mixture was filtered with Whatman No.1 filter paper (Whatman International Ltd, Maidstone, UK) and adjusted to 100 ml with distilled water. The mixture was titrated with standardized 0.1 M KSCN until the solution became permanent brownish red. The salt content was then calculated as follows:

$$NaCl\ (\%) = 5.8 \times [(V_1 \times N_1)-(V_2 \times N_2)]/W$$

where, V_1 =volume of $AgNO_3$ (ml); N_1 =concentration of $AgNO_3$ (M); V_2=volume of KSCN (ml); N_2=concentration of KSCN (M); W= weight of sample (g)

4.3.4.5.4 Determination of thermal property

Thermal transition of shrimp meat proteins was measured using the differential scanning colorimetry (DSC) (Perkin-Elmer, Model DSCM, Norwalk, CT, USA). The samples (15-20 mg wet weight) were placed in the DSC hermetic pans, assuring a good contact between the sample and the pan bottom. An empty hermetic pan was used as a reference. The samples were scanned at 10 °C /min over the range of 20-100 °C. T_{max} was measured and the denaturation enthalpies (ΔH) were estimated by measuring the area under the DSC transition curve.

4.3.4.5.5 Determination of microstructure

Microstructure of cooked shrimp was analyzed as described by Ratanasatein *et al*. (2008). Samples were immersed in liquid nitrogen and was allowed to stand at room temperature for 5 min. Thereafter, the prepared sample was then cut into a cube (4x4x4 mm) with a razor blade. The prepared sample was fixed in 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 20-100% 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.

4.3.4.6 Determination of volatile compounds

4.3.4.6.1 Extraction of volatile compounds by SPME fiber

To extract volatile compounds, 3 g of cooked sample were homogenized at a speed of 13,500 rpm for 2 min with 8 mL of ultrapure water. The mixture was centrifuged at 2000 g for 10 min at 4 °C. The supernatant (6 mL) was heated at 60 °C with equilibrium time of 10 h in a 20-mL headspace vial (Agilent Technologies, Palo Alto, CA, USA). The SPME fiber (75 mm Carboxen™/PDMS StableFlex™) (Supelco, Bellefonte, PA, USA) was conditioned at 270 °C for 15 min before use and then exposed to the headspace. The 20 mL-vial containing the sample extract and the volatile compounds were allowed to absorb into the SPME fiber at 60 °C for 30 min. The volatile compounds were then desorbed in the GC injector port for 10 min at 260 °C.

4.3.4.6.2 GC-MS analysis

GC-MS analysis was performed in a Trace Ultra gas chromatograph coupled with a TSQ Quantum XLS triple quadrupole mass spectrometer (Thermo Scientific Inc., San Jose, CA, USA) and equipped with a splitless injector. Compounds were separated on a HP-Innowax capillary column (Hewlett Packard, Atlanta, GA, USA) (30 m ± 0.25 mm ID, with film thickness of 0.25 mm). The GC oven temperature program was: 35 °C for 3 min, followed by an increase of 3 C min^{-1} to 70 °C, then an increase of 10 C min^{-1} to 200 °C, and finally an increase of 10 °C min^{-1} to a final temperature of 260 °C and holding for 5 min. Helium was employed as a carrier gas with a constant flow of 1.5 mL min^{-1}. The injector was operated in the splitless mode and its temperature was set at 260 °C. Transfer line temperature was maintained at 265 °C. The quadrupole mass spectrometer was operated in the electron ionisation (EI) mode and source temperature was set at 200 °C. Initially, fullscan-mode data was acquired to determine appropriate masses for the later acquisition in scan mode under the following conditions: mass range: 10-200 amu and scan rate: 0.220 s $scan^{-1}$. All analyses were performed with ionization energy of 70 eV, filament emission current at 150 mA, and the electron multiplier voltage at 500 V.

4.3.4.6.3 Analysis of volatile compounds

Identification of the compounds was done by consulting ChemStation Library Search (Wiley 275.L). Identification of compounds was performed, based on the retention time and mass spectra in comparison with those of standards from ChemStation Library Search (Wiley 275.L). Quantification limits were calculated to a signal-to-noise (S/N) ratio of 10. The identified volatile compounds including aldehydes, alcohols, ketones, etc., were presented in the term of abundance of each identified compound.

4.3.4.7 Sensory evaluation

The cooked samples 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, odor, taste, texture and overall likeness. Samples were presented in the plates coded with three-digit random numbers.

4.3.5 Statistical analysis

A completely randomized design (CRD) was used for all 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).

4.4 Results and discussion

4.4.1 Weight gain, cooking loss and cooking yield of Pacific white shrimp treated with alkaline soaking solution containing sugar or sugar alcohol at various concentrations

Weight gain of Pacific white shrimp treated with alkaline soaking solution (ASS; 0.75% NaOH containing 2.5% NaCl, pH 11.5) in the presence of glucose, sorbitol and glycerol at different concentrations is illustrated in **Figure 15a.** Weight gain of all samples subjected to treatments was higher than the control (without treatment) ($P<0.05$), regardless of sugar or sugar alcohols used. In the presence of sugar and sugar alcohols water molecules were likely bound more strongly with proteins, in which water could be bound with proteins via sugar or sugar alcohols rich in hydroxyl groups. Those hydroxyl groups could form hydrogen bonds with water. During heat exposition, the transition of the water molecules from liquid to gas in the presence of sugar is retarded (Russ *et al.*, 2014). It was noted that weight gain varied with treatments. Due to differences in their chemical structure regarding the positions and orientation of their hydroxyl groups, different sugars could bind water differently (Gharsallaoui *et al.*, 2008). Among all samples, that treated with ASS containing 1% glucose showed similar weight gain to that treated with mixed phosphate ($P<0.05$). Addition of all sugar alcohols had no enhancing effect on weight gain in comparison with that treated with ASS ($P\geq0.05$). In general, sugar or sugar alcohols had no impact on improvement of weight gain. When concentration of sugar alcohols increased, no increase in weight gain of all samples was observed ($P<0.05$). For glucose treatment, weight gain increased with increasing concentrations ($P<0.05$). The dynamic state of water molecules around the sugar molecules in solution depends on the polar interaction between water and sugar molecules, which is affected by the number of equatorial hydroxyl groups (n(-OH)) (Russ *et al.*, 2014). Glucose might interact with water more effectively in the shrimp muscle, in comparison with sugar alcohols sorbitol and glycerol. Uedaira *et al.*, (1989) proposed that the sugar molecules, possessing a higher number of -OH groups per molecule, have a stronger stabilizing effect on the water structure.

Cooking loss of Pacific white shrimp treated ASS different sugar and sugar alcohols at various concentrations is shown in **Figure 15b**. Cooking loss of shrimp

treated with sorbitol and glycerol decreased ($P<0.05$). When high level (1%) was used. Nevertheless, no differences in cooking loss were found for samples treated with ASS with glucose at various levels ($P\geq0.05$) The water is probably lost due to heat induced denaturation of protein during cooking of the meat, which causes less water to be entrapped within the protein structures held by capillary forces (Analyng *et al.*, 2003). In general, treatment of shrimp using ASS containing all sugar and sugar alcohols had no impact on cooking loss. It was noted that all treatments showed the lower effect on prevention of cooking loss induced by heat, compared with mixed phosphates ($P<0.05$).

Cooking yield of Pacific white shrimp treated with ASS as influenced by sugar and sugar alcohols at various concentrations is shown in **Figure 15c.** No marked differences in cooking yield were found in shrimp treated with different solutions. Cooking yield slightly increased when the concentrations of sugar and sugar alcohols in ASS increased ($P<0.05$). The highest cooking yield was found in shrimp treated with mixed phosphate and the lowest cooking yield was obtained in the control (no treatment). Phosphates have been known to enhance the solubility of meat protein, and the soluble proteins are able to hold the water, leading to the decrease in the loss of water while cooking (Erdogdu *et al.*, 2007). The results suggested that sugar and sugar alcohols used in the present study had no pronounced effect on improving cooking yield. Thus, sugar and sugar alcohols were not the potential additive for shrimp treatment and they were not used for further study.

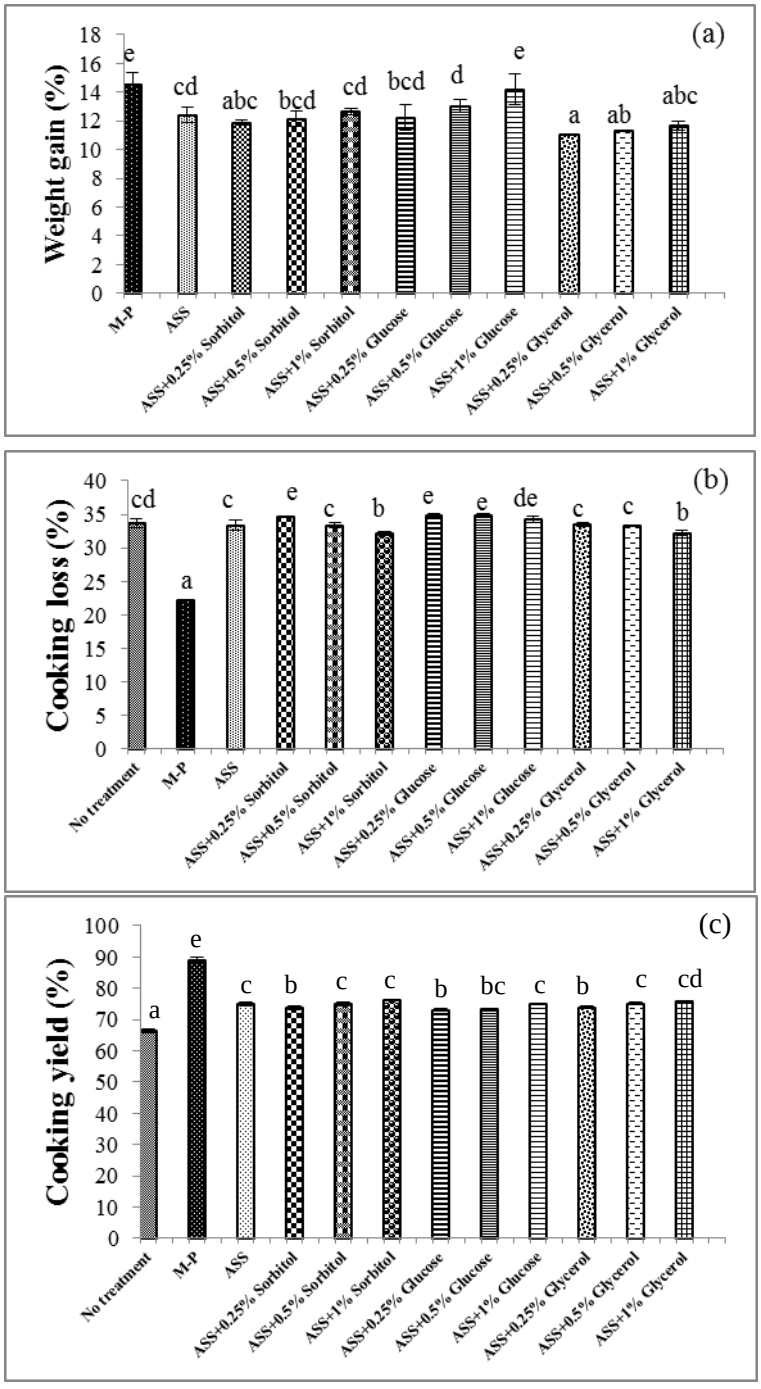


Figure 15.Weight gain (a), cooking loss (b) and cooking yield (c) of Pacific white shrimp treated with 0.75% NaOH containing 2.5% NaCl (pH 11.5) in the presence of sugar and sugar alcohols at different concentrations. Note: M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1, (w/w)), ASS: 0.75% NaOH containing 2.5% NaCl (pH 11.5). Different lowercase letters on the bars indicate significant differences ($P<0.05$). Bars represent the standard deviation (n=3).

4.4.2 Weight gain, cooking loss and cooking yield of Pacific white shrimp treated in alkaline solution containing amino acids at various concentrations

Weight gain of Pacific white shrimp treated with ASS (pH 11.5) in the presence of amino acids at different concentrations (1, 2 and 3%) is shown in **Figure 16a.** Weight gain of the treated shrimp increased, when concentrations of amino acids increased in ASS ($P<0.05$), except those treated with ASS containing glutamic acid, in which weight gain decreased with increasing concentration ($P<0.05$). Among all amino acids, glutamic acid showed the higher increasing effect on weight gain at low level (1%). Under the alkaline condition, carboxyl groups, both at α-carbon and γ-carbons, were deprotonated and COO^- became dominant. Those negatively charged residues might penetrate into the swollen muscle along with soaking solution. Some COO^- groups of glutamic acid could interact with positively charged domain of proteins via ionic interaction, while the rest of COO^- groups were able to bind with water. As a result, the water could be retained in the muscle after treatment. Nevertheless, in the presence of an excessive glutamic acid, those COO^- groups in the solution (aqueous phase) more likely compete with muscle protein in binding with water. As a consequence, the less water was retained in the shrimp muscle as indicated by the lowered weight gain, when the level of glutamic acid in soaking solution was higher than 1%. Glycine has been known as the smallest amino acid and has H atom as the side chain. Due to its small molecule, it could migrate easily into shrimp muscle and subsequently interacted with water by hydrogen bonding via side chains within muscle. Additionally, carboxyl groups at α-carbon of glycine, which was deprotonated under the alkaline condition, could interact with water via ionic interaction. Consequently, water could be more imbibed, particularly when the level of glycine increased. This was reflected by increasing weight gain of shrimp after being treated with glycine at higher concentrations. For shrimp treated with soaking solution containing arginine, weight gain increased as the levels of arginine increased. The side chain of arginine comprises several NH_2. At pH 11.5, some carboxyl groups of arginine (pI=10.76) became deprotonated. Those groups could interact with muscle proteins and simultaneously bound with water. It was noteworthy that pH of solution (11.5) was close to pI of arginine. Therefore, COO^- group of arginine in soaking solution was present at low intensity. Owing to the less abundance of COO^- group in

solution involving arginine, water was not competitively bound with solution rich in COO^- group as found in that containing glutamic acid. Wolfenden *et al.* (1981) found that hydration potential of arginine was high at pH 7. At neutral pH, NH_2 group mostly became protonated, in which NH_3^+ was formed. Those positively charged groups effectively bound with water. However, in the present study, the pH of solution was 11.5, which was above pI (10.76). As a result, NH_3^+ was not present in soaking solution. Furthermore, arginine is abundant in NH_2 group at the side chain. Those NH_2 groups preferably interacted with water via hydrogen bonding during soaking, especially when the higher levels were used. This was evidenced by the higher weight gain of shrimp treated with soaking solution containing arginine at higher concentrations. Arginine is also reported frequently to hydrogen bond to other side-chain heteroatoms and to water molecule (Borders *et al.*, 1994). Thus, charge of amino acid under the alkaline condition and the way those amino acids interacted with water and proteins in muscle might be the prime factor governing weight gain of treated shrimp.

Differences in weight gain among all treatments were more likely governed by the differences in water binding or water holding capacity of amino acids under the alkaline conditions. It was found that weight gain of shrimp treated with ASS containing 3% arginine was higher than those treated with mixed phosphates. Furthermore, ASS containing 3% arginine resulted in the significant increase in weight gain, compared with other treatments ($P<0.05$). It is noteworthy that treatment of shrimp with ASS containing 1% glycine, arginine and 3% glutamic acid led to the lower weight gain, compared with ASS without amino acids. Also, those treated with ASS containing 3% glutamic acid also showed the lower weight gain than that treated with ASS alone ($P<0.05$). Thus, amino acid in alkaline solution had varying impact on weight gain of shrimp after treatments.

Cooking loss and cooking yield of shrimp treated with ASS containing different amino acids at various concentrations are shown in **Figure 16b and 16c**, respectively. Cooking loss of shrimp decreased when the concentration of glutamic acid in ASS ($P<0.05$). However, no differences in cooking yield were noticeable, when glycine at different levels were used ($P\geq0.05$). Overall, when shrimp were

treated with ASS in the presence of all amino acids, the decrease in cooking loss was obtained, in comparison with those treated with only ASS ($P<0.05$). The weight loss was in the descending order in samples treated with ASS containing glycine, arginine and glutamic acid. It was noted shrimp treated using ASS compromising 3% glutamic acid had the higher cooking yield that those treated with M-P ($P<0.05$). The lowest cooking loss was found with the sample treated with ASS containing 3% glutamic acid ($P<0.05$). When heat was applied, denaturation and coagulation of proteins were augmented, which in turn lowered water-holding capacity. Moreover, the increased protein-protein interaction was obtained (Niamnuy *et al.*, 2007).

After cooking, cooking yield of shrimp with various treatments varied **(Figure 16c)**, in which water molecules might be bound with amino acids or proteins in different fashions. The highest cooking yield was obtained with shrimp treated with ASS containing glutamic acid, compared with other treatment ($P<0.05$). It is noteworthy that glutamic acid had potential to bind water in shrimp muscle, when heat was applied. The efficacy in water holding during heating was dependent on concentrations used ($P<0.05$). Efficiency in increasing cooking yield was in the descending order: glutamic acid, arginine and glycine. The negatively charged residues, especially carboxyl group at α-carbon and γ-carbon of glutamic acid are strongly hydrated (Collins *et al.*, 2007). The two amino acids that have the highest water-binding ability are aspartic acid and glutamic acid (Low *et al.*, 1978). An ionic side chain of aspartic acid, glutamic acid and lysine has been claimed to bind 4-7 water molecules (Zayas, 1997). However, the concentration of glycine had no effect on cooking yield of shrimp ($P<0.05$). When comparing with the sample treated with ASS alone, all samples treated with amino acids showed the increased cooking yield ($P<0.05$). Only sample treated with ASS containing 3% glutamic acid had the higher cooking yield than that treated in M-P ($P<0.05$) and the sample treated with ASS having 2% glutamic acid had similar cooking yield to those treated with mixed phosphate ($P\geq0.05$). Therefore, glutamic acid in ASS was shown to play a vital role in increasing the cooking yield by holding water in muscle during heating.

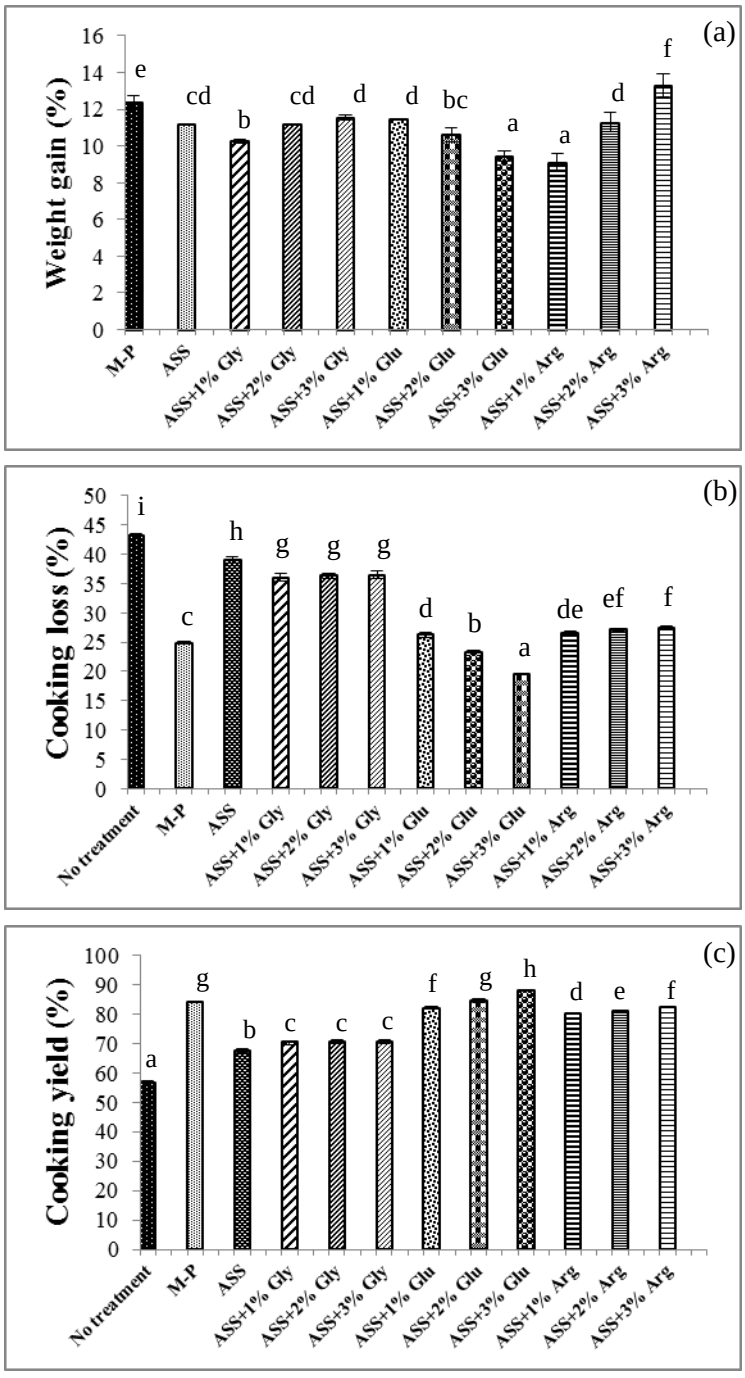


Figure 16. Weight gain (a), cooking loss (b) and cooking yield (c) of Pacific white shrimp treated with 0.75% NaOH containing 2.5% NaCl (pH 11.5) in the presence of amino acids at different concentrations. Note: M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1, (w/w)), ASS: 0.75% NaOH containing 2.5% NaCl (pH 11.5), Gly: glycine Glu: glutamic acid, Arg: arginine. Different lowercase letters on the bars indicate significant differences ($P<0.05$). Bars represent the standard deviation (n=3).

4.4.3 Weight gain, cooking loss, cooking yield and physical properties of Pacific white shrimp treated with alkaline soaking solution containing glutamic acid with various concentrations at different pHs

Weight gain, cooking loss and cooking yield of Pacific white shrimp after soaking in ASS in the presence of glutamic acid at various concentrations with different pHs (7.0 and 11.5) are shown in **Figure 17**. Weight gain and cooking yield of shrimp soaked in ASS, pH 11.5 were higher than those treated with ASS at pH 7.0, for all concentrations of glutamic acid used. At pH above pI or very alkaline pH, proteins had more negative charge, in which protein molecules repulsed each other, resulting in the swollen muscle structure (Chantarasuwan *et al.*, 2011b). As a consequence, water could be more uptaken (Chantarasuwan *et al.*, 2011b). Simultaneously, glutamic acid could be penetrated into the muscle along with NaCl. Glutamic acid might favor the water binding via its COO^- group. As a result, the weight gain was slightly decreased at pH 7.0 and 11.5 as the concentrations of glutamic acid used increased ($P<0.05$). Generally, weight gain of all samples was lower than that of samples treated with ASS and with M-P ($P<0.05$). Among Pacific white shrimp treated with sodium carbonate and sodium bicarbonate at pH 5.5, 7, 8.5, 10 and 11.5, the highest weight gain and cooking yield was observed in those treated at pH 11.5 (Chantarasuwan *et al.*, 2011b). Therefore, pH of soaking solution was the prime factors determining weight gain of shrimp.

In the presence of glutamic acid at all levels, no differences in cooking yield were obtained, compared with the sample treated with ASS alone ($P<0.05$), when pH of soaking solution was 7.0 **(Figure 17c).** On the other hand, cooking yield of samples treated with ASS containing glutamic acid at all levels were higher than that of sample treated with ASS alone, when pH was 11.5 ($P<0.05$). The result indicated the paramount role of pH in holding water of muscle when heating was implemented. When the shrimp were treated with ASS (pH 11.5) containing glutamic acid at levels higher than 1%, similar cooking yield was obtained, compared to that found in the sample treated with mixed phosphates ($P<0.05$). Therefore, the use of glutamic acid in ASS showed the potential in improving the cooking yield, and it efficacy was comparable to mixed phosphates.

Cooking loss **(Figure 17b)** of shrimp decreased, when the concentration of glutamic acid in ASS increased, regardless of pH used. Lower cooking loss was found as ASS had pH of 11.5 than pH 7.0. The decrease in cooking loss was in accordance with the increase in cooking yield. The lowest cooking loss was observed in shrimp soaked with ASS containing 3% glutamic acid (pH 11.5). Glutamic acid with negative charge could provide the charged environment, which enhanced the water binding with muscle proteins. Those water was held tightly during heating as indicated by the lowered cooking loss and increase cooking yield. Aaslyng *et al*. (2003) suggested that a higher cooking loss was found in the sample with the low pH, whereas high water holding capacity was achieved at medium and high pH. When comparing the cooking loss of shrimp treated with mixed phosphates, the sample treated with ASS containing glutamic acid at levels of 2 or 3% (pH 11.5) had the lower cooking loss ($P<0.05$). This reflected the high efficiency of ASS containing glutamic acid at a sufficient amount in lowering the cooking loss. The enhances repulsion of muscle compartment, which allowed glutamic acid with the high negative charge to bind more water along with muscle proteins, led to the lowered cooking loss.

4.4.4 Comparative study of alkaline soaking solution containing glutamic acid and monosodium glutamate on shrimp treatment

Glutamic acid with high efficacy in increasing cooking yield was selected as the potential additive in ASS. However, glutamic acid had low solubility. Conversely, MSG, a salt form, was cheaper than and soluble with ease in water. MSG was used at the same mole equivalent to glutamic acid for treatment of shrimp.

4.4.4.1 Weight gain, cooking loss and cooking yield

Weight gain of shrimp treated with ASS containing glutamic acid at the levels of 1%, 2% and 3% or MSG at the levels of 1%, 2% and 3% mole equivalent of glutamic acid is shown in **Figure 18a**. Sample treated with ASS containing 1% glutamic acid and 1 or 2% MSG had similar weight gain to that treated with mixed phosphate (positive control) ($P\geq0.05$). It was postulated that the negatively charged amino acid residues were able to bind tightly with water molecule via ionic interaction within protein network, leading to the increase water holding in shrimp

muscle. It was found that MSG showed higher ability in water holding than glutamic acid. When MSG was dissolved, Na^+ was generated in the solution. Na^+ was able to penetrate into muscle and subsequently interacted with water. This resulted in higher weight gain of shrimp treated with ASS containing MSG.

For cooking loss **(Figure 18b)**, shrimp treated with ASS containing MSG had the higher cooking loss than those treated with glutamic acid counterpart ($P<0.05$). The cooking loss was lower in samples treated with ASS comprising both glutamic acid and MSG at higher concentrations ($P<0.05$). For samples treated with mixed phosphate and ASS alone, the cooking losses of 19.19% and 31.07%, respectively were observed. The cooking loss of 13.76-19.62% was obtained for sample treated with ASS having glutamic acid, while cooking loss of 17.51-20.41% was obtained for the sample treated with ASS containing MSG. During cooking, muscle proteins underwent denaturation to a higher extent, while the amount of water retained in shrimp meat decreased with coincidental increase in fat and protein content (Manheem *et al.*, 2012 and Benjakul *et al.*, 2008). For the sample treated with ASS containing MSG, the interaction between Na^+, dissociated from MSG, and water might be destroyed during cooking process. This plausibly led to the lower ability of muscle in water holding after cooking as indicated by higher cooking loss. The result suggested that shrimp muscle could retain more water when glutamic acid and MSG were incorporated in ASS. However, glutamic acid showed the slightly higher ability in lower cooking loss of shrimp, compared with MSG, especially at level of 2-3%.

For cooking yield **(Figure 18c)**, the opposite results were observed, in comparison with cooking loss. The lowest cooking yield was observed in the control (without treatment). Cooking yield of treated shrimp increased, when the concentrations of both glutamic acid and MSG increased ($P<0.05$). Similar cooking yield was found in shrimp treated with ASS containing 1% glutamic acid or 2% MSG, compared to that of sample treated with mixed phosphates ($P<0.05$). It was found that shrimp treated with ASS containing 3% glutamic acid had the higher cooking yield than that of sample treatment with mixed phosphates ($P<0.05$). Since MSG was soluble and cheaper than glutamic acid, it was selected for treatment of shrimp. The appropriate concentration of MSG in ASS was 3%.

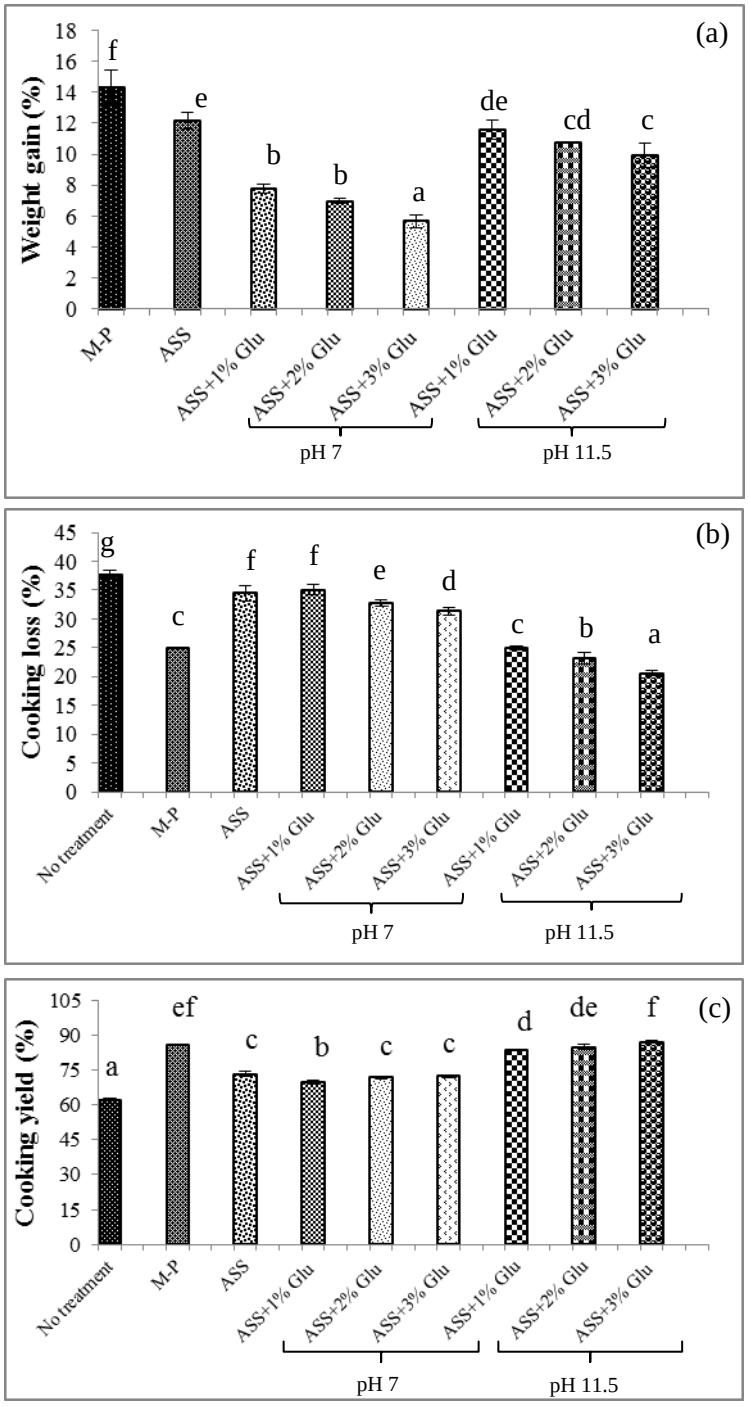


Figure 17. Weight gain (a), cooking loss (b) and cooking yield (c) of Pacific white shrimp treated with 0.75% NaOH containing 2.5% NaCl with pHs 7.0 and 11.5 in the presence of glutamic acid at various concentrations. Note: M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1, (w/w)), ASS: 0.75% NaOH containing 2.5% NaCl (pH 11.5), Glu: glutamic acid. Different lowercase letters on the bars indicate significant differences ($P<0.05$). Bars represent the standard deviation (n=3).

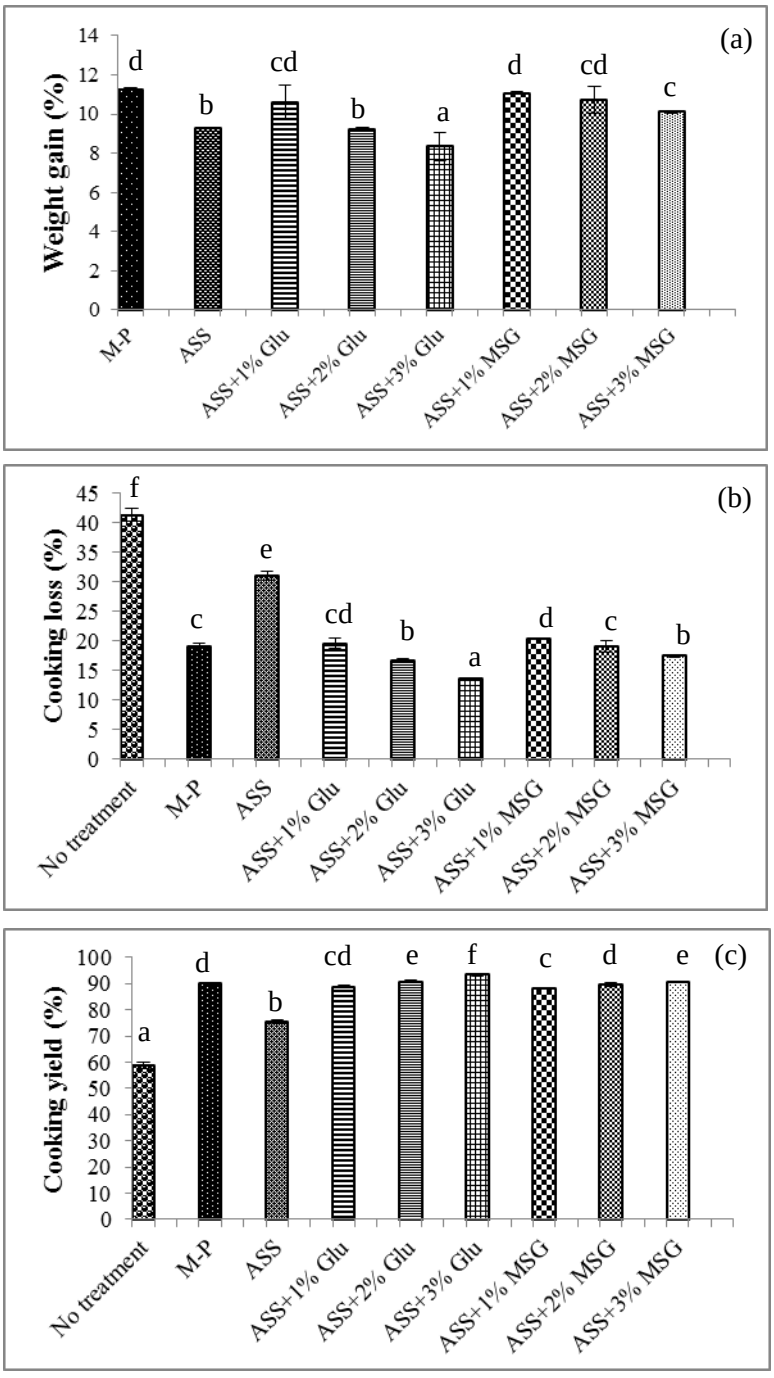


Figure 18. Weight gain (a), cooking loss (b) and cooking yield (c) of Pacific white shrimp treated with 0.75% NaOH containing 2.5% NaCl (pH 11.5) in the presence of glutamic acid and MSG at various concentrations. Note: M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1, (w/w)), ASS: 0.75% NaOH containing 2.5% NaCl (pH 11.5), Glu: glutamic acid. MSG: monosodium glutamate. Different lowercase letters on the bars indicate significant differences ($P<0.05$). Bars represent the standard deviation (n=3).

4.4.4.2 Shear force

Shear force of raw and cooked Pacific white shrimp treated with ASS (pH 11.5) in the presence of glutamic acid and MSG at different levels (1%, 2% and 3%) is presented in **Table 11.** Shear force is related with texture, which is important parameter in shrimp quality. For raw shrimp, all treatments had no impact on shear force of raw shrimp. It was noted that shrimp treated with ASS containing glutamic acid and MSG at all levels (1-3%) had similar shear force ($P \geq 0.05$). Generally, shrimp tended to have the non-significant decrease in shear force after treatment, particularly with increasing concentrations of glutamic acid and MSG in ASS used for treatment. When proteins imbibed water within their structure, the muscle had the lower resistance to the force applied.

For cooked shrimp, the decrease in shear force was observed in all treated samples, in comparison with those without treatment and those treated with ASS alone. Nevertheless, all samples had the similar shear force, compared to those treated with M-P ($P<0.05$). When heat were applied, protein denaturation and aggregation took place. Those phenomena resulted in the toughness as well as high resistance to force. No changes in shear force was found in shrimp treated with ASS containing glutamic acid and MSG at different concentrations used ($P<0.05$). Myofibrillar proteins with increased negative charge favored the repulsion of polypeptide chains, which resulted in the swelling of muscle and became less resistant to shear force applied. When the concentration of glutamic acid or MSG in ASS increased, shear force had the trend to decrease. This was in agreement with the increased cooking yield as the concentration of glutamic acid or MSG in ASS used for shrimp treatment increased **(Figure 18c)**

Therefore, treatment of shrimp using ASS containing glutamic acid or MSG did not had the negative impact on texture property and their shear force was comparable to that of shrimp treated with mixed phosphates.

Table 11. Shear force of raw and cooked of Pacific white shrimp treated with 0.75% NaOH (pH 11.5) containing 2.5% NaCl in the presence of glutamic acid and MSG at various concentrations.

Treatments	Shear force (g)	
	Raw	**Cooked**
No treatment	1970 ± 177 †,abc	2194 ± 326 b
M-P	2057 ± 212 bc	1578 ± 341 a
ASS	1890 ± 416 abc	2031 ± 345 b
ASS+1% MSG	2127 ± 219 c	1549 ± 130 a
ASS+2% MSG	1995 ± 284 abc	1525 ± 334 a
ASS+3% MSG	1794 ± 275 abc	1472 ± 83 a
ASS+1% glu	1972 ± 329 abc	1555 ± 205 a
ASS+2% glu	1665 ± 253 ab	1515 ± 126 a
ASS+3% glu	1618 ± 243 a	1449 ± 118 a

†Mean±SD (n=3).

Note: M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1, (w/w)), ASS: 0.75% NaOH containing 2.5% NaCl (pH 11.5), Glu: glutamic acid. MSG: monosodium glutamate. Different lowercase superscripts in the same column indicate significant differences ($P<0.05$).

4.4.4.3 Color

Color parameters (L^*, a^* and b^*) of raw and cooked shrimp treated with ASS (pH 11.5) in the presence of glutamic acid or MSG at different concentrations (1, 2 and 3%) are shown in **Table 12.** For raw shrimp, L^* value increased when concentrations of MSG in ASS increased (3%) ($P<0.05$). The a^* value generally increased as the concentrations of both glutamic acid and MSG in ASS increased ($P<0.05$). Basically, a^* value indicates the reddish color. No changes in b^* value were observed as the level of glutamic acid or MSG in ASS increased ($P<0.05$).

Table 12. Color of raw and cooked of Pacific white shrimp treated with 0.75% NaOH (pH 11.5) containing 2.5% NaCl in the presence of glutamic acid and MSG at various concentrations.

Samples	**Treatments**	***L****	***a****	***b****
Raw	No treatment	46.46±1.58 ^{ab}	-1.64±1.05 ^{a}	0.12±1.91 ^{cd}
	M-P	46.33±3.43 ^{ab}	-1.69±0.36 ^{a}	-3.23±1.79 ^{a}
	ASS	44.96±2.10 ^{a}	-1.66±0.34 ^{a}	-2.01±1.61 ^{ab}
	ASS+1% MSG	45.42±2.58 ^{a}	0.27±0.96 ^{b}	-1.13±0.48 ^{bc}
	ASS+2% MSG	46.81±2.10 ^{ab}	1.28±1.13 ^{bc}	-0.88±1.28 ^{bc}
	ASS+3% MSG	48.87±2.51 ^{b}	2.30±1.15 ^{d}	0.16±1.96 ^{cd}
	ASS+1% glu	45.22±2.39 ^{a}	0.89±1.51 ^{b}	-0.02±1.63 ^{cd}
	ASS+2% glu	46.09±0.88 ^{a}	2.01±0.74 ^{cd}	0.08±1.95 ^{cd}
	ASS+3% glu	46.35±1.22 ^{ab}	2.77±0.87 ^{d}	1.19±2.71 ^{d}
Cooked	No treatment	70.02±4.04 ^{c}	13.18±4.40 ^{d}	16.34±3.98 ^{b}
	M-P	65.50±4.30 ^{ab}	8.55±2.45 ^{ab}	12.37±4.63 ^{a}
	ASS	69.35±5.37 ^{bc}	11.85±4.02 ^{cd}	13.39±3.20 ^{ab}
	ASS+1% MSG	65.09±2.40 ^{a}	11.17±2.77 ^{abcd}	14.34±3.86 ^{ab}
	ASS+2% MSG	64.77±3.95 ^{a}	8.86±1.31 ^{ab}	11.28±3.63 ^{a}
	ASS+3% MSG	63.17±4.55 ^{a}	8.27±1.94 ^{a}	10.92±3.18 ^{a}
	ASS+1% glu	65.50±3.75 ^{ab}	11.32±1.91 ^{bcd}	15.64±3.06 ^{ab}
	ASS+2% glu	64.94±2.38 ^{a}	9.69±1.60 ^{abc}	14.40±3.13 ^{ab}
	ASS+3% glu	64.44±2.05 ^{a}	9.04±1.30 ^{abc}	11.78±2.18 ^{a}

†Mean±SD (n=3).

Note: M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1, (w/w)), ASS: 0.75% NaOH containing 2.5% NaCl (pH 11.5), Glu: glutamic acid. MSG: monosodium glutamate. Different lowercase superscripts in the same column under the same state of sample indicate significant differences ($P<0.05$).

Generally, raw shrimp turned to be slightly reddish as the levels of glutamic acid or MSG in ASS increased **Figure 19**. Such a change led to the lower consumer acceptance. Development of reddish color is commonly associated with the loss in freshness, in which carotenoid released could contributed to the discoloration. Appearance of the product and the resulting quality implications play a significant role in maintaining high consumer acceptance (Bono *et al.*, 2012). Glutamic acid or MSG in ASS might induce the change of proteins associated with carotenoid, named carotenoprotein, This led to the enhancement of exposed free carotenoids, especially astaxanthin, as evidenced by more reddish color. Astaxanthin was reported as the major pigment in shrimp meat (Niamnuy *et al.* 2008).

After cooking, no difference in color was observed among shrimp treated with ASS containing glutamic acid or MSG at all levels ($P \geq 0.05$). However, the pale color was found in shrimp treated with ASS containing either glutamic acid or MSG, compared with shrimp without treatment as shown in **Figure 20.** This result was in accordance with the lower *a**- and *b**- values of treated samples, compared with the control (no treatment). However, no differences in color and appearance were observed between samples treated with all ASS and those treated with mixed phosphates (positive control). It was noted that higher redness was found in the control (no treatment), compared with those with all treatments. With heat treatment, the control more likely underwent thermal denaturation, in which the loss of water was more pronounced than the samples with treatment. Furthermore, treatment of shrimp with ASS, or mixed phosphates enhanced the water holding capacity of muscle as evidenced by lower cooking loss **(Figure 18)**. As a result, pigments, particularly astaxanthin, became more concentrated in the control. This resulted in the more reddish color **(Figure 20)**. Furthermore, during soaking, some proteins including carotenoprotein were partially solubilized or leached out. As the results, less pigment were retained in the meat. Coincidentally, the soaking solution was more reddish in color as the glutamic acid or MSG concentrations in ASS increased. There was no difference in all color parameters between shrimp treated with ASS containing glutamic acid or MSG at all levels and those treated with mixed phosphates. It was obvious that *L**, *a** and *b**-values increased, in comparison with raw counterparts.

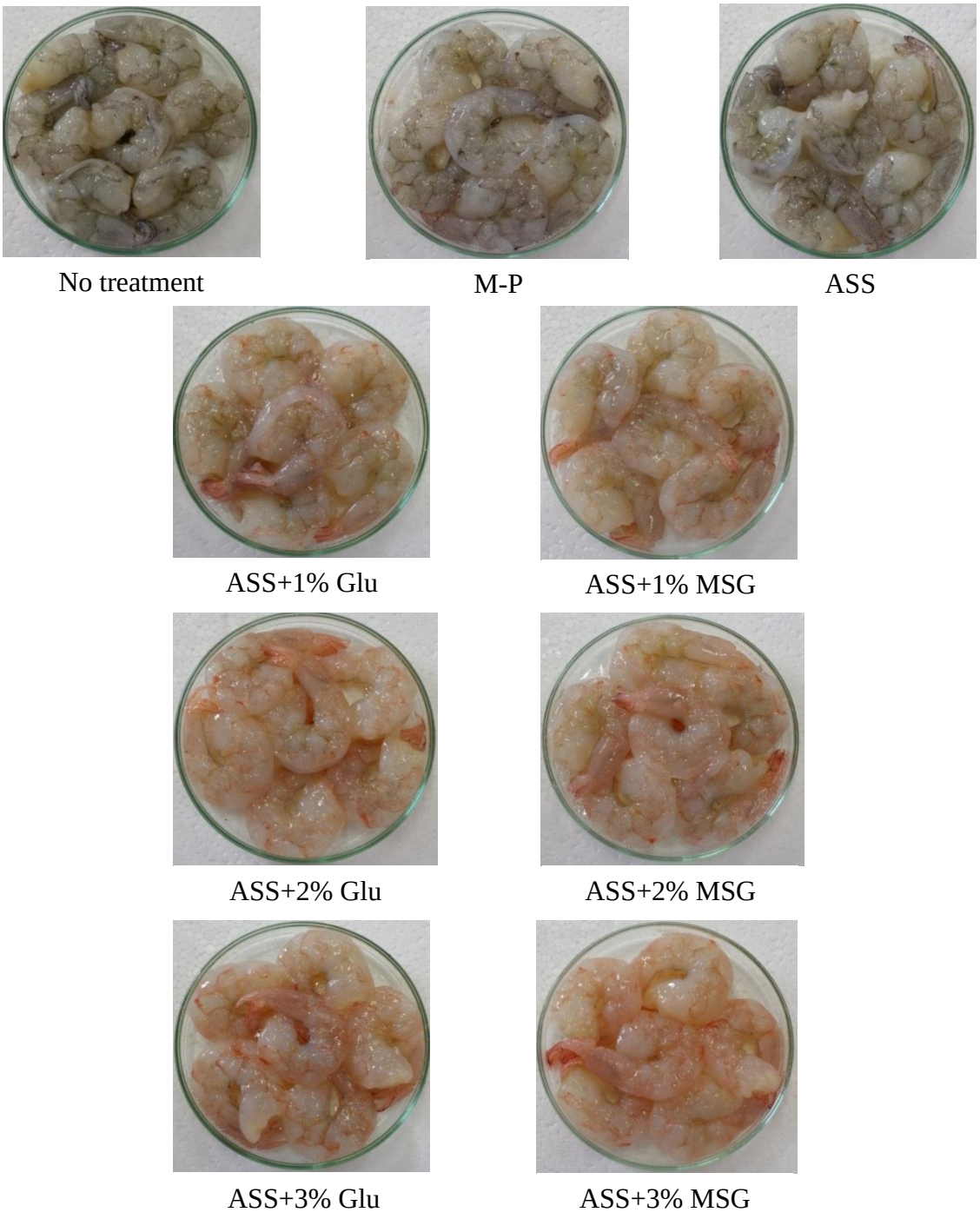


Figure 19. Raw shrimp of Pacific white shrimp treated with 0.75% NaOH (pH 11.5) containing 2.5% NaCl in the presence of glutamic acid and MSG at various concentrations. Note: M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1, (w/w)), ASS: 0.75% NaOH containing 2.5% NaCl (pH 11.5), Glu: glutamic acid. MSG: monosodium glutamate.

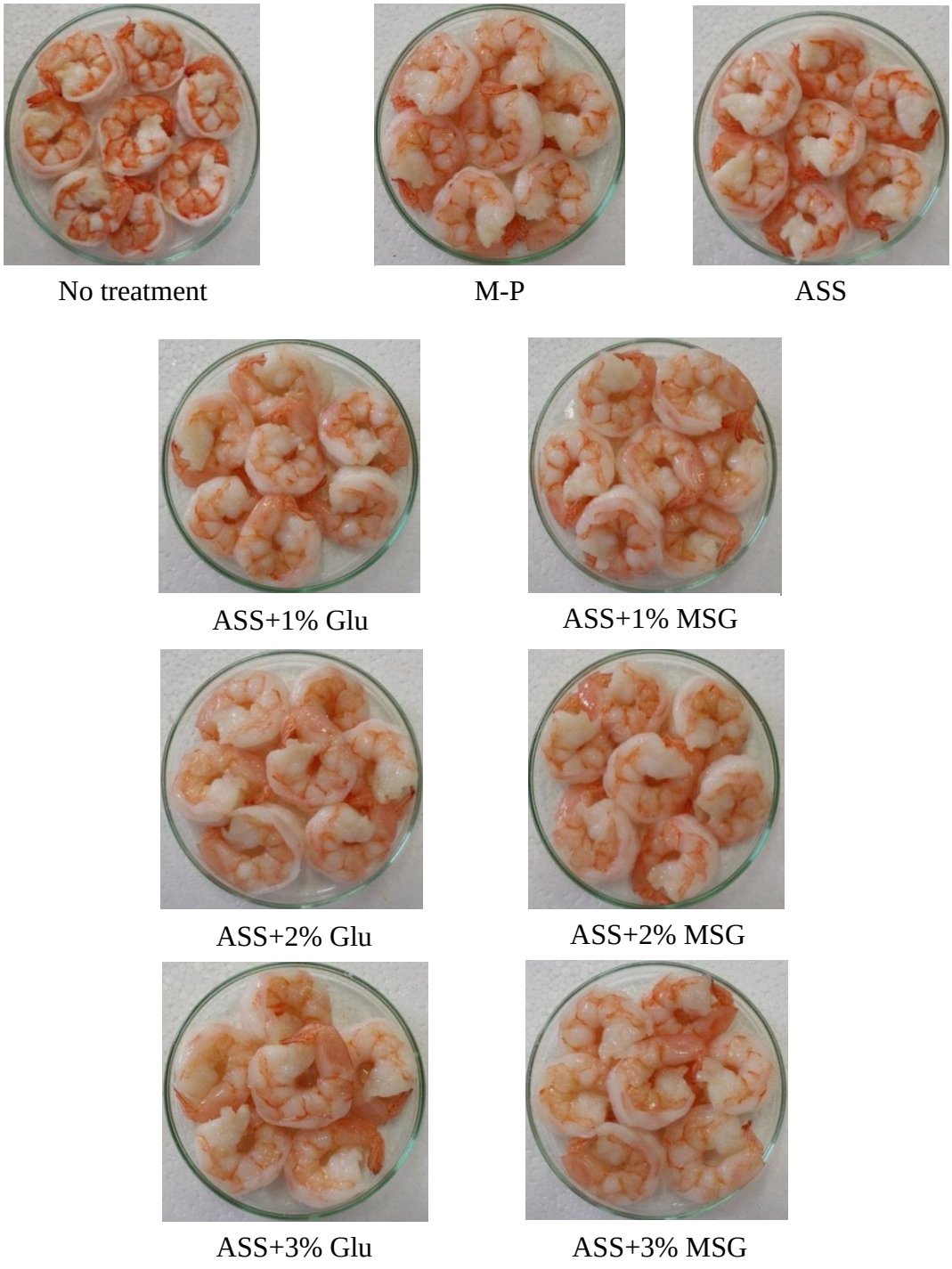


Figure 20. Cooked shrimp of Pacific white shrimp treated with 0.75% NaOH (pH 11.5) containing 2.5% NaCl in the presence of glutamic acid and MSG at various concentrations. Note: M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1, (w/w)), ASS: 0.75% NaOH containing 2.5% NaCl (pH 11.5), Glu: glutamic acid. MSG: monosodium glutamate.

The increase the intensity of the red or yellow color during cooking is caused by the muscle protein denaturation and the release of carotenoid pigment bound to the protein (carotenoproteins) (Latscha 1989 and Niamnuy *et al.* 2008).

4.4.4.4 Protein pattern of soaking solutions

Protein patterns of soaking solutions after shrimp treatments are shown in **Figure 21.** Band intensity of myosin heavy chains (MHC) and actin slightly increased as concentration of both glutamic acid and MSG in ASS increased. The result suggested that more proteins, particularly MHC and actin, were solubilized and leached out to solution as glutamic acid and MSG levels increased. The increase in MHC band intensity in soaking solutions was in agreement with the higher cooking yields **(Figure 18c**). Protein extraction and dissociation of myofibrillar protein were mainly due to the ionic effect and pH alteration (Bendall, 1954). Apart from MHC and actin, protein with MW of 25.64 and 18.22 kDa also increased with increasing levels of glutamic acid and MSG in ASS. Chantarasuwan *et al.*, (2011) reported that Pacific white shrimp soaked in sodium bicarbonate and sodium carbonate solution had the increase in band intensity of MHC as pH of soaking solution increased. Protein pattern of soaking solutions (mixed phosphates or ASS) were similar, in which actin and protein with MW of 18.22 kDa were dominant. After those proteins were leached out from shrimp muscle, the soaking solution could be more penetrated and embedded inside the muscle as indicated by higher weight gain and cooking yield.

From the study, the use of 0.75% NaOH (pH 11.5) containing 2.5% NaCl in the presence of 3% MSG showed the potential for treatment of shrimp.

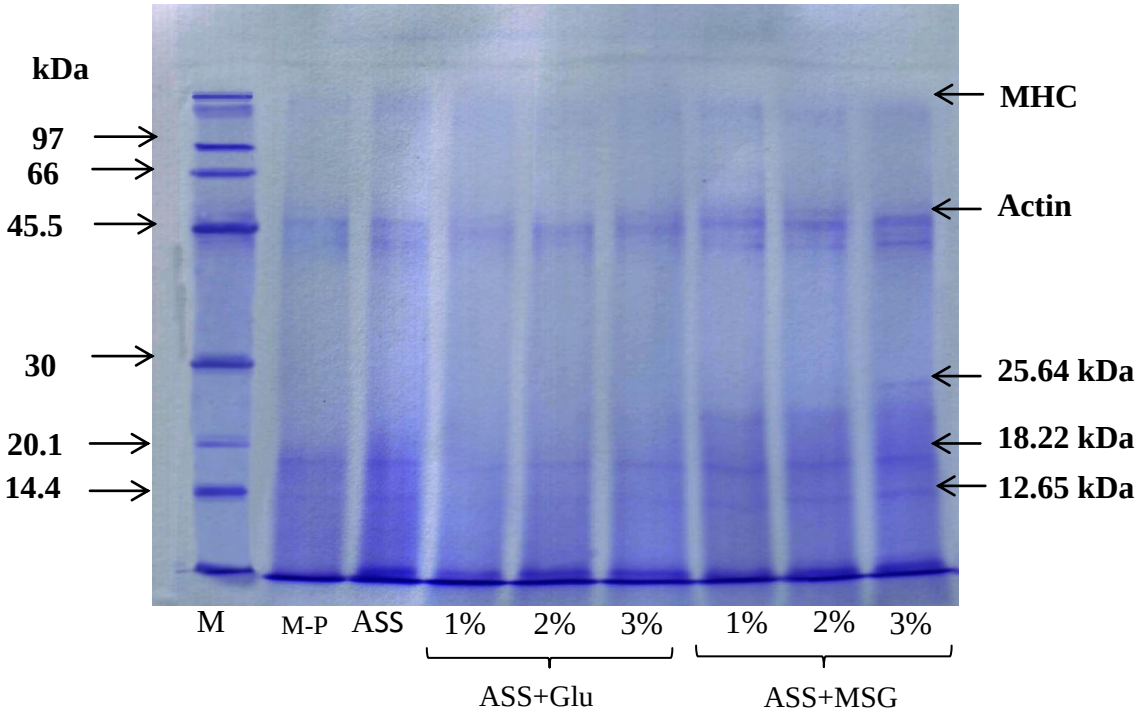


Figure 21. Protein patterns of soaking solution (0.75% NaOH and 2.5% NaCl, pH 11.5) in the presence of glutamic acid and MSG solutions at different concentrations after soaking with shrimp.

M, Standard marker; M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1 (w/w)), ASS: 0.75% NaOH containing 2.5% NaCl (pH 11.5), MSG: monosodium glutamate, MHC: myosin heavy chain. Numbers indicate the concentration of Glu or MSG (%).

4.4.5 Characterization of shrimp treated with alkaline soaking solution containing MSG

Shrimp treated with ASS (pH 11.5) containing 3% MSG (pH 11.5) were subjected to characterization in comparison with those treated with ASS alone and those control (without treatment), those treated with M-P.

4.4.5.1 pH of raw and cooked shrimp

The pHs of raw and cooked Pacific white shrimp treated with ASS containing 3% MSG (pH 11.5) (ASS+3% MSG) are shown in **Figure 22**. For raw shrimp, the control (without treatment), those treated with ASS alone (ASS) and those treated with mixed phosphates (M-P) were 7.14, 7.53, 7.25 and 8.25, respectively **(Figure 22a).** In general, the pH value of shrimp muscle was in the range of 7-8 (Mendes *et al.* (2002) and Manheem (2012)). 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). Although soaking solutions used were alkaline in pH, pH of treated shrimp muscle was not increased drastically. This was due to the buffering capacity of muscle proteins (Chantarasuwan *et al.*, 2011b). For the sample treated with ASS+3% MSG, the solution could penetrate into the shrimp muscle to a higher extent. The solutions absorbed in the muscle more likely contribute to the higher pH of shrimp meat treated with ASS containing 3% MSG (ASS+3% MSG).

For cooked sample **(Figure 22b),** the sample without treatment, M-P and ASS samples had pH of 6.87, 7.40 and 7.10, respectively. ASS+3% MSG sample had higher pH (8.20). The different pH of shrimp meat directly affected weight gain and cooking yield. ASS+3% MSG sample showed the higher pH than others. This was associated with the higher negative charge, compared with others. Increasing charge was related with the increasing repulsion of proteins, thereby providing the space for water to be retained in sample treated with ASS and ASS+3% MSG sample. The retained or bound water was imbibed in the muscle, even when heat was applied as indicated by the higher cooking yield **(Figure 18c)**.

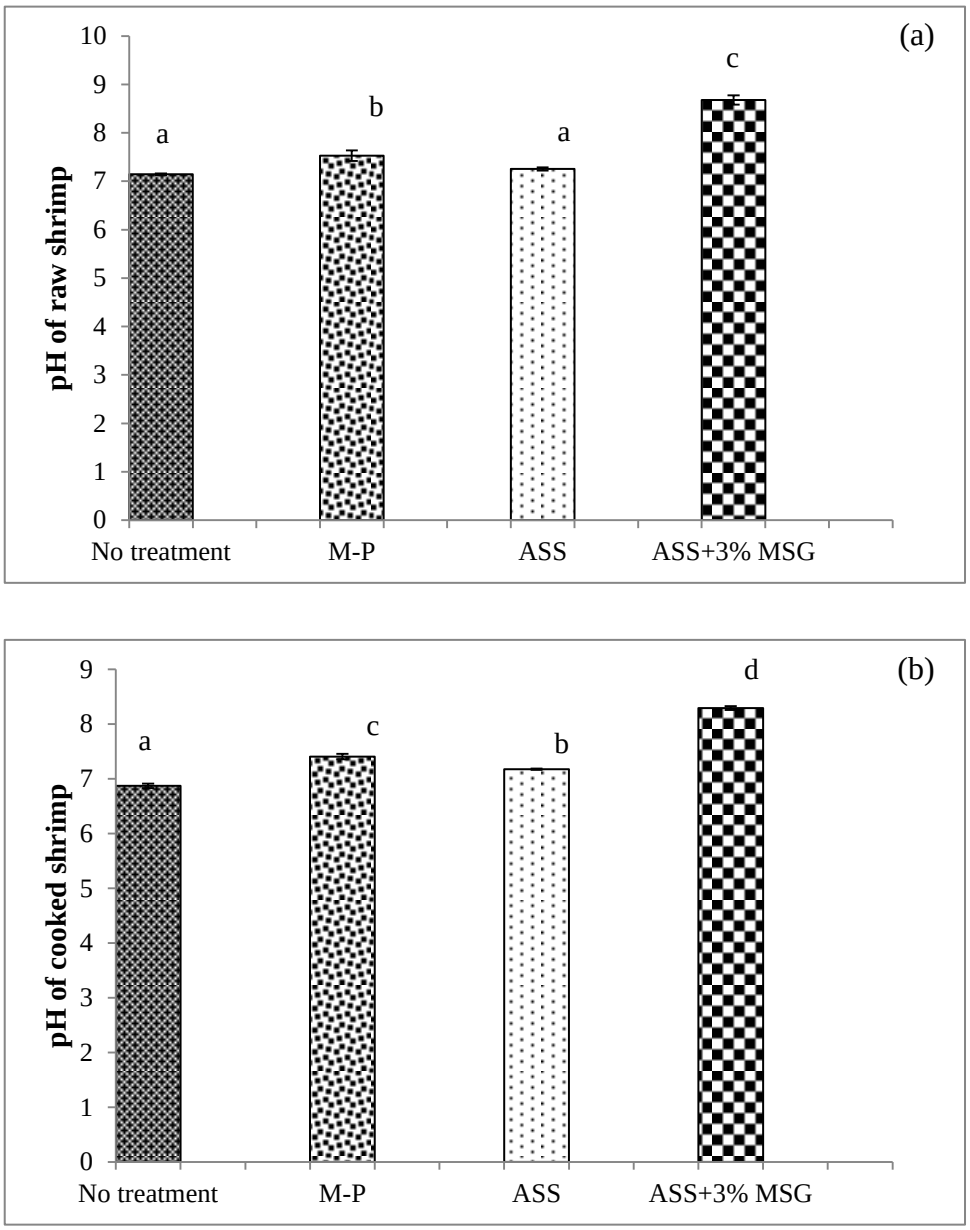


Figure 22. pH of raw (a) and cooked (b) Pacific white shrimp meat. Note: M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1, (w/w)), ASS: 0.75% NaOH containing 2.5% NaCl (pH 11.5), ASS+3% MSG: 0.75% NaOH containing 2.5% NaCl (pH 11.5) in the presence of 3% monosodium glutamate. Different lowercase letters on the bars indicate significant differences ($P<0.05$) Bars represent the standard deviation (n=3).

4.4.5.2 Moisture content

Moisture contents of cooked ASS+3% MSG sample in comparison with other samples are shown in **Table 13.** The highest moisture content (82.02%) was found in ASS+3% MSG sample ($P<0.05$), followed by M-P sample (81.19%). Without any treatment, muscle protein more likely underwent thermal aggregation during cooking, leading to the poorer water holding capacity. Moisture content can be used as an indicator of water holding capacity in shrimp after cooking and it could reflect the juiciness of the product. The lowest moisture content of the control (without treatment) was in accordance with the highest cooking loss. Enhanced denaturation and coagulation of proteins in turn resulted in the lower water holding capacity (Niamnouy *et al.*, 2007); the water was mainly lost as a result of heat-induced denaturation of proteins during cooking of meat (Manheem 2012). This resulted in less water entrapped within the protein structures (Aaslyng *et al.*, 2003). This result indicated that alkaline treatment in combination with MSG (ASS+3% MSG) could effectively increase the water uptake into the shrimp muscle and the water was more retained when heating was employed.

4.4.5.3 NaCl content

NaCl contents of cooked shrimp subjected to different treatments are shown in **Table 13.** Shrimp without treatment had the lowest NaCl content, compared with others having various treatments ($P<0.05$). The highest NaCl content was found in ASS+3% MSG sample, compared with other treatments ($P<0.05$). The higher NaCl content in shrimp meat indicated that NaCl was co-penetrated with NaOH and water. This was coincidental with the highest moisture content of ASS+3% MSG sample **(Table 13)** The increase in NaCl in the muscle might enhance the solubilization of myofibrillar proteins in shrimp meat (Chantarasuwan *et al.*, 2011b). When NaCl was dissociated, Na^+ ions form ion pairs with the negatively charged residues in proteins such as aspartic acid and glutamic acid, minimizing the interaction of protein molecules and enhancing the swelling of myosin filaments. Consequently, the migration of water into the muscle was facilitated and water was retained in the structure via ionic interaction. Cl^- ion has been suggested to be bound with the positively charged residues, e.g. arginine, histidine, and lysine residues of the myosin

filament, thereby introducing swelling of proteins (Puolanne and Halonen, 2010). M-P sample had the lower NaCl content than ASS and ASS+3% MSG sample ($P<0.05$). It postulated that phosphate ion might occupy around the protein molecules. As a consequence, the less surface of protein could interact with chloride ion. Generally, NaCl has been employed in soaking solution to enhance the water uptake into shrimp muscle (Chantarasuwan *et al.*, 2011b). Rattanasatherin *et al.* (2011) found that NaCl content in shrimp soaked in phosphate solution in the presence of 2.5% NaCl was lower than that of shrimp without phosphate treatment. Thus, NaCl content could be used to indicate the migration of soaking solution into shrimp meat and NaCl also determined the characteristics of shrimp meat.

Table 13. Moisture content and NaCl of content of cooked Pacific white shrimp meat treated with 0.75% NaOH (pH 11.5) containing 2.5% NaCl and 3% monosodium glutamate (MSG)

Treatments	Moisture content (wet weight basis)	NaCl content (dry weight basis)
No treatment	74.01± 0.01 †,a	5.38 ± 0.26 a
M-P	81.19 ± 0.50 c	7.34 ± 0.12 b
ASS	78.18 ± 0.41 b	7.91 ± 0.32 c
ASS+3% MSG	82.03 ± 0.10 d	8.98 ± 0.19 d

†Mean±SD (n=3).

Note: M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1, (w/w)), ASS: 0.75% NaOH containing 2.5% NaCl (pH 11.5), ASS+3% MSG: 0.75% NaOH containing 2.5% NaCl (pH 11.5) in the presence of 3% monosodium glutamate. Different lowercase superscripts in the same column indicate significant differences ($P<0.05$).

4.4.5.4 Thermal property

Thermal transitions of shrimp muscle protein as determined using DSC and expressed as T_{max} and ΔH are shown in **Table 14.** DSC analysis was used to determine the thermal transition or unfolding temperature of protein and also to quantify the enthalpy of conformational transition (John and Shastri, 1998; Sriket, *et al.*, 2007a). The transition represents point where the conformational changes occur in protein structure due to denaturation and is generally expressed as peak maximum temperature (T_{max}). For fresh raw shrimp, two major peaks were obtained, corresponding to myosin and actin peaks. The control (without treatment) had T_{max} of MHC and actin at 48.83 °C and 68.50 °C, respectively. DSC thermogram of Pacific white shrimp meat revealed two major endothermic peaks with T_{max} of 50.1 and 71.3 °C, corresponding to myosin and actin peaks (Rattanasatheirn, 2008).

Table 14. T_{max} and enthalpy of content of cooked Pacific white shrimp meat treated with 0.75% NaOH (pH11.5) containing 2.5% NaCl and 3% monosodium glutamate (MSG)

Treatments	T_{max}I (°C)	ΔH (J/g)	T_{max}II (°C)	ΔH (J/g)
No treatment	48.78±0.05 †,d	1.29±0.04 b	69.60±0.16 d	0.33±0.02 d
M-P	45.47±0.18 c	1.14±0.01 a	65.49±0.44 b	0.20±0.02 c
ASS	46.13±0.10 b	1.17±0.01 a	68.56±0.13 c	0.16±0.01 b
ASS+3% MSG	43.85±0.22 a	1.16±0.03 a	64.38±0.0.2 a	0.12±0.01 a

†Mean±SD (n=3).

Note: M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1, (w/w)), ASS: 0.75% NaOH containing 2.5% NaCl (pH 11.5), ASS+3% MSG: 0.75% NaOH containing 2.5% NaCl (pH 11.5) in the presence of 3% monosodium glutamate. Different lowercase superscripts in the same column indicate significant differences (P<0.05).

Sriket *et al.* (2007a) reported that myosin from Pacific white shrimp had T_{max} of 50.13 °C and T_{max} of actin was 71.17 °C. After treatment, T_{max} of both MHC and actin shifted to the lower values. Additionally, ΔH was also decreased. This suggested that both myosin and actin underwent denaturation to some extent during soaking.

The results were in accordance with Rattanasatheirn (2008) who reported that T_{max} of actin and myosin peaks of fresh shrimp shifted to the lower temperature. When protein denatures, both inter- and intramolecular bonds are disrupted and its conformation is changed from a highly order structure. The lowest T_{max} of MHC and actin were found in ASS+3% MSG sample, compared with other treated sample ($P<0.05$). However, no differences in ΔH of MHC were observed between all treated samples. For actin peak, the lowest ΔH was observed in ASS+3% MSG sample ($P<0.05$). Under alkaline condition, MSG could be dissociated and free glutamic acid was released. Glutamic acid was deprotonated and COO^- could provide the negative charge, which further attach to protein, thereby inducing conformational change to high degree. This was evidenced by the solubilization of protein, which was further leached out to soaking solution **(Figure 21).** The decreases in T_{max} and ΔH confirmed that alkaline solution containing MSG (ASS+3% MSG) induced the conformational changes of protein associated with the lowered thermal stability of protein in the treated samples.

4.4.5.5 Microstructures

Microstructures of cooked shrimp subjected to various treatments are illustrated in **Figure 23 and 24.** For the transverse section **(Figure 23)**, the control shrimp (without treatment) had more compact structure, compared with M-P, ASS and ASS+3% MSG sample. When the proteins underwent the thermal denaturation, the water was less imbibed or bound in their structure (Rattanasatheirn *et al.*, 2008). Simultaneously, protein fibrils more likely underwent aggregation, thereby repelling water from muscle compartment. The release of water from protein molecules might facilitate the muscle fiber to align closely, leading to the more compact structure (Rattanasatheirn *et al.*, 2008). Rattanasatheirn *et al.* (2008) reported that cooked shrimp without treatment had more compact structure, compared with shrimp treated with ProfixO and mixed phosphates. The more compact fibers might be associated with the increased shear force **(Table 11)** of cooked shrimp. When shrimp treated with mixed phosphate (M-P) and ASS containing 3% MSG (ASS+3% MSG), the gap between muscle bundles was enlarged, indicating the swelling of muscle fibers induced by treatment. Through muscle fiber expansion (swelling) caused by

electrostatic repulsions, water could more immobilized in the myofibril lattices (Offer and Knight, 1988). The swollen fiber and bundle were related well with higher cooking yield **(Figure 18c).** Under alkaline conditions muscle fibers had negatively charge domain, thus facilitating repulsion or selling.

For the longitudinal section of cooked samples **(Figure 24)**, the destruction of Z-lines was noticeable after treatments. Myofibrils were less attached with the loss of Z-lines. In general, the looser structure was obtained after treatments. Z-line disruption and the destroyed cytoskeletal proteins by the action of proteases, releasing α-actin, nebuli and titin, were related meat tenderization (Busconi *et al*., 1989; Hernandez-Herrero, *et al*., 2003; Luther and Squire, 2002; Olafsdottir *et al*., 1997; Pearson and Young, 1989). On the other hand, compact structure was obtained for the control (without treatment). Heating process caused the shrinkage of muscle of shrimp with concomitant tougher texture **(Table 11).** For M-P samples, M-line disappeared to some degree. M-line might be solubilized or removed by phosphates. Proteins associated with M-line are M-protein, myomesin and creatine kinase. (Pearson and Young, 1989). Myomesin in M-line was successfully extracted with the aid of Na-pyrophosphate (Masaki and Takaiti, 1972). Rattanasatheirn *et al.* (2008) also reported the disappearance of M-line in Pacific white shrimp treated with mixed phosphates. When comparing between ASS and ASS+3% MSG samples, the latter had the larger and more swollen filaments than the former. The results reconfirmed that MSG incorporation in alkaline solutions (ASS+3% MSG) could induce the repulsion of protein in shrimp meat, in which the swelling of muscle fibrils could be achieved. More retained water in swollen shrimp meat contributed to the increased cooking yield **(Figure 18c).**

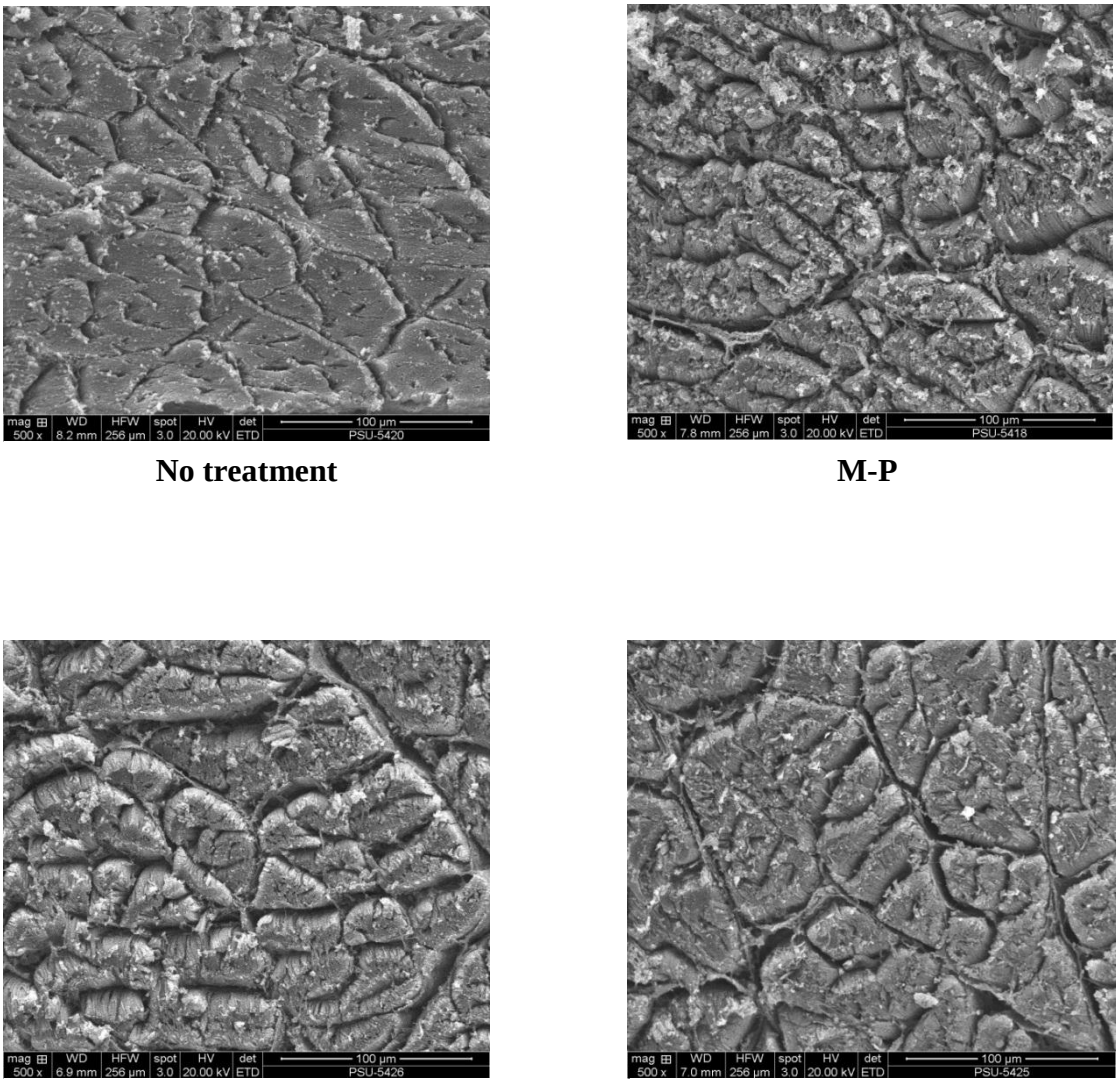
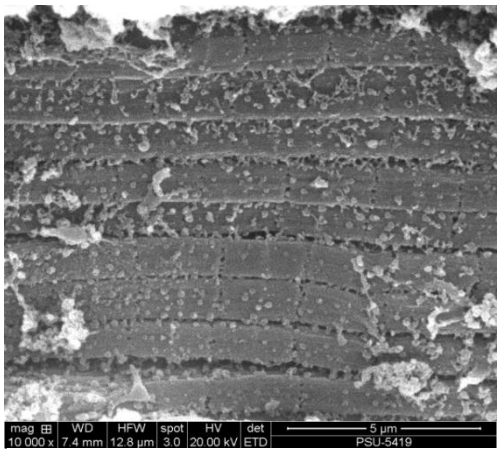
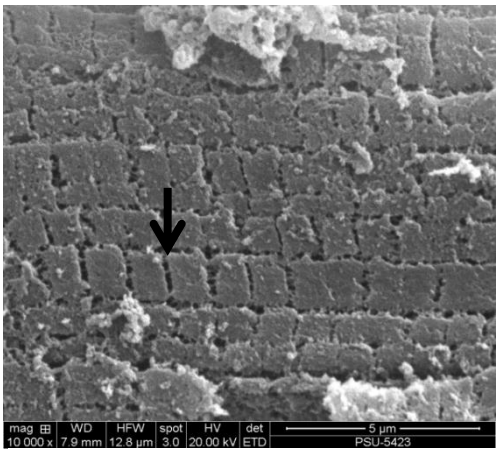


Figure 23. SEM micrographs of transverse sections of cooked shrimp muscle treated with different treatments. Magnificent 5,000x.

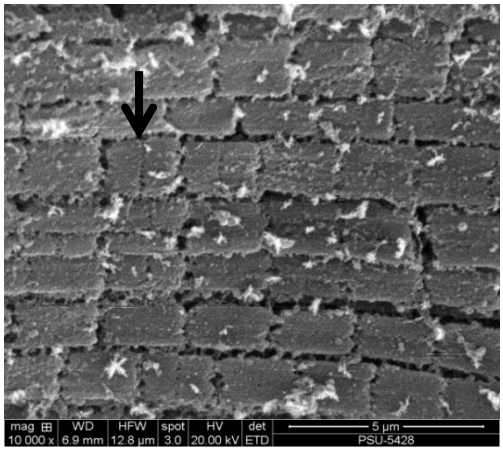
Note: M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1 (w/w)), ASS: 0.75% NaOH containing 2.5% NaCl (pH 11.5), ASS+3% MSG: 0.75% NaOH containing 2.5% NaCl (pH 11.5) in the presence of 3% monosodium glutamate.



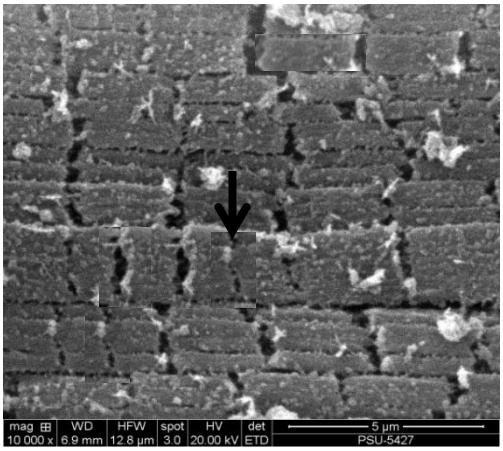
No treatment



M-P



ASS



ASS + 3% MSG

Figure 24. SEM micrographs of longtitudinal sections of cooked shrimp muscle treated with different treatments. Magnificent 10,000x.

*Arrow indicates M-line

Note: M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1 (w/w)), ASS: 0.75% NaOH containing 2.5% NaCl (pH 11.5), ASS+3% MSG: 0.75% NaOH containing 2.5% NaCl (pH 11.5) in the presence of 3% monosodium glutamate.

4.4.5.6 Volatile compounds

Volatile components of cooked shrimp with different treatments are presented in **Table 15.** Aldehydes and alcohols were found as the major volatile compounds in cooked shrimp but the abundance varied with treatments. Aldehydes play an important role in meat aroma (Chen and Zhang, 2006). Alcohols are generally minor contributors to the food flavor because they have higher thresholds unless they are present at high concentrations or are unsaturated enolic constituents (Chen and Zhang, 2006). After cooking, the control shrimp (without treatment) had four major components including pentanol, 2-ethyl hexanol, 1-octaen-3-ol and nonanol, in which pentanol was the dominant volatile. For M-P sample, similar volatiles were found, compared with the control sample, except that no 1-octen-3-ol was detected. Seven volatile compounds were found in ASS and ASS+3% MSG samples as shown in **Table 15.** A total of 94 volatile compounds were found in whole crab and crab meat. Those consisted of aldehyde, alcohol, ketone and ester (Chen and Zhang, 2006). Liu *et al.* (2009) reported that volatile compounds were significantly increased by cooking. The volatile profile of cooked samples by different methods was significantly different, due to the different temperatures used for cooking procedures which affect fatty acid composition (Liu *et al.*, 2009). Generally, aldehydes and alcohols may be produced by the thermal oxidation and degradation of polyunsaturated fatty acid. Thermal degradation of lipid provides the compounds, which determine the flavor of the different species (Gu and Zhao, 2008). Aldehydes have been reported to contribute to green, fruity, nutty and cheesy and sweet, flavor or smell, depending on the concentration (Chen and Zhang, 2006).

In the present study, nonanal and hexanal were higher in abundance in ASS +3% MSG sample than ASS sample. Thus, the use of ASS+3% MSG for shrimp treatment more likely enhanced the development of aldehydes in cooked shrimp.

Nonanal provides geranium, plastic, marine odors to the flavor of cooked meat (Ning-Ping *et al.*, 2014). (E)-2-nonenal yields fishy, cucumber, fatty and green odors. It has been reported that (E)-2-nonenal is a lipid peroxidation product derived from the oxidized n-6 polyunsaturated fatty acids. It could be considered as the off-flavor

compound, which was described as having earthy and wet earth odors (Selli *et al.*, 2006). Hexanal contributes to fishy, grassy, leafy and green odors (Ning-Ping *et al.*, 2014). Hexanal may be responsible for the aldehydic aroma note and was also detected with a distinct coarse, plant-like odor in immediately harvested finfish (Josephson *et al.*, 1983). It has been also reported that hexanal is mainly derived from the oxidation of linoleic acid and provides green and fatty character to fish and other seafood (Caprino *et al.*, 2008).

Table 15. Volatile compounds of cooked Pacific white shrimp meat with different treatments

Compounds	**Peak area (abundance) x 10^7**			
	No treatment	**M-P**	**ASS**	**ASS+ 3% MSG**
Alcohol				
Pentanol	37.58	12.92	30.72	18.25
2-ethyl hexanol	5.46	2.47	3.56	25.50
1-octen-3-ol	5.45	ND	4.19	6.70
1-penten-3-ol	ND	ND	25.87	10.80
2-penten-3-ol	ND	ND	25.88	10.80
Aldehyde				
Nonanal	6.50	4.29	3.37	13.56
Hexanal	ND	ND	3.56	25.50

*ND: not detected

Note: M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1 (w/w)), ASS: 0.75% NaOH containing 2.5% NaCl (pH 11.5), ASS+3% MSG: 0.75% NaOH containing 2.5% NaCl (pH 11.5) in the presence of 3% monosodium glutamate.

Alcohols have been reported to contribute to mushroom-like order, fishy and grassy (Ning-Ping *et al.*, 2014). Among all samples, ASS+3% MSG sample had the highest abundance of 2-ethyl hexanol, while ASS sample showed the higher abundance in pentanol, 1-penen-3 –ol and 2-penten-3-ol. Therefore, glutamic acid derived from MSG directly had the impact on the formation of alcohol in cooked shrimp. 1-Penten-3-ol contributes to fishy, grassy odors and was generated from oxidation of PUFAs catalyzed by lipoxygenase or hydroperoxidase (Ning-Ping *et al.*, 2014). It was found in refrigerated sardine *(Sardinops melanostica)* and identified as the compound that provides a major contribution to the paint-like and chemical-like odors (Shi *et al.*, 2013). 1-octen-3-ol contributed to fishy, fatty, mushroom, grassy odors, and was mainly derived from enzymes involved in the biosynthesis by a lipoxygenase and a hydroperoxide lyase of linoleic acid (Josephson *et al.*, 1983). It is a major volatile alcohol in the seafood such as sea bream, crayfish, prawns, mussels and crab (Boonsumrej *et al.*, 2007). 1-octen-3-ol is an important contributor to off-flavors due to its low-odor threshold and it is also increased during frozen storage (Iglesias *et al.*, 2009). 2-Ethyl-1-hexanol was described as having raw fish and green odors (Ning-Ping *et al.*, 2014). This alcohol was also detected in raw oyster (Pennarun *et al.*, 2002), cooked catfish (Grimm *et al.*, 2000), sardine (Chouinard *et al.*, 1983), and fresh and smoked salmon (Varlet *et al.*, 2006). ASS+3% MSG sample generally had the higher fishy odor than others (data not shown). Due to high abundance of 2-ethyhexanol, this alcohol more likely contributed to such an off-flavor, in conjunction with aldehydes. The results suggested that glutamic acid in alkaline solution might interact with lipoprotein in muscle via COO^- group. This might lead to the conformational changes, in which the lipid domain was more exposed and susceptible to oxidation. As a results, lipid oxidation could be generated and provide fishy off-odor in ASS+3% MSG sample.

4.4.5.7 Sensory properties

Sensory properties of cooked shrimp treated without and with different treatments are shown in **Table 16.** The lowest score for all attributes tested except flavor was found in the control sample (without treatment) ($p<0.05$). During heating process, the shrinkage of muscle of shrimp occurred, resulting in more compact in microstructure of shrimp with less juiciness **(Figure 19 and 20).** Among all samples, ASS+3% MSG sample showed the highest score for all attributes except for flavor that showed the lowest score ($p<0.05$). However, flavor likeness score of ASS+3% MSG sample was non-significant lower than ASS sample ($P<0.05$). This might be associated with fishy odor/flavor of ASS+3%MSG sample. MSG is sodium salt of glutamic acid and provides a flavoring function similar to naturally occurring free glutamate in foods (Yamaguchi and Ninomiya, 2000). Monosodium L-glutamate (MSG) has been used as a flavor enhancer since 1908, when it was identified as the source of umami taste (pleasant savory taste) (Imada *et al.*, 2014). Since ASS+3% MSG samples had the high water holding capacity, in which more water was retained in shrimp meat **(Table 13),** the less firmness or toughness were obtained **(Table 11).** Decreased toughness with high juiciness contributed to the higher likeness score for texture and appearance. For color likeness, the highest score was observed for ASS+3% MSG sample ($P<0.05$). Treatment of shrimp using 0.75% NaOH containing 2.5%NaCl and 3% MSG (ASS+3% MSG) rendered the resulting cooked shrimp with the highest overall likeness score ($P<0.05$).

Table 16. Likeness score of cooked Pacific white shrimp with different treatments

Attributes	No treatment	M-P	ASS	ASS+3% MSG
Appearance	5.50±1.25 †,a	7.76±0.66 c	7.00±0.98 b	8.00±0.74 c
Color	6.03±1.56 a	7.76±0.66 c	7.07±1.01 b	7.87±0.82 c
Flavor	7.10±1.03 b	7.31±1.13 b	6.80±1.19 ab	6.40±1.57 a
Texture	5.57±2.10 a	7.55±0.81 bc	7.00±1.74 b	7.80±0.85 c
Taste	5.80±1.85 a	7.83±0.73 b	7.73±0.78 b	8.13±0.73 b
Overall	5.77±1.45 a	7.76±0.85 bc	7.40±0.81 b	8.07±0.69 c

†Mean±SD (n=3).

Note: M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1 (w/w)), ASS: 0.75% NaOH containing 2.5% NaCl (pH 11.5), ASS+3% MSG: 0.75% NaOH containing 2.5% NaCl (pH 11.5) in the presence of 3% monosodium glutamate. Different lowercase superscripts in the same row indicate significant differences ($P<0.05$).

4.5 Conclusion

The use of 0.75% NaOH containing 2.5% NaCl (pH 11.5) (ASS) in the presence of sugar (glucose) and sugar alcohols (sorbitol and glycerol) at various levels (0.25-1%) had no combined effect on improvement of water holding capacity. Conversely, ASS containing amino acid, particularly glutamic acid, could enhance water uptaken and water holding in treated shrimp. Alkaline pH (pH 11.5) was the key factor for quality improvement of shrimp as evidenced by lower capacity at pH 7.0. When shrimp were treated with ASS having 3% monosodium glutamate (glutamic acid mole equivalent), higher weight gain and cooking yield were obtained, in comparison with those treated with 3% glutamic acid. For cooked shrimp, treatment using ASS containing MSG had no effect on color and shear force, but the treated shrimp had the highest overall likeness score. Therefore, ASS containing 3%MSG could be used as the potential phosphate and bicarbonate replacers for shrimp treatment.

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CHAPTER 5

EFFECT OF PHOSPHATE AND BICARBONATE REPLACERS ON QUALITY CHANGES OF RAW AND COOKED PACIFIC WHITE SHRIMP *(LITOPENAEUS VANNAMEI)* AS INFLUENCED BY THE REPEATED FREEZE-THAWING

5.1 Abstract

Quality changes of raw and cooked Pacific white shrimp treated with different soaking solutions, including 1) 0.75% NaOH, pH 11.5 (ASS), 2) ASS with 3% monosodium glutamate (MSG) (pH 11.5) (ASS+3% MSG) and 3) 2.5% NaCl containing mixed phosphate (M-P) after freeze-thawing with various cycles were investigated. Higher protein solubility was observed in raw shrimp treated with ASS+3% MSG, compared with other treatments ($P<0.05$), regardless of freeze-thaw cycles. Raw shrimp treated with ASS+3% MSG or M-P showed the lowest drip loss after 5 freeze-thaw cycles. No α- glucosidase (AG) as well as β-N-acetyl-glucosaminidase (NAG) activities were found in raw shrimp treated with ASS+3% MSG and M-P at all freeze-thaw cycles tested. As freeze-thaw cycles increased, *a**-value of raw shrimp increased ($P<0.05$). On the other hand, *a** value of cooked shrimp treated with ASS containing 3% MSG decreased with increasing freeze-thaw cycles ($P<0.05$). Shear force of both raw and cooked shrimp with all treatments increased when freeze-thaw cycles increases up to 3 cycles ($P<0.05$), however, it was drastically decreased after 5 freeze-thaw cycles ($P<0.05$). Therefore, treatment of shrimp with 0.75% NaOH containing 2.5% NaCl and 3% MSG (pH 11.5) could retard the deteriorative change induced by freeze-thawing process.

Keywords: Quality changes, Freeze-thawing, Pacific white shrimp

5.2 Introduction

Quality of fish and shellfish is of paramount concern for processor and consumers. To increase the shelf-life and reduce the rate of biochemical and microbial degradation, different preservative methods, mainly based on low temperatures, particularly, freezing, etc. have been used for distribution and storage of products (Gallart-Jornet *et al.*, 2007). It has been known that the extended frozen storage is associated with quality changes, mainly attributed to protein denaturation and lipid oxidation (Kitthiphattanabawon *et al.*, 2012). Additionally, thawing is necessary for frozen food before any additional subsequent food processing or cooking (Xia *et al.*, 2012). Repeated freeze–thawing is a common practice in retail shop, restaurant or home (Boonsumrej *et al.*, 2007). However, quality deterioration takes place during freezing and thawing process, especially texture, flavor and color due to osmotic removal of water, mechanical damage, as well as cross-linking and aggregation of myofibrillar protein (Benjakul *et al.*, 2003). The freeze-thaw process is found to be detrimental to overall physicochemical and textural quality and affects the thermal properties of freshwater prawn (Srinivasan *et al.*, 1997a, 1997b; Benjakul and Friedrich, 2001). Rattanasatheirn *et al.* (2008) demonstrated that freezing and thawing disrupt muscle cells and cause the release of some enzyme such as AG and NAG. Freeze-thawing induced shrimp protein denaturation, tissue disruption and damage to muscle fibers (Sriket *et al.*, 2007). Furthermore, increasing freeze–thaw cycles of shrimp increased the thiobarbituric acid reactive substances and cutting force, but decreased salt-soluble protein. The spacing between the muscle fiber increased and the muscle fibers were disrupted as the number of freeze-thaw cycles increased (Boonsumrej *et al.*, 2007). From the previous study, shrimp treated with 0.75% NaOH containing 2.5% NaCl in the presence of 3%MSG (pH 11.5) provided the highest cooking yield and overall likeness score. Nevertheless, no information regarding the quality changes of those shrimp in both raw and cooked forms as affected by freeze-thawing process has been reported. Therefore, the objective of this work was to investigate the quality changes of shrimp with different treatments after being subjected to multiple freeze-thawing.

5.3 Materials and methods

5.3.1 Preparation of shrimp with different treatments

5.3.1.1 Collection and preparation of shrimp

Pacific white shrimp (*Litopenaeus vannamei)* (55-60 shrimp/kg) were purchased from a local market in Hat Yai, Songkhla province, Thailand. Shrimp with storage time less than 6 h after capture were stored in the insulated box containing 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. Prepared shrimp were placed in polyethylene bag and stored in ice until used.

5.3.1.2 Treatments of shrimp

Shrimp (peeled and deveined) were mixed with different soaking solutions including 1) 0.75% NaOH containing 2.5% NaCl (pH 11.5) (ASS), 2) ASS containing 3% MSG (pH 11.5) (ASS+3% MSG) and 3). 2.5% NaCl containing 3% mixed phosphates (sodium tripolyphosphate+tetrasodium pyrophosphate; 1:2, w/w) (M-P). The mixtures were stirred gently for 30 min at 4 °C and allowed to stand at 4 °C for 30 min. After treatment, the shrimp were placed on the plastic screen for 5 min (4 °C) to drain off solution. Sample without soaking was used as the control.

After treatments, those shrimp were divided to two portions. The first portion was used as raw shrimp. Another portion was cooked 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.

5.3.2 Quality changes of raw and cooked shrimp subjected to various freeze-thaw cycles

Raw and cooked shrimp without and with different treatments (100 g) were packed in the polyethylene bag and heat-sealed. The samples were frozen at -18° C, using an air blast freezer for 24 h. Thereafter, the frozen samples were thawed using a running water (25-26°C). The thawed samples were frozen as previously described, followed by thawing. The freeze-thaw cycles were 0, 1, 3 and 5 cycles. Both raw and cooked shrimp were ground until the uniformity was obtained and used for all

analyses, except for color and shear force, in which the whole shrimp were used. Raw prepared samples were analyzed as follows:

5.3.2.1 Determination of protein solubility

Solubility was determined according to the method of Rattanasatheirn (2008). One gram of sample was mixed with 20 ml of 0.6 M KCl. The mixture was homogenized for 1 min at a speed of 12,000 rpm using an IKA homogenizer (Salangor, Malaysia). The homogenate was stirred at 4°C for 4 h, followed by centrifuging at 8,500*xg* for 30 min at 4°C. To 10 ml of supernatant, cold 50% (w/v) trichloroacetic acid was added to obtain the final concentration of 10%. The precipitate was washed with 10% trichloroacetic acid and solubilized in 0.5 M NaOH. The sample was also directly solubilized by 0.5 M NaOH and used for total protein determination. Protein content was determined using the Biuret method (Robinson and Hodgen, 1940) and expressed as the percentage of total protein in the sample.

5.3.2.2 Assays of α–glucosidase (AG) and β-N-acetyl-glucosaminidase (NAG) activities

Shrimp (25 g) were chopped into small pieces, followed by centrifuging at 10,000*xg* for 60 min at 4 °C using a centrifuge (Beckman Coulter, Avanti J–E Centrifuge, Beckman Coulter, Inc., Palo Alto, CA, USA). The exudate formed was collected using a Pasteur pipette and the volume was measured. The exudate was brought to 25 ml with distilled water before enzyme assay. The resulting exudate was used as the source of AG and NAG. The protein content in exudate was determined by the Lowry method (Lowry *et al.*, 1951).

AG (E.C. 3.2.1.20) and NAG (E.C. 3.2.1.30) activities were determined according to the method of Benjakul and Bauer (2000) with a slight modification. For AG activity assay, the activity was measured spectrophotometrically using ρ-nitrophenyl-α-glucopyranoside as a substrate. The reaction mixture consisted of 0.3 ml of 0.1 M Na-citrate buffer (pH 4.0), 0.2 ml of 1.0 M NaCl, and 1 ml of diluted exudate. The reaction mixture was pre-incubated at 37 °C for 10 min. The reaction was initiated by adding 1 ml of 4.2 mM ρ-nitrophenyl-α-glucopyranoside. After 60 min, the reaction was terminated by adding 1 ml of 0.3 M KOH. The absorbance was measured at 405 nm. The blank was performed using distilled water instead of the

exudate. The negative control was carried out by adding the stopping reagent prior to the addition of substrate.

NAG activity was measured using ρ-nitrophenyl-N-acetyl- β-D-glucose amide as a substrate. The reaction mixture included 0.3 ml of 0.1 M Na-citrate buffer (pH 4.5), 0.2 ml of 0.6 M KCl and 0.2 ml of diluted exudate. The reaction was started by adding 0.2 ml of ρ-nitrophenyl-N-acetyl-β-D-glucose amide. The mixture was incubated at 37 °C for 10 min. The reaction was stopped by adding 1 ml of 0.3 M KOH. The blank and negative controls were performed as described above. The absorbance was measured at 405 nm.

The amount of ρ-nitrophenol released was monitored at 405 nm and calculated using a molar extinction coefficient of 19,500 M^{-1} cm^{-1}. One unit of enzyme is defined as the enzyme, which releases 1 mmol of ρ-nitrophenol per min under the assay condition.

Both raw and cooked shrimp were subjected to further analyses.

5.3.2.3 Determination of drip loss

Drip loss was determined according to the method of Hasegawa (1987). Shrimp samples (A g) was weighed and placed on 2 pieces of filter paper. Samples were allowed to stand in a petri dish with a cover in refrigerator (4 °C) for 1 h. The sample was taken from filter paper and weighed (B g). The analysis was conducted in five determinations. The drip loss was then calculated as follows:

$$\text{Drip loss (\%)} = ((A-B)/A) \times 100$$

5.3.2.4 Determination of moisture content

Moisture content was determined using an oven according to the method of AOAC (2000).

5.3.2.5 Determination of shear force

Shear force of 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.

5.3.2.6 Determination of color

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

5.3.3 Statistical analysis

A completely randomized design (CRD) was used for the whole 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).

5.4 Results and discussion

5.4.1 Protein solubility

Protein solubility in 0.6 M KCl of raw Pacific white shrimp treated with different soaking solutions including ASS, ASS+3% MSG and M-P is shown in **Figure 25.** The solubility of all samples decreased with increasing freeze-thaw cycles ($P<0.05$). Formation of disulfide bonds and hydrophobic interaction during frozen storage was associated with protein aggregation (Jiang *et al*., 1988). The result was in agreement with Rattanasatherin (2008) who reported that solubility of shrimp muscle protein decreased when the freeze-thaw cycles increased. Kittiphattanabawon *et al.* (2012) also reported that solubility of surimi containing blackstip shark skin gelatin hydrolysate decreased when the freeze-thaw cycles increased. The decrease in solubility might be caused by protein aggregation during frozen storage or freeze-thaw process (Cheung *et al*., 2009). White shrimp and black tiger shrimp had the decrease in solubility as the freeze-thaw cycles increased (Sriket *et al*., 2007). After treatment (cycle 0), all samples showed the higher solubility, compared with the sample without the treatment. Among all treated samples, that treated with ASS+3% MSG had the highest solubility, followed by that with M-P and ASS treatments, respectively. Alkaline solution (pH 11.5), especially that containing MSG, more likely modified the muscle protein structure via inducing the repulsion or dissociation of muscle fibrils. This was mediated by the alkaline pH, which yielded the pH far away from pI of proteins. Additionally, glutamic acid in MSG could provide the negatively charged carboxyl group, thereby altering the net charge of muscle proteins. M-P also contributed to the protein modification by pH changes as well as anionic phosphate groups. Those more likely led to the looser structure of proteins, which were more readily solubilized in salt solution.

After 5 freeze-thaw cycles, solubility of shrimp without treatment, those treated with ASS, M-P and ASS+3% MSG were 71.01, 76.97, 81.07% and 87.94%, respectively. The rate of decrease in solubility was slightly slower in the sample treated with ASS+3% MSG, compared with others. ASS+3% MSG might reduce the denaturation of protein via increasing water binding or holding. As a consequence, the formation of ice crystals from free water could be lowered. Along with the enhanced repulsion among proteins, the retarded losses in protein solubility of samples

mediated by aggregation were achieved. The decrease in protein solubility is a primary criterion of protein denaturation during frozen storage, resulting from hydrophobic interaction, disulfide bond and ionic interaction (Kittiphattanabawon *et al.*, 2012; Xiong, 1997).

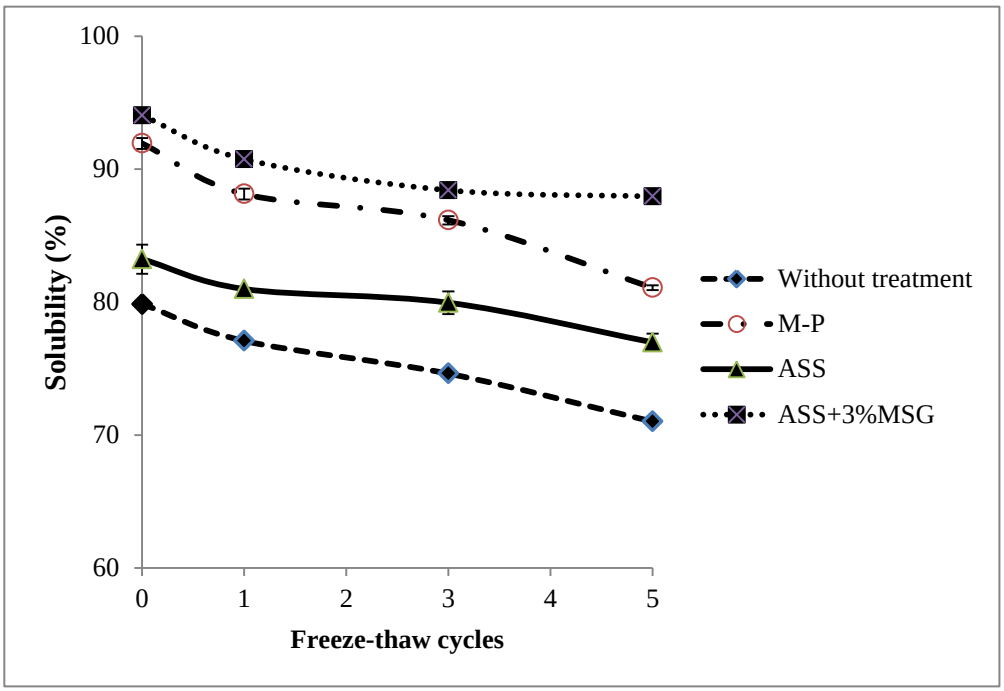

Figure 25. Solubility of raw Pacific white shrimp without and with different treatments as affected by various freeze-thaw cycles. Note: ASS: 0.75% NaOH containing 2.5% NaCl (pH 11.5), ASS+3% MSG: 0.75% NaOH containing 2.5% NaCl (pH 11.5) in the presence of 3%MSG and M-P: 2.5% NaCl with 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1 (w/w)). Bars represent the standard deviation (n=3).

5.4.2 α–glucosidase (AG) and β-N-acetyl-glucosaminidase (NAG) activities

AG and NAG activities in the exudate of raw shrimp with different freeze-thaw cycles are shown in **Figure 26a and 26b,** respectively, the activities of AG and NAG of shrimp treated with M-P and ASS+3% MSG were not detected for all freeze-thaw cycles used. AG and NAG activities of the sample without treatment increased continuously with increasing freeze-thaw cycles. The increases in activities of AG and NAG of the shrimp treated with ASS were found in shrimp after 5 freeze-thaw cycles. AG and NAG detected in exudate indicated the disintegration of membrane structure caused by freeze-thawing. Freezing and thawing can disrupt muscle cells, resulting in the release of enzymes from lysosomes and mitochondria into the sarcoplasm (Hamm, 1979). AG and NAG have been used as the marker of freezing and thawing process of fish muscle (Sriket *et al.,* 2007). Sriket *et al.* (2007) found that AG and NAG activities of white shrimp and black tiger shrimp in the exudate formed increased when the freeze-thaw cycles increased. Mitochondrial and lysosomal enzymes have been used to differentiate fresh and frozen muscle (Sriket *et al.*, 2007). The highest AG and NAG activities were found in shrimp without treatment, indicating the drastic disintegration of cells induced by freeze-thaw process. Nevertheless, the damage of cell could be reduced when shrimp were treated with ASS+3% MSG or M-P. Additionally cell disruption induced by repeated freeze-thawing could be retarded to some degree by treatment with ASS. It was noted that no exudate was obtained in shrimp treated with ASS+3% MSG or with M-P after centrifugation of ground sample. This was related with the negligible AG and NAG detected in both samples. Rattanasatheirn (2008) found no exudate in Pacific white shrimp treated with mixed phosphates, either without and with ProfixO after 5 freeze-thaw cycles. Therefore, treatment of shrimp with ASS+3% MSG showed similar efficacy in prevention of cell damage induced by freeze-thawing to M-P.

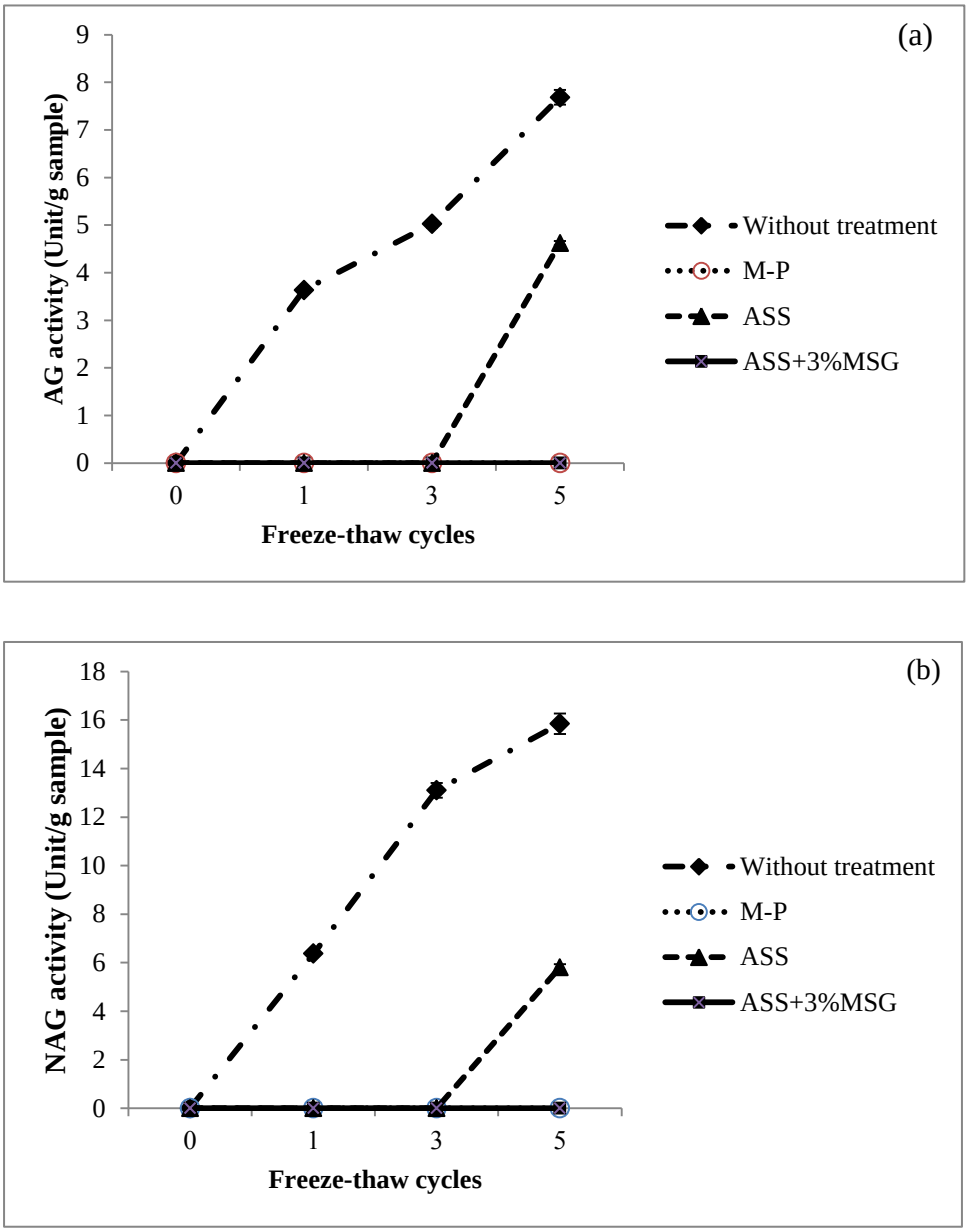

Figure 26. Changes in α-glucosidase (a) and β-N-acetyl-glucosaminidase (b) activities of raw Pacific white shrimp without and with different treatments as affected by various freeze-thaw cycles. Note: ASS: 0.75% NaOH containing 2.5% NaCl (pH 11.5), ASS+3% MSG: 0.75% NaOH containing 2.5% NaCl (pH 11.5) in the presence of 3%MSG and M-P: 2.5% NaCl with 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1 (w/w)). Bars represent the standard deviation (n=3).

5.4.3 Drip loss

Drip loss of raw and cooked shrimp without and with different treatments subjected to multiple freeze-thaw cycles is presented in **Figures 27.** Both raw and cooked shrimp had the increases in drip loss when the freeze-thaw cycles increased ($P<0.05$), irrespective of treatments. When the number of freeze-thaw cycles increased, the muscle fiber and cells were more disrupted and lacked of continuity as evidenced by the increased spacing between muscle fiber (Boonsumrej *et al.*, 2007).

For raw shrimp, drip loss was much greater in sample without treatment, compared with those with all treatments ($P<0.05$). However, no differences in drip loss were observed between the sample without treatment and those treated with ASS at 3 and 5 freeze-thaw cycles ($P\geq0.05$). During freezing, ice crystals were formed from intracellular or extracellular water, resulting in mechanical damage caused by irregular ice crystals protruding through and disrupting the cell walls (Xiong, 1997). Drip loss has been linked to partial denaturation of proteins taken place during freezing, which leads to decreased WHC (Rattanasatherin, 2008). Drip loss of shrimp treated with ASS markedly increased with increasing freeze-thaw cycles. ASS might augment the water holding in treated shrimp via protein repulsion. Water in the cavity might migrate to form ice crystal with ease. Subsequently, the ice crystals formed could be molten and the drip could be released to a higher extent. On the other hand, shrimp treated with ASS in the presence of 3% MSG showed very low drip loss. ASS+3%MSG might retard the formation of ice crystal. As a result the damage of cell was lowered with coincidentally low AG and NAG activities detected in exudate **(Figure 26)**. Mixed phosphate might exhibit the similar mechanism to ASS+3%MSG. The result suggested that M-P and ASS+3%MSG could prevent disintegration of muscle cell during freeze-thawing process as indicated by lowered drip loss. Those compounds plausibly enhanced electrostatic repulsion of protein molecules, in which water could be entrapped tightly in the modified muscle. Alternatively, M-P and ASS+3%MSG might impede the formation and growth of ice crystals in the muscle by introducing the high proportion of bound water.

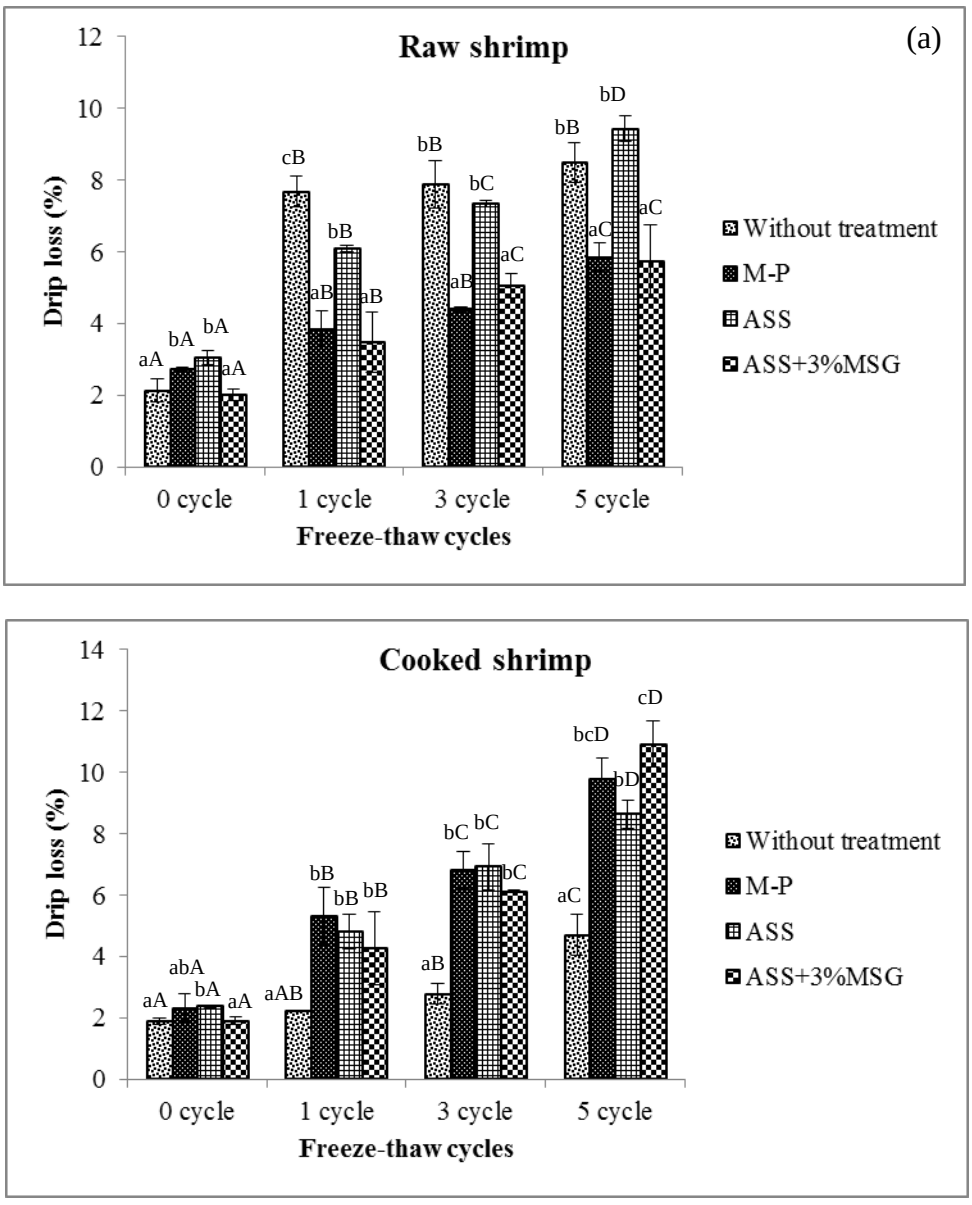

Figure 27. Drip loss of raw (a) and cooked (b) Pacific white shrimp meat without and with different treatments as affected by various freeze-thaw cycles. Note: ASS: 0.75% NaOH containing 2.5% NaCl (pH 11.5), ASS+3% MSG: 0.75% NaOH containing 2.5% NaCl (pH 11.5) in the presence of 3%MSG and M-P: 2.5% NaCl with 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1 (w/w)). Different lowercase letters on the bars within the same freeze-thaw cycle indicate significant differences ($P<0.05$). Different uppercase letters on the bars within the same treatment indicate significant differences ($P<0.05$). Bars represent the standard deviation (n=3).

For cooked shrimp, the lowest drip loss was observed in shrimp without treatment at all freeze-thaw cycles ($P<0.05$). The result suggested that water of those shrimp was more likely lost during cooking caused by thermal denaturation. Conversely, those with treatments contained more water retained in the muscle. When being subjected to freeze-thawing, the water might get involved in formation of exudate. It was noteworthy that proteins in cooked shrimp were mostly denatured and lost in functionalities, especially water holding. When the freeze-thaw cycles increased up to 3 cycles, drip loss of cooked shrimp treated with ASS, M-P and ASS+3%MSG was similar ($P\geq0.05$). However, the highest drip loss was found in the sample treated with ASS+3% MSG and M-P after 5 freeze-thaw cycles ($P<0.05$), indicating more release of free water. Drip loss of muscle can lead to less acceptability due to the loss of tasteful constituents, e.g. some amino acids or nucleotides (Sriket *et al.*, 2007)

5.4.4 Moisture content

Moisture contents of raw and cooked shrimp without and with different treatments are shown in **Table 17.** For raw shrimp, the treated shrimp showed the higher moisture content than those without treatment ($P<0.05$). During soaking, water along with alkali and salt could penetrate into shrimp muscle and more water was retained in their structures. Freezing and thawing alter the distribution of moisture in meat tissue. (Leygonie *et al.*, 2012). When freeze-thaw cycles were increased up to 3 cycles, no differences in moisture content were observed ($P\geq0.05$). Moisture content of those shrimp decreased after 5 freeze-thaw cycles ($P<0.05$). During freezing and thawing process, the proteins underwent denaturation along with the formation of ice crystal. As a result, cells were more damaged and some fluids were released to a higher extent. Melting during thawing and reformation of ice crystals during freezing in multiple freeze-thaw situations was detrimental to muscle tissues by causing mechanical damage to cell membranes and the loss of water holding capacity (Manheem, 2012). At all freeze-thaw cycles used, shrimp treated with ASS+3% MSG showed the highest moisture content, compared with others ($P<0.05$), suggesting that such a treatment could retain moisture in shrimp more effectively during the repeated freeze-thawing process.

For cooked shrimp, the highest moisture content was observed in shrimp treated with ASS+3%MSG ($P<0.05$) and those without treatment had the lowest moisture content ($P<0.05$). COO^- group of glutamic acid in solution might show stronger stabilizing effect on the water within shrimp proteins. When heat was applied denaturation and coagulations of protein took place and in turn lowered water holding capacity and enhanced protein-protein interactions (Niamnuy *et al.*, 2007). This led to less water entrapped within the protein structure (Aaslyng *et al.*, 2003). Moisture content of cooked shrimp decreased, especially, after 3 freeze-thaw cycle ($P<0.05$). However, no marked changes in moisture content were noticeable after 5 freeze-thaw cycles ($P\geq0.05$). Thus freeze-thawing had impact on moisture content and treatments could help in retaining more moisture in the cooked shrimp.

Table 17. Moisture content of raw and cooked Pacific white shrimp without and with different treatments as affected by various freeze-thaw cycles

Samples	Moisture content (% wet weight basis)			
	0 cycle	1 cycle	3 cycle	5 cycle
Raw				
Without treatment	81.36±0.14 ^{aB}	81.48±0.21 ^{aB}	81.16±0.33^{aAB}	80.91±0.03 ^{aA}
M-P	83.36±0.19 ^{cC}	82.80±0.16 ^{bB}	81.21±0.07 ^{aA}	81.14±0.40 ^{abA}
ASS	82.35±0.24 ^{bB}	82.34±1.20^{abB}	81.51±0.12 ^{abAB}	80.94±0.10 ^{aA}
ASS+3%MSG	83.02±0.68^{bcC}	82.16±0.10 ^{abB}	81.67±0.14 ^{bAB}	81.41±0.04 ^{bA}
Cooked				
Without treatment	72.40±0.01 ^{aB}	72.01±0.33 ^{aA}	71.94±0.05 ^{aA}	71.70±0.24 ^{aA}
M-P	80.06±0.99 ^{bcB}	78.63±0.19 ^{bA}	78.53±0.17 ^{cA}	77.71±0.41 ^{bA}
ASS	79.10±0.77 ^{bB}	78.56±0.10 ^{bB}	77.44±0.31 ^{bA}	77.58±0.47 ^{bA}
ASS+3%MSG	81.63±1.91^{cB}	79.25±0.12 ^{cA}	78.41±0.23 ^{cA}	77.78±0.08 ^{bA}

†Mean±SD (n=3).

Note: ASS: 0.75% NaOH containing 2.5% NaCl (pH 11.5), ASS+3% MSG: 0.75% NaOH containing 2.5% NaCl (pH 11.5) in the presence of 3%MSG and M-P: 2.5% NaCl with 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1 (w/w)). Different lowercase superscripts in the same column under the same state indicate significant differences ($P<0.05$). Different uppercase superscripts in the same row indicate significant differences ($P<0.05$).

5.4.5 Shear force

Shear force of shrimp without and with different treatments, M-P, ASS and ASS+3% MSG after 0, 1, 3 and 5 freeze-thaw cycles is presented in **Table 18.** Non-significantly lower shear force was observed in raw treated shrimp, compared with shrimp without treatments ($P \geq 0.05$). After treatment, the muscle compartments became less compact, due to the repulsion mediated by alkaline pH as well as phosphates. The looser structure was less resistant to force applied as indicated by the lower shear force. When shrimp were freeze-thawed up to 3 cycles, shear force of all samples increased markedly ($P<0.05$) and the sample without treatment showed the lowest shear force ($P<0.05$), compared with others. It was postulated that the more compact muscle fibers might be formed with increasing freeze-thawing cycles. This was related with the increased drip loss **(Figure 25)**. When the water was removed from muscle as induced by freezing-thawing, the muscle fibrils became more concentrated, thereby resulting in higher aggregation. Those aggregates led to the tougher texture as shown by the increased shear force. Boonsumrej *et al.* (2007) found that cutting force of tiger shrimp *(Penaeus monodon)* increased as the number of freeze-thaw cycles increased. However, shear force of all samples decreased after 5 freeze-thaw cycles ($P<0.05$). The decrease in shear force suggested the loss in integrity of muscle fibers, leading to the weakening of muscle. Repeated melting and reformation of ice-crystals more likely disrupted the muscle compartment, in the way that weaker structure with lower resistance to force was developed. Shear force correlated well with the diameter (width) of the muscle portion sheared or with the weight of the shrimp (Srinivasan *et al.*, 1997). Shear force of shrimp treated with M-P and ASS+3% MSG had the lowest shear force after 5 freeze-thaw cycles ($P<0.05$). The result suggested that M-P and ASS+3% MSG more likely retarded the loss in integrity of muscle fibers via maintaining the water in the muscle and lowering the drip loss.

For cooked shrimp, the highest shear force was observed in shrimp without treatment, compared to those treated with M-P, ASS and ASS+3% MSG ($P<0.05$). Without treatment, muscle proteins were more likely aggregated as induced by heat to high degree. Those aggregated proteins were more resistant to the force applied. The

bound water in shrimp muscle treated with M-P, ASS and ASS+3 MSG might play a role in preventing the coagulation of protein during heating. This resulted in the lower shear force of treated samples. Erdogdo *et al.* (2004) reported that protein aggregation was induced by heating and the connective tissue shrinkage was also induced. Shear force of all treatments increased as the number of freeze-thaw cycle increased up to 3 cycle ($P<0.05$). Thereafter, the decrease in shear force was found after 5 freeze-thaw cycles ($P<0.05$). This was similar to the result of raw shrimp. Sriket *et al.* (2007) also reported that protein denaturation and disruption of endomysium induced by freeze-thawing, possibly resulted in a less compact structure. In general, freeze-thawing process showed the direct effect on textural properties of both raw and cooked shrimp. Treatment of shrimp, in the way which the water was retained in the muscle compartment, could retard the toughness of shrimp as induced by multiple freeze-thawing.

Table 18. Shear force of raw and cooked Pacific white shrimp without and with different treatments as affected by various freeze-thaw cycles

Samples	Shear force (g)			
	0 cycle	1 cycle	3 cycle	5 cycle
Raw				
Without treatment	1782±151 ^{aA}	1815±137 ^{bA}	2521±171 ^{bB}	2093±64 ^{bA}
M-P	1652±43 ^{aA}	1702±159 ^{abA}	2303±171 ^{aB}	1753±84 ^{aA}
ASS	1623±44 ^{aA}	1639±88 ^{aA}	2250±147 ^{aC}	2030±124 ^{bB}
ASS+3% MSG	1623±143 ^{aA}	1771±75 ^{abAB}	2280±127 ^{aC}	1877±90 ^{abB}
Cooked				
Without treatment	2351±144 ^{bA}	2422±99 ^{cA}	2919±337 ^{cB}	2620±281 ^{cAB}
M-P	1700±97 ^{aA}	1719±161 ^{aA}	2204±141 ^{aB}	1817±82 ^{aA}
ASS	1852±72 ^{aA}	1902±129 ^{bA}	2576±237 ^{bcC}	2239±200 ^{bB}
ASS+3% MSG	1729±131 ^{aA}	1861±74 ^{abA}	2520±244 ^{bcB}	1716±54 ^{aA}

†Mean±SD (n=3).

Note: ASS: 0.75% NaOH containing 2.5% NaCl (pH 11.5), ASS+3% MSG: 0.75% NaOH containing 2.5% NaCl (pH 11.5) in the presence of 3%MSG and M-P: 2.5% NaCl with 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1 (w/w)). Different lowercase superscripts in the same column under the same state indicate significant differences ($P<0.05$). Different uppercase superscripts in the same row indicate significant differences ($P<0.05$).

5.4.6 Color

Color parameters (*L**, *a** and *b**-value) of raw and cooked shrimp without and with various treatments are shown in **Table 19 and 20,** respectively. For raw shrimp, *L** values of shrimp treated with M-P and ASS+3% MSG were lower than those of shrimp without treatment and treated with ASS ($P<0.05$). It was suggested that shrimp treated with M-P and ASS+3% MSG had more water retained in muscle. The water distributed in the muscle compartment was associated with swollen structure, in which the light could pass through and contributed to the increased transparency. *L**values of all samples increased after 5 freeze-thaw cycles ($P<0.05$) except for the shrimp treated with ASS+3% MSG, which had no changes in *L** values ($P\geq0.05$). The increase in *L** value might be caused by the increase in water released to the surface as well as the denaturation of muscle proteins (Sriket 2007). Lightening is due to an increased light reflection, arising from light scattering by denatured proteins (Young and West, 2001). Protein denaturation and/or shrinkage of the myofibrils tends to increase light scattering, thus giving higher *L**-values (Jeong *et al.*, 2011). Moreover, color changes can occur during frozen storage and thawing due to lipid oxidation and pigment degradation (Dias *et al.*, 1994). The lowest *L**-values of sample treated with ASS+3% MSG was coincidental with the highest moisture content **(Table 17).** Thus, water or moisture in shrimp muscle plausibly affected the lightness of shrimp.

Among all samples, the highest *a** value was observed in shrimp treated with ASS+3% MSG ($P<0.05$). It was suggested that ASS+3% MSG might induce the dissociation of carotenoprotein, resulting in the release of astaxanthins with red color in nature. When shrimp without and with all treatments were subjected to freeze-thawing with increasing cycle, both *a** and *b** values increased ($P<0.05$). Increases in *a** and *b** values might be mediated by the generation of ice crystals and the increase in ionic strength in muscles. Those phenomena induced denaturation and the disruption of the actin-myosin complex, resulting in the pronounced release of free pigments with red color. The increase in *b**-values could be partially due to lipid oxidation. An increase in lipid oxidation of cattle fish muscle during freeze–thaw cycles was coincidental with an increase in *b**-value (Thanonkaew *et al.*, 2006). Temperature abuse attributed to repeated freeze–thawing cycling stimulated lipid

Table 19 Color of raw Pacific white shrimp without and with different treatments as affected by various freeze-thaw cycles

Color values	Treatments	Freeze-thaw cycles			
		0	**1**	**3**	**5**
*L**	Without treatment	52.52±1.27 †,cA	53.06±1.19 cA	53.16±1.47 dA	56.31±3.44 dB
	M-P	47.93±2.39 aA	48.86±1.80 aA	50.01±1.01 bA	52.82±2.74 bB
	ASS	50.33±1.55 bA	50.82±2.67 bA	51.57±1.53 cA	57.28±2.63 cB
	ASS+3% MSG	46.77±2.34 aA	47.88±1.40 aA	46.58±1.56 aA	46.74±2.58 aA
*a**	Without treatment	-1.00±0.54 aA	-0.40±0.54 aA	0.88±0.80 aB	2.11±1.28 abC
	M-P	-0.63±0.33 aA	-0.06±0.58 aA	1.15±0.78 abB	1.16±1.04 aB
	ASS	-1.07±0.37 aA	-0.18±0.70 aA	2.12±1.48 bcB	2.93±1.64 bB
	ASS+3% MSG	1.65±0.65 bA	2.15±0.95 bA	2.38±0.90 cAB	3.27±1.32 bB
*b**	Without treatment	-0.43±1.66 bA	1.04±1.17 bAB	2.01±2.03 bB	4.56±0.86 bC
	M-P	-2.93±1.75 aA	-1.74±2.28 aAB	-0.55±2.29 aBC	0.65±0.97 aC
	ASS	-2.54±1.08 aA	-0.32±1.11 abB	1.05±1.73 abB	2.95±2.58 bC
	ASS+3% MSG	-0.87±1.45 bA	0.58±2.12 bAB	2.22±1.43 bBC	4.06±3.33 bC

†Mean±SD (n=3).

Note: ASS: 0.75% NaOH containing 2.5% NaCl (pH 11.5), ASS+3% MSG: 0.75% NaOH containing 2.5% NaCl (pH 11.5) in the presence of 3%MSG and M-P: 2.5% NaCl with 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1 (w/w)). Different lowercase superscripts in the same column under the same color value indicate significant differences ($P<0.05$). Different uppercase superscripts in the same row indicate significant differences ($P<0.05$).

oxidation and accelerated surface discoloration in meat (Hansen *et al.*, 2004; Moore, 1990; Moore and Young, 1991). Xia *et al.* (2009) suggested that yellow pigment formation in pork could be due to non-enzymatic browning reactions between lipid oxidation products and the amine in the phospholipid head groups or the amine in protein. After 5 freeze-thaw cycles, the lowest *a**- and *b**-values were observed in the sample treated with M-P ($P<0.05$). Phosphates have been known to be capable of metal chelation, especially prooxidant. As a consequence, lipid oxidation could be lowered. Additionally, phosphates might exhibit different mechanism toward

carotenoproteins in shrimp from glutamic acid in ASS. Thus the changes in redness and yellowness of raw shrimp treated with various soaking solution were different.

For cooked shrimp, *L**, *a** and *b**-values of all treatments showed the differences when the number of freeze-thaw cycle increased ($P<0.05$). The highest *L** value was found in shrimp without treatment and those treated with ASS ($P<0.05$). When heat was applied, protein underwent aggregation, resulting in more turbidity and opaqueness. Cooked shrimp without treatment also showed the highest *a** value ($P<0.05$). With the lowest water retained, the pigments were more concentrated as indicated by the highest *a** value. However, there was no difference in *b** value among all treated samples ($P<0.05$). When the number of freeze-thaw cycles increased, no changes in *L**-values were found ($P<0.05$). However, *a**- value was decreased with increasing freeze-thaw cycles ($P<0.05$). During thawing process, exudate containing pigments could be released. As a result, less pigment was retained in the shrimp, as shown by the lower *a**-value. The loss in pigment was pronounced as the freeze-thaw cycles increased. The lowest *a**-value after 5 freeze-thaw cycles was obtained in sample treated with M-P and ASS+3% MSG ($P<0.05$). This coincidental with the high water adsorption of those samples, in which repeated freeze-thawing could induce the liberation of water along with leached pigment. No changes in *b**-values were observed in all treated samples, regardless of freeze-thaw cycles ($P\geq0.05$). It was found that slight increases in *b**-value occurred in sample without treatment. With lower water retained, lipid in muscle might be more prone to oxidation, in which yellowness could be developed to a higher degree. Therefore, treatment directly determined the color of cooked shrimp as influenced by freeze-thawing process.

Table 20 Color of cooked Pacific white shrimp without and with different treatments as affected by various freeze-thaw cycles

Color values	Treatments	Freeze-thaw cycles			
		0	**1**	**3**	**5**
*L**	Without treatment	73.28±4.31 †,bA	73.21±2.14 bA	72.61±2.94 cA	72.25±2.99 bA
	M-P	68.71±1.73 aA	67.82±3.07 aA	68.04±3.38 abA	68.03±3.05 aA
	ASS	73.82±3.02 bA	73.17±2.53 bA	71.34±3.08 bcA	71.14±1.07 bA
	ASS+3% MSG	67.61±2.87 aA	67.81±4.21 aA	67.30±3.73 aA	65.17±3.87 aA
*a**	Without treatment	15.78±2.06 bA	13.83±3.26 bA	13.76±0.83 dA	13.50±1.76 cA
	M-P	9.28±2.39 aB	8.26±2.69 aB	7.59±1.83 bB	4.98±2.03 aA
	ASS	10.58±2.09 aA	10.14±1.70 aA	10.09±1.55 cA	9.53±2.85 bA
	ASS+3% MSG	9.67±1.33 aC	8.13±2.50 aBC	7.10±1.62 aAB	6.01±1.76 aA
*b**	Without treatment	13.81±1.84 aA	17.36±3.05 bB	18.49±2.42 bAB	20.21±1.99 bB
	M-P	12.33±2.47 aA	13.19±2.99 aA	14.89±4.47 abB	14.92±4.27 aA
	ASS	13.58±3.29 aA	15.41±2.80 aA	15.20±4.79 abA	15.69±3.56 aA
	ASS+3% MSG	13.15±3.26 aA	13.12±2.79 aA	13.47±3.87 aA	15.59±2.64 aA

†Mean±SD (n=3).

Note: ASS: 0.75% NaOH containing 2.5% NaCl (pH 11.5), ASS+3% MSG: 0.75% NaOH containing 2.5% NaCl (pH 11.5) in the presence of 3% MSG and M-P: 2.5% NaCl with 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1 (w/w)). Different lowercase superscripts in the same column under the same color value indicate significant differences ($P<0.05$). Different uppercase superscripts in the same row indicate significant differences ($P<0.05$).

5.5 Conclusion

Shrimp treated with ASS+3% MSG (pH 11.5) had the reduced drip loss, which was associated with more retained moisture content and no AG and NAG activities was detected when subjected to the repeated freeze-thawing process. Shear force of both of raw and cooked shrimp with all treatments increased when freeze-thaw cycles increases up to 3 cycles, but the drastic decrease was found after 5 freeze-thaw cycles. Freeze-thawing process caused the protein denaturation, tissue disruption and the damage of muscle fiber as indicated by the release of AG and NAG. Thus, soaking shrimp with 0.75% NaOH containing 2.5% NaCl and 3% MSG able to retard quality changes induced by freeze-thawing process.

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CHAPTER 6

SUMMARY AND FUTURE WORKS

6.1 Summary

1. The alkaline (NaOH or KOH) treatment had the 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. Shrimp soaked in 0.75% NaOH containing 2.5% NaCl solution showed the highest weight gain and cooking yield, which were concomitant with the lowest cooking loss. However, those treated shrimp had poor sensory properties caused by the extremely high pH (pH 13.04).

2. pH of alkaline solution played an important role to in weight gain, cooking yield and cooking loss of Pacific white shrimp. Treatment of shrimp using extremely high pH (pH 13) led to the decreased sensorial property of resulting shrimp. The use of 0.75% NaOH containing 2.5% NaCl at pH 11.5 (ASS) could therefore be an alternative means for shrimp treatment.

3. Alkaline treatment led to dissociation of actomyosin complex and could induce the protein conformational change. Those alterations were more pronounced as the pH of solution increased.

4. Addition of sugar and sugar alcohols into ASS had no pronounced effect on improving cooking yield however, amino acids, especially glutamic acid, exhibited the positive effect. ASS+3% MSG (mole equivalent of glutamic acid) was recommended as the phosphate or bicarbonate replacer, especially for cooked shrimp. Nevertheless, raw shrimp turned to be slightly reddish after being soaked in ASS+3% MSG.

5. Shrimp treated with ASS+3% MSG had the retarded quality changes as induced by the repeated freeze-thawing. Mainly, much a treatment could lower the drip loss and maintain the sensory properties of shrimp after repeated freeze-thawing process.

6.2 Future works

1. Prevention of reddish discoloration in raw shrimp treated with 0.75% NaOH containing 2.5% NaCl and 3%MSG (pH 11.5) should be further studied.
2. Shelf-life and storage stability of shrimp treated with 0.75% NaOH containing 2.5% NaCl and 3%MSG (pH 11.5) during frozen storage should be investigated.
3. Other natural hydrophilic substances such as oligosaccharide should be used to enhance the efficacy of alkaline solution for shrimp treatment.

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List of Proceeding

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