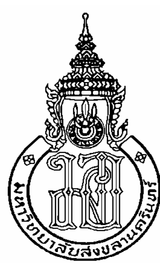

Effect of Mixed Phosphates and Non-Phosphate Compounds on the Quality of Pacific White Shrimp (*Litopenaeus vannamei*)

Nithiruch Rattanasatheirn

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Science in Food Technology

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Thesis Title	Effect of Mixed Phosphates and Non-Phosphate Compounds on the Quality of Pacific White Shrimp (*Litopenaeus vannamei*)
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ชื่อวิทยานิพนธ์	ผลของสารประกอบฟอสเฟตผสมและสารที่ไม่ใช่สารประกอบฟอสเฟตต่อคุณภาพของกุ้งขาวแวนนาไม (*Litopenaeus vannamei*)
ผู้เขียน	นางสาวนิธิรัช รัตนเสถียร
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บทคัดย่อ

การศึกษาผลของสารประกอบฟอสเฟตชนิดต่างๆ และสารประกอบฟอสเฟตผสมซึ่งประกอบด้วย โซเดียมแอซิดไพโรฟอสเฟต (SAPP) โซเดียมไตรพอลิฟอสเฟต (STPP) เตตระโซเดียมไพโรฟอสเฟต (TSPP) โซเดียมเฮกซะเมตาฟอสเฟต (SHMP) ที่ระดับความเข้มข้นร้อยละ 3.5 ร่วมกับโซเดียมคลอไรด์ที่ระดับความเข้มข้นร้อยละ 2.5 ต่อคุณภาพของกุ้งขาวแวนนาไม (*Litopenaeus vannamei*) พบว่าการใช้ SAPP ที่ระดับความเข้มข้นร้อยละ 0.875 ผสมกับ TSPP หรือ STPP ที่ระดับความเข้มข้นร้อยละ 2.625 ร่วมกับการใช้โซเดียมคลอไรด์ที่ระดับความเข้มข้นร้อยละ 2.5 ทำให้กุ้งขาวมีน้ำหนักภายหลังการแช่และผลผลิตที่ได้ภายหลังการให้ความร้อนเพิ่มสูงขึ้น รวมทั้งสามารถลดการสูญเสียน้ำหนักภายหลังการให้ความร้อน โดยกุ้งที่ได้มีลักษณะใสน้อยกว่ากุ้งที่แช่ในชุดการทดลองอื่นๆ ประสิทธิภาพของฟอสเฟตผสมในการปรับปรุงคุณภาพกุ้งที่เก็บในน้ำแข็งนาน 7 วันต่ำกว่าเมื่อเปรียบเทียบกับกุ้งสด ส่วนการผ่าหลังส่งผลให้กุ้งมีน้ำหนักภายหลังการแช่เพิ่มขึ้น ($p<0.05$) กุ้งที่เก็บรักษาในน้ำแข็งนาน 7 วันและผ่านการแช่สารประกอบฟอสเฟตผสมมีการแยกออกของ M-line เมื่อนำไปผ่านการให้ความร้อนซึ่งการเปลี่ยนแปลงดังกล่าวไม่พบในกุ้งสดสำหรับชุดการทดลองเดียวกัน การแช่ด้วยสารประกอบฟอสเฟตผสมมีผลทำให้อุณหภูมิสูงสุดในการสูญเสียสภาพธรรมชาติ (T_{max}) ของไมโอซินลดลงและเป็นสาเหตุให้ไม่พบพีคของแอกติน

การศึกษาการใช้สารเติมแต่งที่ไม่ใช่สารประกอบฟอสเฟต (ProfixO) ที่ระดับความเข้มข้นร้อยละ 1 3 และ 5 ในสภาวะที่มีหรือไม่มีการใช้สารประกอบฟอสเฟตผสม (0.875% SAPP and 2.625% TSPP) ร่วมในการแช่กุ้งสดและกุ้งที่ผ่านการเก็บในน้ำแข็งนาน 7 วัน พบว่าทั้งกุ้งสดและกุ้งที่เก็บในน้ำแข็งนาน 7 วันที่ผ่านการแช่ในสารประกอบฟอสเฟตผสมร่วมกับโซเดียมคลอไรด์ที่ระดับความเข้มข้นร้อยละ 2.5 ในสภาวะที่มี ProfixO ที่ระดับความเข้มข้นร้อยละ 3 ส่งผลให้ค่าความขุ่นและผลผลิตของกุ้งภายหลังการให้ความร้อนของกุ้งสูงขึ้นอีกทั้งสามารถลดน้ำหนักที่สูญเสียภายหลังการให้ความร้อนของกุ้งได้ดีเมื่อเปรียบเทียบกับการใช้ ProfixO เพียงอย่างเดียว ความเข้มข้นของเกลือ (ร้อยละ 0-2.5) ซึ่งใช่ร่วมกับสารประกอบฟอสเฟตผสม และระยะเวลาใน

การแช่ (60-360 นาที) มีผลต่อคุณภาพของกุ้งที่แช่ในสารละลายที่มีประกอบด้วยโซเดียมคลอไรด์ที่ระดับความเข้มข้นร้อยละ 1 ร่วมกับสารประกอบฟอสเฟตผสมนาน 240 นาทีมีความใสต่ำในขณะที่ยังมีน้ำหนักภายหลังการแช่ และผลผลิตที่ได้ภายหลังการให้ความร้อนที่สูงขึ้นโดยทั้งนี้ไม่ขึ้นกับการใช้ร่วมกับ ProfixO กุ้งที่ผ่านการเก็บในรักษาในน้ำแข็งนาน 7 วัน มีการสูญเสีย Z-disks แต่ M-line มีการสูญเสียลดลงเมื่อแช่กุ้งในสารประกอบฟอสเฟตผสมร่วมกับ ProfixO

ระหว่างการเก็บรักษาในสภาพแช่เยือกแข็งที่อุณหภูมิ -20 °ซ เป็นระยะเวลา 12 สัปดาห์ พบว่ากุ้งที่แช่ในสารละลายโซเดียมคลอไรด์ที่ระดับความเข้มข้นร้อยละ 1 ร่วมกับสารประกอบฟอสเฟตผสมและ ProfixO ที่ระดับความเข้มข้นร้อยละ 3 มีการอัตราลดลงของกิจกรรม Ca^{2+}, Mg^{2+}, Mg^{2+}-Ca^{2+} -ATPase และ Ca^{2+}-sensitivity ต่ำและสามารถชะลอการเพิ่มขึ้นของกิจกรรม Mg^{2+}-EGTA-ATPase เมื่อเปรียบเทียบกับกุ้งที่ไม่ผ่านการแช่และกุ้งที่แช่ในสารสารละลายที่มีประกอบด้วยโซเดียมคลอไรด์ที่ระดับความเข้มข้นร้อยละ 1 ร่วมกับสารประกอบฟอสเฟตผสม กุ้งที่แช่ในสารละลายฟอสเฟตผสม มีอัตราการเกิดพันธะไดซัลไฟด์และการเพิ่มขึ้นของไฮโดรโฟบิซิตีลดลงทั้งนี้ไม่ขึ้นกับการใช้ ProfixO ร่วม นอกจากนี้สามารถชะลอการสูญเสียการละลาย การสูญเสียจากการทำละลาย และ น้ำหนักที่สูญเสียหลังการให้ความร้อน การใช้สารประกอบฟอสเฟตผสมร่วมกับ ProfixO สามารถลดการเปลี่ยนแปลงทางเคมีกายภาพของกล้ามเนื้อกุ้งขาวที่ผ่านการแช่แข็ง-ทำละลายจำนวนหลายๆ รอบ (1 3 และ 5 รอบ) ดังนั้นการแช่กุ้งในสารละลายโซเดียมคลอไรด์ที่ระดับความเข้มข้นร้อยละ 1 ร่วมกับสารประกอบฟอสเฟตผสม (1% NaCl + 2.625% TSPP +0.875% SAPP) ร่วมกับ ProfixO ที่ระดับความเข้มข้นร้อยละ 3 มีผลในการเพิ่มประสิทธิภาพในการอุ้มน้ำ เพิ่มผลผลิตภายหลังการให้ความร้อน และป้องกันการสูญเสียสภาพระหว่างการแช่เยือกแข็งและการแช่แข็ง-ทำละลาย

Thesis Title	Effect of Mixed Phosphates and Non-Phosphate Compounds on the Quality of Pacific White Shrimp (*Litopenaeus vannamei*)
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ABSTRACT

The quality of Pacific white shrimp (*Litopenaeus vannamei*) treated with various single phosphates including tetrasodium pyrophosphate (TSPP), sodium hexametaphosphate (SHMP), sodium tripolyphosphate (STPP), sodium acid pyrophosphate (SAPP) and different mixed phosphates at the concentration of 3.5% in combination with 2.5% sodium chloride was studied. The uses of 0.875% SAPP+2.625% TSPP or 0.875% SAPP+2.625% STPP in combination with 2.5% NaCl resulted in the increases in weight gain and cooking yield with lowered cooking loss. Additionally, such treatments yielded the shrimps with lower translucence, compared with cooked shrimp in other treatments. Efficacy of mixed phosphates in quality improvement of ice-stored shrimp was lower than fresh shrimp. Deveining resulted in the increased weight gain and yield ($p<0.05$). Disintegration was observed at M-line in ice-stored shrimps treated with mixed phosphates after cooking, while such a phenomenon was not found in the cooked fresh sample with the same treatment. Treatment with mixed phosphates decreased the maximum transition temperature (T_{max}) of myosin and caused the disappearance of actin peak.

The use of ProfixO, non-phosphate compound, at different levels (1, 3 and 5%) in combination with mixed phosphates (0.875% SAPP and 2.625% TSPP) on quality of white shrimp, fresh shrimp and shrimp stored in ice for 7 days, was investigated. The use of mixed phosphates in combination with 3% ProfixO increased the opacity score, cooking yield, but decreased cooking loss of fresh and ice-stored shrimp, in comparison with the use of only ProfixO. Salt concentrations (0-2.5%) used in conjunction with mixed phosphates and soaking time (60-360 min) had the impact on the quality of resulting shrimps. Shrimps soaked in solution containing 1% NaCl and mixed phosphates for 240 min possesses the lower translucence, while still had the high weight gain and cooking yield, regardless of ProfixO incorporated. Loss of

Z-disks of ice-stored shrimp was more pronounced but the destruction of M-line was much lowered when shrimp was treated with mixed phosphates in combination with ProfixO.

During frozen storage at -20 °C up to 12 weeks, shrimps treated with 1% NaCl containing mixed phosphates and 3% ProfixO had the lower rate of decrease in Ca^{2+}, Mg^{2+}, Mg^{2+}-Ca^{2+} -ATPase activities and Ca^{2+}-sensitivity and retarded rate of increase in Mg^{2+}-EGTA-ATPase activity, compared with those with no treatment or treated only with mixed phosphates. Regardless of ProfixO incorporated, shrimps treated with mixed phosphates had the lower rates of decrease in disulfide bond and the increase in surface hydrophobicity. Loss in solubility, drip loss and cooking loss were also retarded. Mixed phosphates in combination with ProfixO also lowered the physicochemical changes in shrimp muscle subjected to multiple freeze-thawing (1, 3 and 5 cycles). Therefore, soaking the shrimps in 1% NaCl containing mixed phosphates (1% NaCl + 2.625% TSPP + 0.875% SAPP) and 3% ProfixO effectively increased the water holding capacity and cooking yield and prevented the losses in quality changes during frozen storage and freeze-thawing process.

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Nithiruch Rattanasatheirn

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Future works

1. Effect of size and muscle position on quality of Pacific white shrimp treated with mixed phosphates should be further studied.
2. M-line protein in shrimp muscle should be further characterized and the role of non-phosphates and phosphates compounds on disintegration of this protein should be elucidated.
3. Other non-phosphate compounds such as carbonate salt or protein hydrolysate should be used to improve quality of Pacific white shrimp or other shrimps.

CHAPTER 1

INTRODUCTION

Shrimp is one of the seafood products, which have become economically important for Thailand. Thailand has exported seafood and seafood products to different countries such as America, Europe and Asia. In 2005, Thailand exported 279,347 tons of shrimp and shrimp products with a value of 71,358 million bahts. Among the products, frozen shrimp and shrimp products accounted for 57.21% and the remainders are the processed seafood products (The Customs Department, 2006). Seafood industry of Thailand is well known for its long-standing excellent reputation worldwide, owing to its outstanding quality, freshness, variety, and taste. In general, freezing technology has been successfully applied to maintain the quality and prevent the spoilage of shrimp and shrimp products. Despite microbial spoilage can be terminated effectively, quality deteriorations, especially in texture, flavor and color, still take place during frozen storage (Benjakul *et al.,* 2003). Freeze-thaw process can also promote protein denaturation and lipid oxidation, which may affect the texture of fish muscle (Benjakul and Bauer, 2000; Srinivasan *et al.,* 1997). Deterioration of muscle proteins during frozen storage depends on many factors including species, storage temperature, time and enzymatic degradation (Ang and Hultin, 1989; Badii and Howell, 2001; Hsieh and Regenstein, 1989). To minimize those negative effects associated with the frozen storage, some additives particularly phosphate compounds have been widely used in shrimp and shrimp products.

Phosphates have been accepted as the potential additives in fish and seafood to improve the functional properties of those products by increasing water retention in fresh fish, reducing the thaw loss in frozen fish, modifying the texture, yielding the better color and reducing cooking loss (Dziezak, 1990; Chang and Regenstein, 1997a). Salt in combination with phosphates has a synergistic effect in improving water holding capacity and cooking yield (Young *et al.*,

1987). Phosphates can be applied to shrimp by soaking or by vacuum tumbling. However, overtreatment generally results in the formation of a translucent and slimy texture. Therefore, much attention has been paid to minimize the translucence as well as to improve the quality of Pacific white shrimp treated with phosphates. Non-phosphate compounds should be an alternative to lower the use of phosphates and to obtain the better shrimp quality. Additionally, some factors such as freshness and deveining may have the influence on quality and yield of shrimps treated with phosphates. To lower translucence and improve the yield, basic knowledge on the uses of phosphate or non-phosphate compounds in shrimps should be more understood. Also, the quality changes of shrimps treated with either phosphate or non-phosphate compounds should be elucidated.

Literature Review

1. Chemical composition of fish and shellfish

Fish and shellfish are the aquatic foods rich in nutrients. The main constituents of fresh fish are water (65-80%), protein (15-24%), fat (0.1-22%), carbohydrate (1-3%) and inorganic substances (0.8-2%) (Suzuki, 1981). The composition of fish meat varies with species, age, part of body, pre- or post-spawning season and the food condition also affect the fish composition (Suzuki, 1981). The relative amounts of these components are generally within the range found in mammals (Mackie, 1994). Protein is a major composition of fish muscle with the range of 15-20% (wet weight) depending upon muscle type, feeding period and spawning, etc. Generally, protein content is decreased in spawning period (Almas, 1981). Shrimp meats is an excellent source of protein (Yanar and Celix, 2006), consists of high protein content (50% dry weight) (Barelay *et al.,* 1983). However, chemical compositions of shrimps were reported to change seasonally (Yanar *et al.,* 2005). Karakoltsidis *et al.* (1995) reported that the changes in chemical compositions of shrimp (*Aristeus antennatus*) and Norway lobster were influenced by seasons (Table 1). Slight differences in proximate composition between 2 species of penaeid shrimp cultured in Thailand were reported. Black tiger shrimp contained slightly higher moisture content but lower protein content than white shrimp. Black tiger shrimp consisted of 80.47% moisture, 17.03% protein, 1.23% lipid and 0.95% ash, whereas white shrimp comprised 77.21% moisture, 18.75% protein, 1.23% lipid, and 0.47% ash (Sriket *et al.* , 2007a).

1.1 Muscle protein composition

There are different proteins in fish muscle. These proteins perform different tasks and have varying properties (Sikorski and Borderias, 1990). Fish muscle proteins can be divided into three major groups on the basis of solubility as follows:

Table 1. Chemical composition (%) of lobster and shrimp at different seasons

Species	Season	Protein	Fat	Moisture	Ash	Carbohydrate
Lobster (Norway)	Spring	19 ± 0.9	0.2 ± 0.1	78 ± 1.9	2.0 ± 0.1	0.8 ± 0.1
	Fall	17 ± 0.8	0.5 ± 0.1	80 ± 0.6	2.0 ± 0.2	0.5 ± 0.2
	Winter	17 ± 0.9	0.2 ± 0.1	79 ± 0.5	1.0 ± 0.2	3.0 ± 0.3
Shrimp	Spring	19 ± 1.3	0.2 ± 0.5	77 ± 0.3	2.0 ± 0.1	2.0 ± 0.4
	Fall	16 ± 1.4	0.4 ± 0.1	80± 0.4	2.0 ± 0.2	2.0 ± 0.2
	Winter	19 ± 0.2	0.6 ± 0.07	79 ± 0.4	1.0 ± 0.1	1.0 ± 0.6

Source: Karakoltsidis *et al.* (1995)

1.1.1 Sarcoplasmic proteins

Sarcoplasmic proteins are located in sarcolemma and are soluble at low salt concentrations (<0.1 M KCl). Sarcoplasmic proteins comprise about 30-35% of the total muscle proteins (Xiong, 1997c). Those proteins also include lipoproteins, as well as the chromoproteins of muscle and blood (Sikorski *et al.*, 1990b). Despite their diversity, sarcoplasmic proteins share many common physicochemical properties. Most are of relatively low molecular weight, high isoelectric pH, and have globular or rod-shaped structures. Sarcoplasmic proteins can be extracted by homogenizing the muscle tissue with water or solutions of neutral salts with ionic strength below 0.15. Sarcoplasmic enzymes influencing the quality of fish include the enzymes of the glycolytic pathway and the hydrolytic enzymes of the lysosomes (Sikorski *et al.*, 1990b).

1.1.2 Myofibrillar protein

Myofibrillar proteins are the major proteins in fish muscle. Due to their association with the textural properties of flesh, these proteins have received a greatest attention. Normally, these proteins account for 65-75% of total protein in muscle, compared with 52-56% in mammals (Mackie, 1994). 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.*, 1990b; Zayas, 1997). Myofibrillar proteins undergo changes during the rigor mortis and extended frozen storage (Shahidi, 1994). The texture of fish products and the gel-forming ability of fish minces and surimi may also be affected by the changes of myofibrillar proteins (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". Kolakowska *et al*. (1976) recommended that NaCl solution at 2.5-3.0% could be used for extraction of myofibrillar proteins. Myofibrillar proteins can be further divided into the subgroups as follows:

1.1.2.1 Contractile proteins

Contractile proteins, which are different in size and location in the muscle, are listed in Table 2 (Ashie and Simpson, 1997).

- Myosin

Myosin is a large fibrous protein with a molecular weight of about 500 kDa (Ogawa *et al.*, 1994). It is the most abundant myofibrillar component, constituting approximately 40-60% of total protein content (Bechtel, 1986). Myosin consists of six polypeptide subunits, two large heavy chains and four light chains arranged into an asymmetrical molecule with two pear-shape globular heads attached to long α-helical rodlike tail (Xiong,

1997c) (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). Enzyme, such as trypsin, may be used to cleave the myosin in the middle region, producing two fractions of the protein: heavy meromyosin (HMM) and light meromyosin (LMM). HMM has been shown to be composed of two regions, sub-fragment 1 (S-1) and sub-fragment 2 (S-2) (Wright *et al.*, 1977). For fish myosin, the rod was found to be as labile as S-1 (Wright and Wilding, 1984). According to Lawrie (1998), HMM section contains all the ATPase and actin-combining properties and these properties depend upon the number of free SH-groups in the molecules. Myosin is susceptible to aggregation due to oxidation of thiol groups (Sikorski, 2001). Myosin exhibits three important functional properties. It is an enzyme of ATPase activity; it forms natural complexes with actin; myosin molecules react with each other and build filaments (Sikorski, 2001).

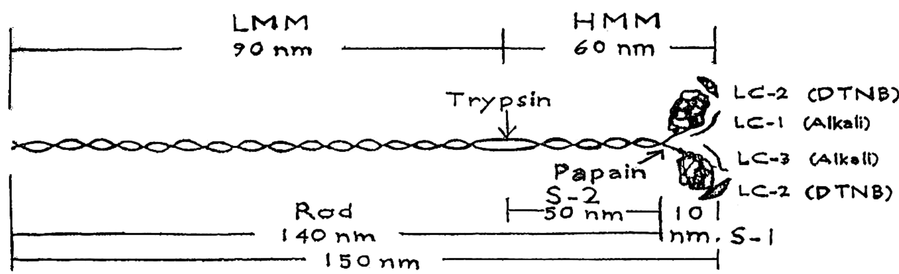


Figure 1. Schematic representation of the myosin molecule

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 (1997c)

- Actin

Actin is the second most abundant myofibrillar protein, constituting about 22% of myofibrillar proteins. This protein has a molecular weight of 42 kDa. 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, 1997c).

- 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). 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). 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. The actomyosin specifically dissociates with ATP, pyrophosphate, and other polyanions. 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).

1.1.2.2 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-, γ-actinin, C-, M-, H-, and X-protein paratropomyosin, and others (Kijowski, 2001; Xiong, 1997c). Those are involved in contraction-relaxation of muscle (Table 2).

Table 2. 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		17-18	
Troponin-I		20-24	
Troponin-T		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 Ashie and Simpson (1997)

- 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, 1997c). 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. 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) (Figure 2a). 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).

- 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, 1997c). Each subunit of troponin has distinct functions. Troponin C is a calcium biding protein and confers calcium regulation to the contractile process via the thin filament. It has four sites to bind calcium ions and 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) (Figure 2b).

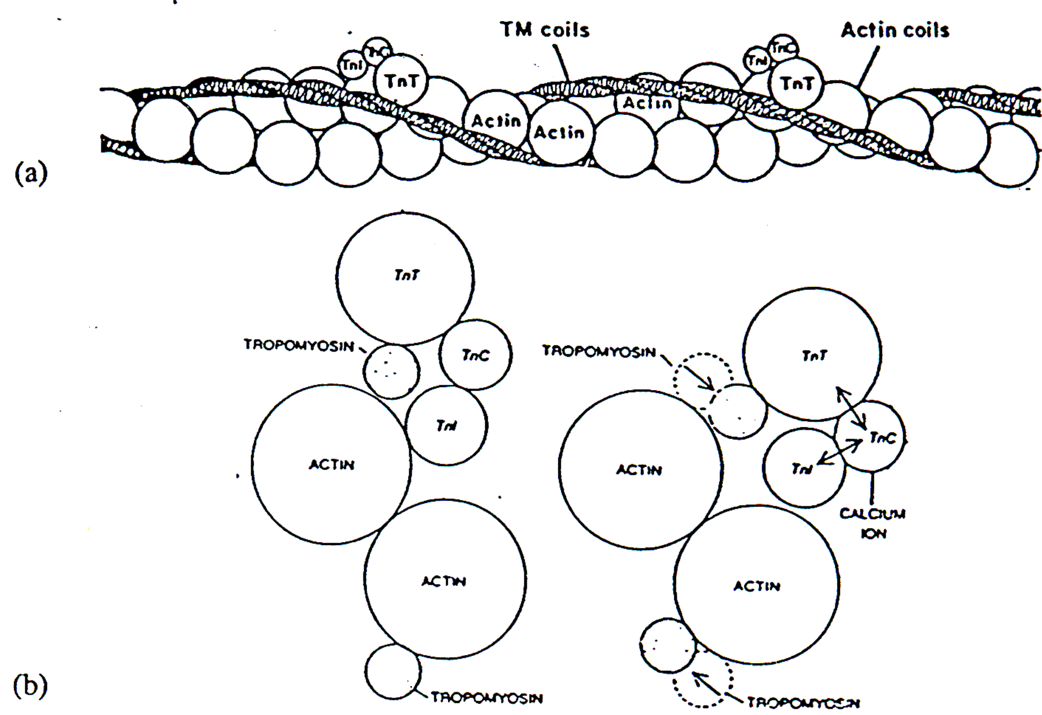


Figure 2. A thin filament of muscle formed by the filament of tropomyosin molecules wound in each of the two grooves of the actin helix (a) and proposed model for configuration of actin, tropomysin and troponin (Tn) subunits(b).

Tn T = troponin-tropomyosin subunit

Tn I = troponin-inhibitory subunit

Tn C = troponin-calcium-binding subunit

Source: McCormick (1994)

- M-line proteins

In the region of M-line, which is located at the middle of the A-band, M-protein, myomesin and creatine kinase can be found. M-protein is a single peptide chain with a molecular weight of 185 kDa. The main function of M- protein is to maintain the thick filaments in register (Kijoski, 2001). Transmission electron microscopy revealed two structural components in the M-line: M-filament and M-bridges (Knappeis and Carlsen, 1968). M-filaments are

positioned in parallel, while M-bridges are positioned transversely to the myofibrils. Hence, it is thought that M-bridges bind neighboring myosin filaments and stabilize them in the line, in the row, longitudinally, and transversely (Knappeis and Carlsen, 1968).

- C-protein

C-protein occurs in thick filaments and the molecule of that protein clasp myosin molecules into a thick filament. One filament consists of around 37 molecules of C-protein. One C-protein ring contains three to five molecules. C-protein contain 97.1% proline, hence, it has the structure of α-helix. Molecular weight is dependent on animal species and kind of muscle and ranges from 135to 150 kDa (Kijoski, 2001). The function of C-protein is not fully understood, although it has been shown to bind to the light meromyosin region of the myosin tail by attaching to the surface of the thick filament shaft (Pearson and Yong, 1989). C-protein could provide a mechanism for regulating the interaction and movement of the actomyosin cross-bridges during contraction. There is also speculation that, by forming bands around the myosin filament shaft, C-protein may provide a structural role in the myosin filament (Xiong, 1997c).

- Z-line proteins

The principal protein of Z-line is α-actinin that amount around 2% of myofibril protein weight. The SDS electrophoresis technique revealed its molecular weight of 95 kDa. The protein stabilizes the thin filaments of the neighboring sarcomeres. The liberation of α-actinin postmortem is associated with Z-line decomposition and with an increase in meat tenderness (Kijoski, 2001).

- H- and X- proteins

H- and X- proteins are associated with myosin at discrete sites on the surface of thick filaments, and they can overlab with the thin filaments on both ends of the A-band. X-protein is bound to C-protein: thus, it may be a part of the C-protein bands surrounding the myosin filaments. X-protein is present in slow red fibers and absence in fast white fibers. H- and X-protein account for only a small percentage (0.18 and 0.2%, respectively) of total myofibrillar protein. H-protein is a relatively small protein with a molecular weight about 69 kDa, compared to X-protein which has a molecular weight of 152 kDa (Starr and Offer, 1983).

1.1.2.3 Cytoskeletal proteins

Cytoskeletal proteins function in maintaining the cytoskeletal structure. They appear to provide support and stabilization of the contractile and regulatory proteins either longitudinally or laterally and therefore are also called "scaffold proteins" (Xiong, 1997c). The cytoskeleton of the muscle cell is formed by titin and nebulin filament and intermediate, while the costamers form the submembrane structure (Kijoski, 2001).

- Titin

Titin is the major constituent of the fine filaments located in the gap between the actin and myosin filaments, and they can be seen in highly stretched muscle fibers (Xiong, 1997c). Titin appears as a double band with an estimated molecular weight of 1,000 kDa. Titin filaments exhibit the ability to bind the rod like fragment of myosin filament with C-proteins and presumably with M-line proteins (Labeit *et al*., 1992; Nave *et al*., 1989). Titin protein extends between two neighboring Z-lines, hence, its role is to ensure sarcomere integrity. It binds the thick filament to Z-disc and maintains the position of the thick filament in the sarcomere center (Horowits *et al*., 1986). Titin is susceptible to proteolysis in meat during the aging process. It can

be degraded by muscle endogenous enzymes such as calpains and carboxyproteases (Koohmaraie, 1992).

- Nebulin

Nebulin has been identified histologically as thin, nebulous, continuous transverse arrays, called N-lines, near the boundary of the A-I zones and each side of the Z-disks (Pearson and Yong, 1989). Nebulin may attached to titin in the gap filament, thereby providing structural and regulatory role in the myofibril assemblage. Nebulin is rapidly degraded in postmortem meat in the initial aging period and is particularly susceptible to calpain. Its possible involvement in meat tenderization during aging has been speculated (Xiong, 1997c). The principal function of nebulin in the muscle is stabilization and regulation of the actin filament length (Kijoski, 2001).

- Desmin

Desmin is another protein of cell cytoskeleton with a molecular weight of 52-55 kDa (Kijoski, 2001). Desmin is arranged in a filamentous form around the periphery of Z-disk. These filaments occupy the space between adjacent myofibrils where they tie the myofibrils into the cytoskeleton of the muscle cell. Desmin is highly susceptible to postmortem proteolysis, and its degradation rate is comparable to that of troponin T. Since the disruption of Z-disks, particulary in fast-twitch fibers, is one of the most noticeable postmortem changes in meat. It seems likely that proteolysis of desmin is involved in meat tenderization during postmortem aging (Xiong, 1997c).

1.1.3 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). 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).

1.2 Muscle structure

The skeletal muscle fibers show very regular transverse striations along their length, the protein-dense A-bands alternating with the less dense I-bands. At moderate to long muscle lengths, there is a lighter zone, the H-zone, within the A-band, and in the center of the A-band, there is a dense line, the M-line. The I-band is bisected by the very dense Z-disc. The structure is not continuous across the width of the muscle fiber but is divided up into roughly cylindrical elements, the myofibrils, which are aligned along the fiber axis. These are separated from one to another by gaps containing membrane-lined channels (the sarcoplasmatic reticulum) whose function is to store Ca^{2+} ions until they are released to trigger muscular contraction. In some muscle fibers, mitochondria are additionally present in these gaps. The structural unit which is repeated between successive Z-discs is called the sarcomere. The striations of the myofibrils are caused by a highly organized array of two kinds of longitudinally-oriented filaments: the thick filaments, confined to the A-band and joined together at their centers by the M-line, and the thin filaments, extending from either side of the Z-disc to the edge of the H-zone and joined together by the Z-disc (Figure 3) (Pearson and Yong, 1989).

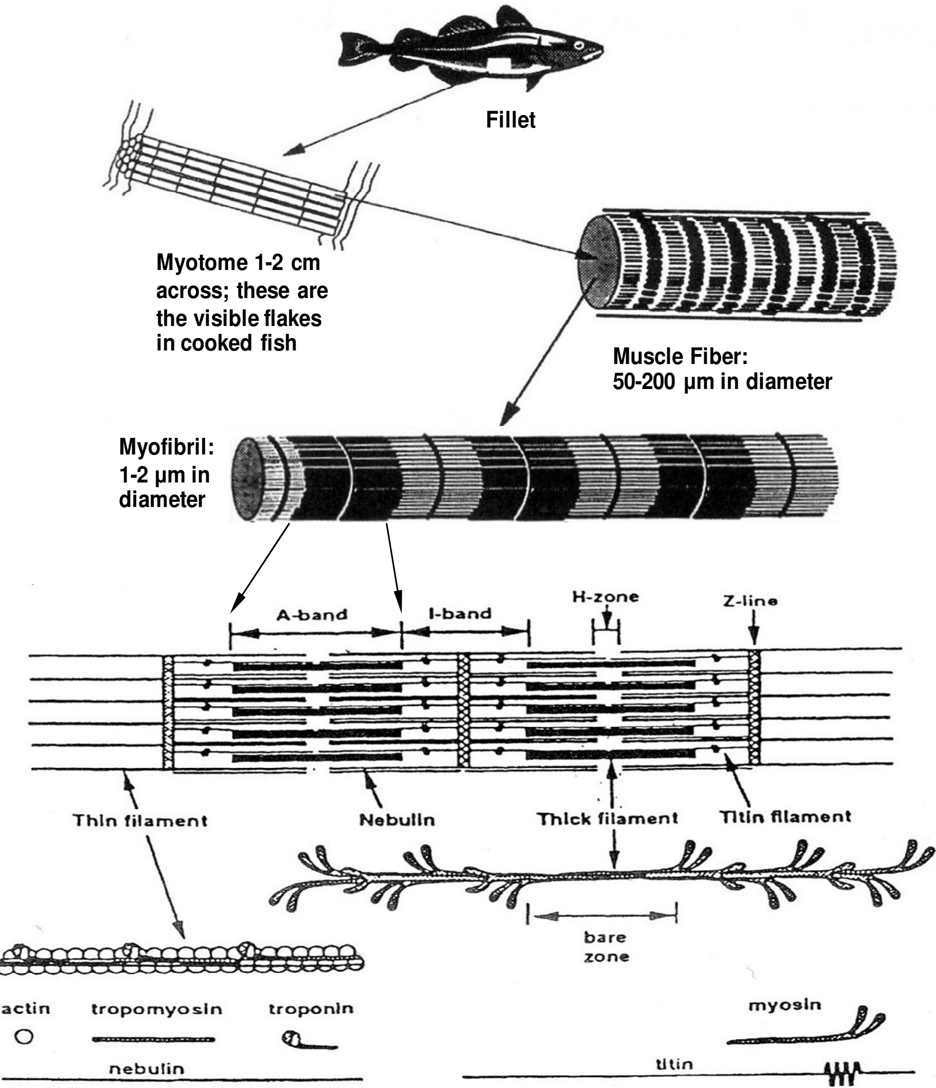


Figure 3. Proteins of myofibrillar structure organization from fish fillet

Sources: Adapted from Goodbano (2002) and Greaser (1997)

1.3 Thermal stability of fish muscle protein

Stability is one of the most important properties of muscle 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 fish 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 stable transition observed in the thermograms and is assumed to correspond to actin denaturation. Fish myosin is very unstable in comparison with that of mammal (Ogawa *et al.*, 1994). Whole cod muscle showed two maximal transitions on DSC thermogram with T_{max} about 45 and 75 °C (Hasting *et al.,* 1985) and whole muscle of fresh hake also showed two endothermic transitions with T_{max} values of 46 and 75 °C (Beas *et al.,* 1990). Differential scanning colorimetric (DSC) study revealed three transitions corresponding to the thermal denaturation of myosin and paramyosin, connective tissues and actin of cuttlefish (*Sepia pharaonis*) muscle, at temperatures of 49.8–50.3, 59.8–60.3 and 74.7–78.8 1 °C, respectively (Thanonkaew *et al.,* 2006). The difference in T_{max} of the transitions among the fish species seems to be correlated with the habitat temperature of the fish (Davies *et al.,* 1988). Thermal denaturation of hake (*Merluccius hubbsi*) myofibrillar proteins of whole muscle showed two T_{max}, 46 °C and 75 °C (Beas *et al.*, 1990). Myosin of cod (*Gadus morhua*) which lived in cold water was more labile, compared with that of snapper (*Lutjanus sebae*) which lived in tropical area (Davies *et al.*, 1988). Yongsawatdigul and Park (2003) reported that the major endothermic transition of threadfin bream actomyosin were at 38.4, 51.0 and 80.7 °C. Poulter *et al.* (1985) reported that the tilapia muscle had T_{max} of 46.4, 57.1 and 73.6 °C.

Thermal denaturation of the myosin was dependent on pH and ionic strength (Stabursvik and Martens, 1980; Wright and Wilding, 1984). Raising and lowering pH from neutral pH reduced T_{max} values and ΔH. Salt treatment of herring muscle led to the decrease in T_{max} values by 5-10 K and the peak areas were also decreased by the salt addition (Hastings *et al.*, 1985). The salt ions are believed to cause weakening of the interaction between oppositely

charged side chains (Aktas and Kaya, 2001; Thorarinsdottir *et al.*, 2002). At higher salt concentration, muscle proteins may denature, resulting in stronger protein-protein bonds, shrinkage of the muscle and dehydration. This is attributed to hydrophobic interactions to the stabilization of the native conformation, probably via a modification of the water structure (Smyth *et al.*, 1998). Park and Lanier (1989) showed that addition of salt shifted the denaturation transitions to lower temperatures and decreased the enthalpies of heat denaturation. These results suggested that addition of salt caused a partial unfolding of proteins, increasing their sensitivity to heat denaturation.

1.4 Water-holding capacity of muscle proteins

Water-holding capacity (WHC) is the ability of meat to retain its water despite the application on force. WHC of meat is of great importance because moisture losses affect the weight, and therefore the financial value of meat. Majority of water in meat is confined within the myofibrils in the spaces between the myosin and actin (Xiong, 1997c). Myofibrils are the primary sites for intracellular water. Other cellular components may also contribute to water binding in the meat. Entrapment and mobility of the water present outside the cell could be affected by many environmental factors, and water content varies with different processing treatments (Xiong *et al.*, 2000). There are two major types of forces that contribute to water retention in meat: polarity, including surface charges, and capillary effects (Xiong, 1997c). 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. Any change in the surroundings of myofibrils that results in increased protein charges or dipoles (high concentrations of salt and pH away from the protein isoelectric point) would lead to increase water retention in meat (Xiong, 1997c).

2. Post mortem changes of fish

2.1 Degradation of proteins

Post mortem tenderization is one of the most unfavorable quality changes in fish muscle. A proteolytic degradation of myofibrillar and connective tissue components occurs during post mortem storage. Myofibrillar proteins fraction in muscle of Monterey sardine was unstable during ice storage (Pacheco-Aguilar *et al*., 2000). The participation of various proteinases in autolytic processes of ice-stored fish depends on location of the enzymes in cytosol and/or factors affecting tissue compartmentization, the presence of activators or inhibitors and the susceptibility of the proteins responsible for muscle integrity to cleavage by the respective enzymes (Ladrat *et al*., 2003). Among post-harvest changes, degradation of fish muscle caused by endogenous proteases is a primary cause of quality losses during cold storage or handling (Haard *et al.,* 1994). The decrease in the relative amount of myosin heavy chain (MHC) and a concomitant increase in the number and intensity of bands of molecular size about 100 kDa were found in *Penaeus borealis* after 24 h and the appearance of a band of slightly less than 50 kDa was observed after 5 h of iced storage (Martinez *et al*., 2001). Benjakul *et al.* (1997) showed that MHC of Pacific whiting muscle was hydrolyzed continuously throughout iced storage. MHC decreased to 45% of the original content within 8 days, whereas no changes in actin were observed.

Proteases are able to hydrolyze the muscle proteins differently. An *et al.* (1994) reported that among the Pacific whiting proteins, MHC was the most extensively hydrolyzed, followed by troponin-T, α- and β-tropomyosin. Apart from myofibrillar protein, collagen and connective tissue are also degraded during the extended storage storage. Eckhoff *et al.* (1998) reported that insoluble collagen in salmon (*Salmo salar,* L) decreased gradually during 15 days of the storage in ice. The relationship between collagen content and texture was further confirmed by Hatae *et al*. (1986), who showed that a high collagen content resulted in a firm meat. Raw fish meat softened rapidly during chilled storage and

histological examinations were shown to be caused by disintegration of collagen (Sato *et al*., 1991). A change in microstructure of *Macrobrachium rosenbergii* during storage in ice was observed (Nip and Moy, 1988). Rowland *et al*. (1982) reported the substantial morphological changes due to proteolysis in the tails of *M. rosenbergii.*

3. Effect of thermal process on fish and shellfish quality

Thermal processing is one of the most effective means of preserving or cooking food (Karel *et al.,* 1975). Prior to consumption, shrimp meat stored for a certain period in frozen or chilled conditions needs processing such as heat-processing (Mizuta *et al.,* 1997). Thermal process causes the denaturation of muscle proteins and degree of denaturation varies with species. Fish myosin is very unstable in comparison with that of mammal (Ogawa *et al.,* 1994). Johnston *et al.* (1973) found a relationship between the thermal stability of fish myosin and the environment temperature in which the species live. Myosin of cod (*Gadus morha*) which lived in cold water was more labile, compared with that of snapper (*Lutjanus sebae*) which lived in tropical area (Davies *et al.,* 1988). Shrimp meat is enhanced in firmness or solidity by heat processing and becomes too solid and unpalatable when its inner temperature is above 100 °C (Mizuta *et al.,* 1999). Japanese professional cooks are careful to keep the central inner temperature of prawn meat below 65 to 70 °C (Mizuta *et al.,* 1999). The texture and taste of the product become rapidly undesirable with excessive firmness and a lack of juiciness once the heating temperature exceeds 70 °C (Mizuta *et al.,* 1999). Denaturation of the shrimp muscle during heat processing enhances the loss of water-holding capacity of shrimp meat (Murakami, 1994). Heat processing enhanced firmness and shrinkage of kuruma prawn *Penaeus japonicus* (Mizuta *et al.,* 1999). The yield of cooked meat under commercial conditions depends on the size and age of the shrimp (Erdogdu *et al.,* 2004). Small shrimp yield less cooked meat through processing, compared with the larger shrimp (Crawford, 1981). The reduction of processing time

showed the beneficial effects on the quality, sensory and textured property of heated shrimp (Mohan *et al.,* 2006).

4. 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 products. There are three accepted theories to explain denaturation of structural 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 and Zotos *et al*., 1985). As freezing progresses, proteins are exposed to increased ionic strength in the nonfrozen aqueous phase that 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, changes occur in protein conformation (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). 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 (Benjakul *et al*., 2003). Total solubility of proteins in neutral 5 % NaCl solution may decrease to about 30%, whereby the main loss regards the contractile proteins, mainly myosin heavy chain, M-proteins, tropomyosin, and troponins I and C in descending order (Owusu *et al*., 1987). Decrease in solubility during frozen storage and loss of ATPase activity was described for myosin in frozen fish (Li-Chen *et al*., 1985). Nambudiri and Gopakumar (1992) found a decrease in ATPase activity of fresh water and brackish fish by 70-90% after 6 months of storage at -20ºC. The 24%

decrease in Ca^{2+}-ATPase activity in Alaska pollock was observed after 226 days of frozen storage at -29ºC (Scott *et al* 1988). 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).

Extended frozen storage causes the severe changes in tertiary conformation of actomyosin (Xiong, 1997b). The denaturation and aggregation of protein started from the formation of disulfide bond, followed by a rearrangement of hydrophobic and hydrogen bonded regions on an intra- and inter-molecular basis (Buttkus, 1974). Badii and Howell (2002) reported the initial increase in surface protein hydrophobicity of cod muscle in the first month before decreasing during the frozen storage at -10 and -30 °C. The level of reactive SH groups decreases considerably during frozen storage. This demonstrates that more disulfide bonds are formed in the muscle during frozen storage (Zayas, 1997). Disulfide bridges are the important covalent bonds, which relates to aggregation of protein (Sikorski *et. al.,* 1990a). 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).

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). Benjakul and Bauer (2001) reported that 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. The loss of Ca^{2+}-ATPase increased with increasing freeze-thaw cycles. 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. 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.

5. Phosphate compounds

5.1 Classification of phosphates

Phosphates are compounds prepared from phosphoric acid where the acid has been partially or fully neutralized with alkali metal ions, predominately sodium, potassium, or calcium (Dziezak, 1990). Phosphates can be divided into two general classes:

5.1.1 Orthophosphates

Orthophosphate consists of one phosphorus atom tetrahedrally surrounded by four oxygens (Table 3). It can form straight-chain and cyclic polymers. These compounds have three valences that can be filled by hydrogen atoms, alkali metal cations, or a combination of hydrogens and metal cations. Monobasic orthophosphates have one alkali metal ion and two hydrogens; dibasic orthophosphates have two metal ions, one hydrogen; and tribasic orthophosphates are fully neutralized with three metal ions (Dziezak, 1990).

5.1.2 Condensed phosphates

Condensed phosphates are produced by heating mixtures of orthophosphates under controlled conditions. They are composed of two or more phosphorus atoms linked through a shared oxygen. This group includes straight-chain phosphates called polyphosphates and rings, termed metaphosphates (Dziezak, 1990).

Table 3. Classes, formulas, pH, solubility, and functions of several phosphates

Class of phosphate and Basic structure[a]	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 dihydrate	$Na_2HPO_4 .2H_2O$	9.1	15	Emulsifier, buffer
	Trisodium phosphate	Na_3PO_4	11.8	14	Emulsifier, buffer
	Monopotassium phosphate	KH_2PO_4	4.6	25	Water binding in meats
	Dipotassium phosphate	K_2HPO_4	9.3	168	Emulsifier, buffer
	Tripotassium phosphate	K_3PO_4	11.9	107	Emulsifier, buffer
	Monocalciun phosphate	$Ca(H_2PO_4) .H_2O$	3.8	-	Acidulant, leaving acid, dough condition, yeast food, nutrient
Condensed phosphates					
pyrophosphate	Sodium acid pyrophosphate	$Na_2H_2P_2O_7$	4.3	15	Emulsifier,buffer,sequestrant Water binding in meats
	Tetrasodium pyrophosphate	$Na_4P_2O_7$	10.3	8	Dispersant, coagulant, crystallization inhibitor in canned tuna
	Tetrapotassium pyrophosphate	$K_4P_2O_7$	10.5	187	Emulsifier, Water binding in meats, suspending agent
tripolyphosphate	Sodium tripolyphosphate	$Na_5P_3O_{10}$	9.9	15	Emulsifier, Water binding in meats
	Potassium tripolyphosphate	$K_5P_3O_{10}$	9.6	193	Emulsifier, Water binding in meats
Long-chain polyphosphates	Sodium polyphosphates, glassy, or Graham's Salt;	$(NaPO_3)_6 .Na_2O$	7.7	40[b]	Sequestrant, emulsifier Water binding in meats, suspending agent
	three chain lengths;	$(NaPO_3)_{13} .Na_2O$	6.9	40[b]	Sequestrant, emulsifier Water binding in meats, suspending agent
	Sodium hexametaphosphate has an average chain length of 13	$(NaPO_3)_{21} .Na_2O$	6.3	40[b]	Sequestrant, emulsifier Water binding in meats, suspending agent
Metaphosphate Tri- Tetra-	Sodium trimetaphosphate	$(NaPO_3)_5$	6.7	23	
	Sodium tetrametaphosphate	$(NaPO_3)_4 .4H_2O$	6.2	18	

[a]M stands for one equivalent of a metal ion or hydrogen

[b]Solubility is higher than 40% but this recommended for ease of preparation and use

Source: Dziezak (1990)

Among all polyphosphates, pyrophosphates are the simplest as they have a two-phosphorus chain. Tripolyphosphates are next in the series with three phosphorus atoms and are followed by long-chain polyphosphates which have four or more phosphorus atoms. Pyrophosphates and tripolyphosphates are crystalline materials unlike long-chain polyphosphates, which are amorphous or glassy. Long-chain polyphosphates are not pure compounds but instead mixtures of many polyphosphates of varying chain lengths (Ellinger, 1977). Polyphosphates can be hydrolyzed, yielding increasing amounts of orthophosphates, pyrophosphates, and tripolyphosphates (Ellinger, 1972a). Metaphosphates are pure crystalline compounds, which are composed of six- or eight- membered rings. There are two metaphosphates, sodium trimetaphosphate and sodium tetrametaphosphate; only the first is used commercially (Dziezak, 1990).

5.2 Functions of phosphates in food application

Certain chemical properties of phosphates enable these compounds to produce a wide variety of effects in food products.

5.2.1 Inactivation of metal ions

The function of phosphate is a metal chelator (Park, 2000). The phosphates can inactivate metallic ions, which enhance the detrimental effect in food products. They inactivate the metallic ions either by precipitating and removing them from interference with the desired food-processing reactions or by complexing and maintaining them in a soluble bound state (Ellinger, 1975).

Polyphosphates are considered to undergo ion-exchange reactions, in which a hydrogen, sodium, or potassium ion is exchanged for one of the alkaline-earth or transition-metal ions (Ellinger, 1975). Very weak, soluble complexes are formed with alkali-metal and ammonium ions. More stable but somewhat dissociated complexes are formed with the alkaline-earth metals,

such as calcium, magnesium. Very stable, soluble complexes are formed with the transition metal ions, such as copper, nickel, and iron. A significant advantage in the use of phosphates to complex nutritionally important ions, such as calcium, magnesium, and iron, is that the ions can still be absorbed through the intestinal walls and utilized by the body, and their absorption and retention may actually be increased in the form of their complexes (Ellinger, 1975). The polyphosphate anion can bind calcium more firmly than sodium; therefore, sodium pyrophosphates exchange their sodium ions for calcium ions when they are present.

5.2.2 Complexing organic polyelectrolyte food constituents

In solution, phosphates are polyvalent anions, as they have more than one negative charge. Orthophosphates have up to three negative charges depending on the pH, and polyphosphates can be even more anionic. By highly charged nature, polyphosphates interact with various food constituents to produce many useful effects. They can become adsorbed onto surfaces of certain constituents and affect the surface charge, thereby promoting deflocculation, dispersion, emulsification, or suspension of the constituent (Van Wazer, 1971). The ability of polyphosphates to attach themselves to positively charged sites of large molecules results in the increased water-binding and gel formation of the proteins, improved whipping properties (by increasing the solubility of the protein), and enhanced precipitation and insolubilization of proteins for separation (Ellinger, 1972b; VanWazer, 1971). The polyelectrolyte properties of polyphosphates generally increase with chain length (Ellinger, 1972b).

5.2.3 Buffering or pH stabilization

The ability to maintain a constant pH after addition of acids or bases is termed ‘buffering’. For the pH ranges of 2 to 3, 5.5 to 7.5 and 10 to 12, the orthophosphates such as mono- and disodium phosphates and pyrophosphates such as sodium acid pyrophosphate have the best buffering capacity (Van Wazer, 1971). Long-chain polyphosphates are generally poor

buffers, and their buffering capacity decreases with increasing chain length. Phosphates can be used to increase or decrease pH to optimum levels. Both the acidic phosphate such as monosodium phosphate, monoammonium phosphate, and sodium acid pyrophosphate, and the alkaline phosphates such as di- and trisodium phosphates, sodium tripolyphosphate, and tetrasodium pyrophosphate are commonly used for this purpose (Dziezak, 1990).

5.3 Applications of phosphates in meat and seafood products

Optimal goals for the use of phosphates in meat and seafoods are retention of natural moisture and flavor, inhibition of fluid losses during shipment and prior to sale, emulsification, inhibition of oxidation of flavors and lipids by chelation of heavy metals, and cryoprotection to extend shelf-life (Lampila and Godber, 1990). The effect of phosphates depends to a large extend on their type and concentration and the ionic strength in the muscle (Peterson *et al.,* 1988; Lindsay, 1996; Xiaowen, 1996). Phosphates may enhance water retention of the muscle by effects on pH, ionic strength, and specific interactions of phosphates with divalent cations and myofibrillar proteins (Lindsay, 1996). Key applications are shown in Table 4. Meat and seafoods are treated with phosphates by dipping in, spraying with, or tumbling in a phosphate solution. Injector needle systems may also be used with and without added tumbling. Dry addition is used in comminuted meat systems, e.g.,surimi and fish sausage formulations (Lindsay, 1996).

The ability of phosphates to improve water-holding capacity in muscle food has led to the increasing uses of their compounds. Phosphates decrease the concentration of sodium chloride required for maximum myofibrillar swelling, but do not result in more swelling than can be reached with salt alone. When used in conjunction with salt (NaCl), phosphates enhanced hydration of raw meat and reduced the cooking loss (Xiong, 1997a). Phosphate anion act as polyelectrolytic to increase ionic strength, resulting in increased water holding capacity by direct binding of water to the phosphate anion and the repulsion of protein groups due to the

predominance of negative charges on the protein groups. This repulsing effect opens up protein structure, and increase the number of binding sites available for water, which allows for more water to be contained in the meat (Xiong, 2005). Trout and Schmidt (1987) concluded that at high ionic strengths (>0.25), pyrophosphate affected hydrophobic interactions which stabilize the protein structure, and thus, the thermal stability of the protein. Water retention is correlated with increased pH and normally associated with the use of alkaline polyphosphates such as sodium tripolyphosphate. Orthophosphates have virtually no effect on water-binding (Offer and Trinick, 1983). Salt 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, 1979), and the sodium ions form an ion “cloud” around the filaments (Offer and Knight, 1983).

Offer and Knight (1988) explained the action of phosphates in 3 ways. First, phosphates are good buffers, which may assist in the depolymerization of thick filaments and increase water uptake and retention. Second, in the presence of Mg^{2+}, pyrophosphate and triphosphate bind to the myosin molecule. Pyrophosphate acts as an analog to ATP and binds to the myosin heads, thus promoting the dissociation of actomyosin. Third, polyphosphate can bind to the myosin tails and promote dissociation of the myosin filaments to myosin molecules. Increases in water binding and hydration in salted meat and muscle fibers are generally attributed to enhanced electrostatic repulsions between myofibril filaments causing the filamental lattices to expand for water entrapment (Offer and Trinick, 1983). This results in local concentration differences which lead to an increased osmotic pressure within the myofibrils which causes the filament lattice to swell. Increased myofibrillar/ cytoskeletal protein extraction by phosphate in the presence of NaCl was associated with increased beef myofibril swelling and increased beef muscle water-holding capacity (Peterson *et al*., 1988). Phosphate treatment produced a transverse expansion of myofibrils with a simultaneous extraction of myosin from the ends of the A-band in sarcomere (Xiong, 2005).

Table 4. Phosphates for uses in seafood

Application	Phosphate functionality
Canned salmon	STPP or STPP/SHMP combinations to inhibit curd formation
Canned tuna	SAPP to inhibit struvite formation
Canned abalone	SAPP and citric acid to inhibit blackening
Fresh or frozen fillets	STPP or phosphate blends to retain natural moisture, inhibit color and lipid oxidation, reduce drip, and protect native protein
Pasteurized crab	SAPP to inhibit blue discoloration of the meat
Smoked fish	STPP or STPP/SHMP blends to retain flavor
Mechanical peeling of shrimp	STPP to assist cleavage of immature collagen and to firm the flesh
Peeled shrimp	STPP treatment before freezing to decrease thaw-drip loss, or STPP treatment prior to cooking to decrease cook– cool loss
Fresh scallops	STPP or phosphate blends to inhibit excessive exudate after harvest
Frozen fish blocks	STPP or STPP/SHMP combinations for solubilizing surface proteins to prevent voids
Kamaboko/surimi-based analogs	Mixtures of SAPP, TSPP, STPP, and SHMP

Source: Adapted from Dziezak (1990) and Henson and Kowalewski (1992)

The efficacy of phosphates in increasing the water-binding capacity varied with the type and concentration used (Trout and Schmidt, 1984, 1986). In general, pyrophosphate was the most effective phosphate for increasing water-binding capacity, as well as binding in restructured meat (Trout and Schmidt, 1984). Phosphates also improved the raw and cooked appearance, binding property and acceptability of restructured steaks (Miller *et al*., 1986). Trout and Schmidt (1986) showed that the effectiveness of phosphates on prevention of cook loss of meat products was in the following order: pyrophosphate > tripolyphosphate > tetrapolyphosphate > hexametaphosphate. The effects of phosphates on increasing water retention of muscle were summarized by Hamm (1970), involving the increases in pH and ionic strength, the binding of phosphates to meat proteins, and the dissociation of actomyosin into actin and myosin. Kiely and Martonosi (1968) suggest that phosphates also react specifically with myosin on the same active binding sites of a molecule as those which react with actin or ATP. Kijowski and Mast (1988) reported that the amount of specifically reacting pyro- or tripoly- phosphate was probably restricted to the number of available binding sites on the myosin head, usually reacting with actin. At the phosphate concentrations greater than 0.5%, the excess ions reacted nonspecifically with myosin molecules, i.e., with oppositely charged groups of this protein.

Polyphosphates, including pyrophosphate, have been used in frozen surimi, together with cryoprotectants. Pyrophosphate neutralized the surimi, in which the protective effect of sorbitol was enhanced (Kumazawa *et al*., 1990). Phosphate is normally added to surimi in combination with cryoprotectants such as sugar or sorbitol (Sultanbawa and Li-Chan, 2001). The addition of cryoprotectants is important to ensure maximum functionality of frozen surimi because freezing induces protein denaturation and aggregation. Cryoprotectants are uniformly incorporated into the dewatered meat using a kneader or silent cutter before formation of the surimi blocks. Sucrose (4%) and sorbitol (4- 5%) serve as the primary cryoprotectants and sodium polyphosphate (0.2-0.3%) is also added as both a chelating agent by making metal ions in surimi inactive and as a pH enhancing agent. Combining theses ingredients together protects fish

myofibrillar proteins and minimizes protein denaturation during long periods of frozen storage (Pipatsattayanuwong *et al*., 1995). Phosphates are added to surimi as cryoprotectant at 0.25 to 0.3%, traditionally as a mixture (1:1) of sodium tripolyphosphate or tetrasodium pyrophosphate (Park and Lin, 2005). Minced fish flesh goes through extensive washing and dewatering, nevertheless, there are still small quantities of metal ions remaining in the dewatered meat. If these metal ions are not completely removed or inactivated (chelated), they can accelerate the denaturation of fish myofibrillar proteins during freezing and frozen storage (Park, 2005). Chemical blends (1:1) of tetrasodium pyrophosphate and sodium tripolyphosphate have been proved to exhibit the cryoprotective effect. Addition of pyrophosphate, in combination with 2.5% NaCl, was found to increase the breaking strain of walleye pollack surimi gel set at temperatures above 40 °C (Matsukawa *et al*., 1992). Julavittayanukul *et al*. (2006) reported that kamaboko and directly heated gels from bigeye snapper surimi added with 0.05% PP had the increases in breaking force and deformation by 17.35% and 11.52%, and 13.54% and 3.53%, respectively, compared with the control gel (without PP addition). PP, which has the lowest molecular weight, might distribute uniformly and solubilize or dissociate the actomyosin complex more effectively, compare with other phosphates used (sodium tripolyphosphate and sodium hexametaphosphate) and its exhibited a greater gel-strengthening effect than others, particularly at the appropriate concentration (Julavittayanukul *et al*., 2006). The raising pH caused by this compound results in the improved water holding/binding of the gel as well as better solubilization of myofibrillar proteins (Park, 2000). Pyrophosphate has been reported to dissociate protein complex, leading to the improved gel forming ability (Matsukawa *et al*., 1995).

CHAPTER 2

MATERIALS AND METHODS

1. Materials

1.1 Shrimp samples

Pacific white shrimps (*Litopenaeus vannamei*) with the size of 55-60 shrimps per kg were obtained from a farm in Songkhla province. Three different batches of shrimps were used for each experiment. Shrimps were kept in ice with a shrimp/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Thailand, within 1 h. Upon arrival, shrimps were washed with clean water, immediately deheaded and the shells were then peeled off. The shrimps were placed in polyethylene bag and kept on ice during preparation and until used.

1.2 Chemicals

1.2.1 Phosphate compounds

Tetrasodium pyrophosphate (TSPP), sodium hexametaphosphate (SHMP) and sodium dodecyl sulfate (SDS) were obtained from Ajax Finechem (Wellington, Auckland, New Zealand). Sodium tripolyphosphate (STPP) and sodium acid pyrophosphate (SAPP) were purchased from Fluka (Buchs, Switzerland).

1.2.2 Non-phosphate compounds

ProfixO® containing 5% egg albumen hydrolysate powder, 10% sodium bicarbonate, 60% trisodium citrate, 10% sodium carbonate and 15% potassium chloride was obtained from Taiyo Kagaku (Yokkaichi, Japan).

1.2.3 Other chemicals

Sodium chloride and potassium chloride were obtained from Merck (Darmstadt, Germany). β-mercaptoethanol (βME), acrylamide, *N,N,N',N'*-tetramethylethylenediamide (TEMED) and bis-acrylamide were procured from Fluka (Buchs, Switzerland). Adenosine 5′-triphosphate (ATP), ammonium molybdate, 5-5′-dithio-bis (2-nitrobenzoic acid) (DTNB), glutaraldehyde, ρ-nitrophenyl-α-glucopyranoside, ρ-nitrophenyl-N-acetyl-ß-d-glucose-amide, 1-anilinonaphthalene-8-sulphonic acid (ANS) and coomassie Brilliant Blue R-250 were purchased from Sigma, St. Louis, MO, USA).

2. Instruments

Instruments	Model	Company/City/Country
pH meter	CG 842	Schott, Mainz, Germany
Magnetic stirrer	BIG SQUID	IKA labortechnik, Stanfen, Germany
Homogenizer	T25 basic	IKA labortechnik, Selangor, Malysia
Water bath	W350	Memmert, Schwabach, Germany
Microcentrifuge	MIKR 020	Zentrifugan, Hettich, Germany
Refrigerated centrifuge	RC-5B plus	Sorvall, Norwalk CT, USA
Double-beam Spectrophometer	UV-16001	Shimadzu, Kyoto, Japan
Electrophoresis apparatus	Mini-Protein II	Bio-Rad, CA, USA
Spectrofluorometer	RF-1501	Shimadzu, Kyoto, Japan
Mixer	MK-K77	National, Tokyo, Japan
Differential scanning calorimeter	DSC 7	Perkin Elmer, Michigan, USA
Scanning Electron Microscope	JSM 5800LV	JEOL, Akishima, Japan

3. Methods

3.1 Optimum condition of mixed phosphates for quality improvement of Pacific white shrimp

Different formulations of mixed phosphates were used as appeared in Table 5. SAPP, TSPP, STPP, SHMP were dissolved in 2.5% of NaCl to obtain different final concentrations. Shrimps were soaked in different mixed phosphate solutions for 2 h at 4 °C with continuous stirring. The ratio of shrimp/solution was 1:2 (w/v). After removal from the mixed phosphate solutions, the samples were placed on plastic screen for 5 min (4 °C) to drain off solution, then placed in polyethylene bag and kept in ice during the analyses. Shrimp meat was analyzed for moisture content, weight gain, cooking loss, cooking yield, salt content and phosphate content. Opacity was also evaluated for cooked shrimps. Protein patterns of soaking solutions were determined using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using 10% running gel and 4% stacking gel as described by Laemmli (1970).

3.1.1 Determination of Opacity

Evaluation of opacity was carried out by ten trained panelists. Trainings of 5 sessions (2 h each) were performed. Different references including 1) 7 days ice-stored shrimp soaked in 3.5% TSPP for 10 h at 4 °C; 2) fresh shrimp soaked in 3.5% TSPP for 2 h at 4 °C and 3) fresh shrimp were steamed for 5 min, followed by cooling in iced water for 1 min were used for training with the scale of 1, 3 and 5, respectively. Cooked shrimps were evaluated for opacity using a point structured scale with a value of 1 for very translucent, 3 for moderately translucent and 5 for turbid or opaque. The opacity score was recorded. The higher values represent the higher opacity or less translucence. All samples were identified by a three digit code. Sensory testing was held in a clean, well lighted and well ventilated room.

Table 5. Composition of mixed phosphates in 2.5% NaCl

Treatment	Types and concentration (%) of phosphates			
	SAPP	TSPP	STPP	SHMP
1	-	3.5	-	-
2	0.875	2.625	-	-
3	1.75	1.75	-	-
4	2.625	0.875	-	-
5	3.5	-	-	-
6	-	-	3.5	-
7	0.875	-	2.625	-
8	1.75	-	1.75	-
9	2.625	-	0.875	-
10	-	-	-	3.5
11	0.875	-	-	2.625
12	1.75	-	-	1.75
13	2.625	-	-	0.875

3.1.2 Determination of weight gain, cooking loss and cooking yield

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

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

where : A = initial weight (before soaking)

B = weight after soaking, followed by draining

Cooking loss and cooking yield were measured by weighing the shrimps before and after steaming. Shrimps were cooked by steaming for 5 min, immediately cooled in iced water for 1 min and drained at 4 °C for 5 min. Cooking loss and cooking yield were calculated as follows:

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

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

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

3.1.3 Chemical analyses

3.1.3.1 Determination of moisture content

Shrimps were finely chopped prior to analyses. Moisture content was determined according to the method of AOAC (2000).

3.1.3.2 Determination of phosphate content

Sample (10 g) was mixed with 20 ml of 10% trichloroacetic acid (TCA). The mixture was homogenized at a speed of 6,500 rpm using an Ultra Turrax homogenizer (IKA Labortechnik, Selangor, Malaysia) for 5 min. The homogenate was filtered using Whatman No. 1 filter paper and the sediment was rinsed with 10 ml of 10% TCA. The filtrate obtained was subjected to determination of phosphate content according to the method of Fiske and Subbarow (1925).

3.1.3.3 Determination of salt content

Salt content was determined by the method of AOAC (2000). Sample (1 g) was added with 10 ml of 0.1 N $AgNO_3$ and 10 ml of conc. HNO_3. The mixture was boiled gently on a hot plate until all samples except $AgCl_2$ were dissolved. The mixture was then cooled using running water. Then 50 ml of distilled water and 5 ml of 5% ferric alum

($FeNH_4(SO_4)_2.12H_2O$) indicator were added. The mixture was titrated with standardized 0.1 N KSCN until the solution became permanent brownish-red. The salt content was then calculated as follows.

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

where : V_1 = volume of $AgNO_3$ (ml); N_1 = concentration of $AgNO_3$ (N);

V_2 = volume of KSCN (ml); N_2 = concentration of KSCN (N); W = weight of sample (g)

3.1.4 Protein patterns

Protein patterns of soaking solutions were determined using SDS–PAGE according to the method of Laemmli (1970). Soaking solution (20 ml) was mixed with 10 ml of 10% (w/v) SDS solution. The mixture was then homogenized using a homogenizer (IKA Labortechnik, Selangor, Malaysia) at a speed of 11,000 rpm for 1 min. The homogenate was incubated in a water bath (85 °C) for 1 h to dissolve the proteins, followed by centrifuging at 7500×g for 15 min to remove undissolved debris. Sample with the protein content of 15 mg, determined by the Biuret method (Robinson and Hodgen, 1940) using bovine serum albumin standard, was loaded onto the gel. After separation by SDS–PAGE made of 4% stacking gel and 10% separating gel using 15 mA/plate, gels were fixed and stained for 3 h in 0.125% Coomassie Brilliant Blue R-250 in 40% methanol and 10% acetic acid. Gels were destained for 15 min with destaining solution I (50% methanol and 7.5% acetic acid) for 15 min and with the destaining solution II (5% methanol and 7.5% acetic acid) for 3 h.

Formulation of mixed phosphates yielding the shrimps with the least translucence and cooking loss, but showing high weight gain and cooking yield, were chosen for further study.

3.2 Effect of freshness and deveining on the properties of Pacific white shrimp

3.2.1 Preparation of ice-stored and deveined shrimp

Shrimps were washed with clean water and were separated into two groups, 1) fresh samples and 2) samples stored in ice for 7 days. For the second group, samples were kept in a styrene foam box containing crushed ice, with a shrimp/ice ratio of 1:2 (w/w) for 7 days. Molten ice was removed and replaced with an equal amount of ice every 2 days. The boxes containing samples and ice were kept at room temperature (28-30 °C). K-value determined by the method of Uchiyama and Kakuda (1984) of fresh and ice-stored shrimps were 0.7 and 38.5%, respectively. Before mixed phosphate treatment, both fresh and iced-stored shrimps were washed with clean water and deheaded; the shells were then peeled off. The shrimps were either deveined or non-deveined. Fresh and 7 days ice-stored shrimps, both deveined and non-deveined, were soaked in 2.5% NaCl in the absence or in the presence of different phosphates including 1) 3.5% TSPP, 2) 0.875% SAPP and 2.625% TSPP, 3) 3.5% STPP, 4) 0.875% SAPP and 2.625% STPP for 2 h at 4 °C. Subsequently, the treated samples were drained at 4 °C for 5 min. Both soaking solutions and resulting shrimps were subjected to analyses.

3.2.2 Analyses

All analyses were performed as described in section 3.1. Additionally, shrimp samples were also determined as follows:

3.2.2.1 Thermal properties of muscle proteins

Thermal transition of shrimp meat proteins was measured using the differential scanning calorimetry (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.

3.2.2.2 Microstructure of Pacific white shrimp meats

Microstructures of raw and cooked Pacific white shrimps were analyzed as described by Jones and Mandigo (1982). Sample was prepared by cutting into a cube (4 x 4 x 4 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 distilled water for 15 min per time and dehydrated with a serial concentration of 50-100% ethanol for 15 min each. All specimens were coated with 100% gold (Sputter coater SPI-Module, PA, USA). The microstructure was visualized using a scanning electron microscopy (JEOL, JSM-5800 LV, Tokyo, Japan).

The formulation of mixed phosphates rendering shrimps with the lowest translucence and cooking loss but having the highest weight gain and cooking yield, were chosen for further study.

3.3 Combination effect of mixed phosphates and ProfixO on quality improvement of Pacific white shrimp

Shrimps were washed with clean water and were separated into two groups, 1) fresh samples and 2) samples stored in ice for 7 days as previously mentioned in section 3.2.1. Before phosphate treatments, both fresh and iced-stored shrimps were washed with clean water and deheaded; the shells were then peeled off. The shrimps were treated with of 2.5% of NaCl containing ProfixO at different levels (0, 1, 3 and 5%) in combination with or without mixed phosphates (selected from section 3.2). The different treatments are shown as follows:

- Sample with no treatment (No treatment)
- 0% ProfixO + 2.5% NaCl
- 1% ProfixO + 2.5% NaCl
- 3% ProfixO + 2.5% NaCl
- 5% ProfixO + 2.5% NaCl
- 0% ProfixO + 2.5% NaCl + selected phosphates from section 3.2
- 1% ProfixO + 2.5% NaCl + selected phosphates from section 3.2
- 3% ProfixO + 2.5% NaCl + selected phosphates from section 3.2
- 5% ProfixO + 2.5% NaCl + selected phosphates from section 3.2

Both soaking solutions and treated shrimps were subjected to analyses as described in section 3.2. The formulation lowering translucence and cooking loss and giving the higher weight gain and cooking yield were used for further study.

3.4 Effect of ProfixO on quality improvement of Pacific white shrimp as affected by salt concentration and soaking time

Fresh shrimps and shrimps stored in ice for 7 days were washed with clean water and deheaded; the shells were then peeled off. Shrimps were soaked in NaCl solution at different concentrations (0, 1 and 2.5%) in combination with or without ProfixO (selected concentration from section 3.3) for different times (60, 120, 240 and 360 min). The different treatments are shown as follows:

- Sample with no treatment (No treatment)
- 0% NaCl with or without ProfixO; soaking time for 60 min
- 1% NaCl with or without ProfixO; soaking time 60 min
- 2.5% NaCl with or without ProfixO; soaking time 60 min
- 0% NaCl with or without ProfixO; soaking time 120 min

- 1% NaCl with or without ProfixO; soaking time 120 min
- 2.5% NaCl with or without ProfixO; soaking time 120 min
- 0% NaCl with or without ProfixO; soaking time 240 min
- 1% NaCl with or without ProfixO; soaking time 240 min
- 2.5% NaCl with or without ProfixO; soaking time 240 min
- 0% NaCl with or without ProfixO; soaking time 360 min
- 1% NaCl with or without ProfixO; soaking time 360 min
- 2.5% NaCl with or without ProfixO; soaking time 360 min

Both soaking solution and treated shrimps were analyzed as described in section 3.2. Solution providing the lowest translucence and cooking loss but maximized weight gain and cooking yield was chosen for further study. Furthermore, shrimps, both fresh and ice-stored shrimps, before and after treatment were determined for moisture, ash, fat and protein contents according to the method of AOAC (2000). The values were expressed as % (wet weight basis).

3.5 Effect of frozen storage on physical and physiochemical properties of Pacific white shrimp treated with mixed phosphates

Fresh shrimp, shrimp treated with 1% NaCl containing 0.875% SAPP and 2.625% TSPP for 4 h (MP) and shrimp treated with 1% NaCl containing 0.875% SAPP, 2.625% TSPP and 3% ProfixO for 4 h (MP+PO) were packed in polyethylene bag and frozen at -20 °C using an air-blast freezer for 18 h. The frozen samples were stored at -20 °C. Samples were taken at week 0, 1, 2, 4, 6, 8, 10 and 12 for analyses. Prior to analyzes, frozen samples were thawed in cool room (4°C) overnight. Thawed samples were then analyzed as follows:

3.5.1 Determination of drip loss

Drip loss was determined according to the method of Hasegawa (1987). Shrimp muscle (A g) was weighed and placed it on 2 pieces of filter paper. Sample placed in a petri dish with cover was kept 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.

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

3.5.2 Determination of cooking loss

Shrimp (A g) was steamed at 100 °C for 5 min. Cooked shrimp was cooled in water for 1 min and drained at 4 °C for 5 min. The sample was then weighed.

Weight loss was calculated with the following equation:

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

where : A = weight before steaming

B = weight after steaming

3.5.3 Determination of protein solubility

Solubility was determined according to the method of Benjakul and Bauer (2000). To 1 g sample, 20 ml of 0.6 M KCl was added and the mixture was homogenized for 1 min at 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 8500*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.

3.5.4 Determination of ATPase activity assay

ATPase activity of natural actomyosin (NAM) was determined as described by Benjakul *el* 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; 2mM $MgCl_2$, for Mg^{2+}-ATPase; 0.1 mM $CaCl_2$, and 2 mM $MgC1_2$, for Mg^{2+}-Ca^{2+}-ATPase; and 2 mM $MgC1_2$, and 0.5 mM EGTA for Mg^{2+}-EGTA-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. A blank solution was prepared by adding chilled trichloroacetic acid prior to addition of ATP. Ca^{2+}-sensitivity was calculated according to Benjakul *et* al. (1997) as follows:

$$Ca^{2+}\text{-sensitivity} = 1 - (Mg^{2+}\text{-EGTA-ATPase activity} / Mg^{2+}\text{-}Ca^{2+}\text{-ATPase activity}) \times 100$$

3.5.5 Determination of surface hydrophobicity

Surface hydrophobicity was determined as described by Benjakul *et al.* (1997) using 1-anilinonaphthalene-8-sulphonic 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. The initial slope of the plot of fluorescence intensity versus NAM concentration was referred to as SoANS.

3.5.6 Determination of total sulfhydryl content

Total sulfhydryl content was determined using 5, 5′-dithio-bis (2-nitrobenzoic acid) (DTNB) 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. Sulfhydryl content was calculated using the extinction coefficient of 13,900 M^{-1} cm^{-1}.

3.5.7 Determination of disulfide bond content

Disulfide bond in NAM was determined by using 2-nitro-5-thiosulfobenzoate (NTSB) assay according to the method of 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. Disulfide bond content was calculated using the extinction coefficient of 13,900 M^{-1} cm^{-1}.

3.5.8 Determination of α–glucosidase (AG) and β-N-acetyl-glucosaminidase (NAG) activity assay

Shrimp muscle exudates was used as the source of enzymes. Shrimp slices (25 g) were chopped into small pieces, followed by centrifuging at 10,000xg for 60 min at 4 °C using a Sorvall RC 26 Plus refrigerated centrifuge (Sorvall, Norwalk, CT, USA). The exudate formed was collected using a Pasteur pipette and the volume obtained was measured. The exudate was brought to 25 ml with distilled water before enzyme assay. 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 assayed 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 contained 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 shrimp muscle 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 shrimp muscle exudate. The negative control was carried out by adding the stopping reagent prior to the addition of substrate.

NAG activity was determined using ρ-nitrophenyl-N-acetyl- β-D-glucose amide as a substrate. The reaction mixture consisted of 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 shrimp muscle exudate. The reaction was initiated by adding 0.2 ml of ρ-nitrophenyl-N-acetyl- β-D-glucose amide and incubated at 37 °C for 10 min. The reaction was stopped by adding 1 ml of 0.3 M KOH. The blank and negative control was 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 was defined as the activity, which released 1 nmol of ρ-nitrophenol per min.

3.6 Effect of freeze-thawing on physical and physiochemical properties of Pacific white shrimp

Fresh shrimp, shrimp soaked in 1% NaCl containing mixed phosphates in the absence or presence of 3% ProfixO for 4 h were packed in polyethylene bag and frozen at -20°C using an air-blast freezer for 48 h. The frozen shrimps were thawed in a cold room (4 °C)

overnight. The freeze-thawing was performed for 0, 1, 3 and 5 cycles. The shrimps were subjected to analyses, as described in section 3.5.

4. Statistical analysis

All experiments were run in triplicate and CRD (Completely Randomized Design) was used. Analysis of variance (ANOVA) was performed and mean comparisons were carried out by Duncan's multiple range tests (Steel and Torrie, 1980). Analyses were conducted using a SPSS package (SPSS 11.0 for windows, SPSS Inc, Chicago, IL, USA).

CHAPTER 3

RESULTS AND DISCUSSIONS

1. Optimum condition of mixed phosphates for quality improvement of Pacific white shrimp

1.1 Effects of different single and mixed phosphates on the physical properties of Pacific white shrimp

Cooked shrimps treated with different mixed phosphates had varying opacity score (Figure 4). Shrimps soaked in different single phosphates (3.5%) had the similar opacity score ($p>0.05$), but showed the lower opacity score than the shrimp soaked in 2.5% NaCl ($p<0.05$). After being treated with different mixed phosphates in combination with 2.5% NaCl, all samples became more translucent as evidenced by the lower opacity scores, compared with those soaked in 2.5% NaCl. Opacity score of all samples treated with TSPP, STPP or SHMP increased as the levels of SAPP used in combination increased ($p<0.05$). Shrimps treated with 2.625% SAPP and 0.875% STPP or TSPP (0.875-1.75%) in combination with 1.75-2.625% SAPP had similar opacity score to the control shrimp which was soaked in 2.5% NaCl. Treatment with SHMP had no impact on opacity score of cooked shrimp, regardless of the SAPP level used. Furthermore, shrimps treated with 3.5% SAPP showed the opacity score similar to that of shrimp soaked in 2.5% NaCl ($p>0.05$). Therefore, use of SAPP in combination with STPP or TSPP at an appropriate level could reduce translucence of Pacific white shrimp. The higher opacity score in shrimp treated with SAPP or mixed phosphates containing SAPP at high level might be associated with the coagulation of protein at acidic pH caused by SAPP.

Weight gain and moisture content of shrimps soaked in 2.5% NaCl or 2.5% NaCl in combination with different single or mixed phosphates solutions are shown in Figure 5A and 5B, respectively. Samples treated with 3.5% TSPP had the higher weight gain and moisture content than those treated with STPP or SHMP at the same level used ($p<0.05$).

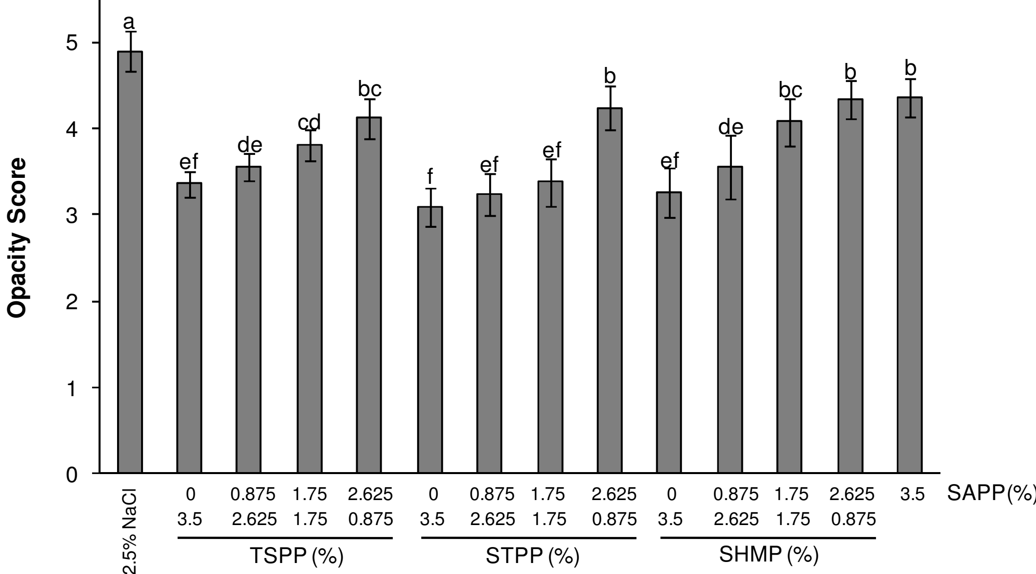


Figure 4. Opacity score of Pacific white shrimp soaked in 2.5% NaCl containing single phosphate and different mixed phosphates. Bars represent the standard deviation from ten determinations. Different letters on the bar indicate the significant differences ($p<0.05$).

(1: Very transparent ; 3: Moderately transparent ; 5: Turbid, dull)

Weight gain and moisture content of shrimps treated with TSPP, STPP or SHMP decreased with increasing concentration of SAPP ($p<0.05$). Use of TSPP or STPP at the 3.5% level or TSPP or STPP at the 2.625% level in combination with 0.875% SAPP showed the higher weight gain than shrimps treated with 2.5% NaCl (control) ($p<0.05$). In the presence of SAPP higher than 0.875%, shrimps treated with TSPP or STPP had the decreases in weight gain ($p<0.05$). Weight gain of shrimp treated with SHMP in the presence or absence of SAPP was lower than that of control ($p<0.05$). The result indicates that SHMP had less or no effect on increasing water holding capacity of shrimp. Phosphates generally enhance water holding capacity ability of shrimp via ionic effect and pH alteration and specific interactions of phosphate anions with divalent cations and myofibrillar proteins (Thorarinsdottir *et al.,* 2004). Pyrophosphate was shown to cause the dissociation of the actomyosin complex, in which the muscle structure become looser and water could penetrate into muscle easily. Increasing weight gain was generally associated with the increase in moisture content. Generally, moisture contents of shrimps treated with TSPP and STPP decreased as the level of SAPP used in combination increased ($p<0.05$). Treatment of shrimps with 2.625% TSPP in combination with 0.875% SAPP yielded the highest moisture content ($p<0.05$). Hamm and Grau (1958) and Hellendoorn (1962) showed an increased water binding capacity of raw muscle after it was treated with the mixture of pyrophosphate and tripolyphosphate solution. The specific activity of both phosphates seemed to be highly dependent on pH. In general, a pH of 6 or higher was required to produce a maximum hydration effect. The result was in accordance with Xiong *et al.* (2000) who reported that pyrophosphate and tripolyphosphate performed similarly in promoting protein extraction, in which orthophosphate (P) had no apparent effect, and hexametaphosphate exhibited an intermediate effect. Pyrophosphate, tripolyphosphate and hexametaphosphate treatments markedly improved protein solubility in 0.3 and 0.4 M NaCl through the release of myosin, but the phosphate effect diminished at concentration greater than 0.6 M NaCl. The enhancing effect of mixed phosphates was in the order: TSPP with SAPP> STPP with SAPP> SHMP with SAPP.

Increased moisture retention ability by the phosphates is achieved through muscle fiber expansion (swelling) caused by electrostatic repulsions, which allows more water to be immobilized in the myofibril lattices (Offer and Trinick, 1983). Increase in water holding capacity of the muscle is attributed to the increased extractability of myofibrillar proteins induced by phosphates (Froning and Sackett, 1985).

Soaking shrimps in 2.5% NaCl containing 3.5% TSPP or 3.5% STPP resulted in the decreased cooking loss and increased cooking yield, compared with the samples without phosphate treatment ($p<0.05$). Nevertheless, efficacy in lowering cooking loss was lower for 3.5% SHMP (Figure 3). Treatment of shrimp with 3.5% SAPP resulted in the similar cooking loss, compared with the control. For white shrimp treated with mixed phosphates, cooking loss of white shrimp increased as the concentration of SAPP increased ($p<0.05$). Increases in cooking loss obtained when the concentration of SAPP increased were in accordance with the decreases in moisture content and weight gain (Figure 5).

From the results, TSPP and STPP were shown to improve weight gain, moisture content and cooking yield more effectively than SHMP and SAPP. Shrimps treated with the mixture of TSPP and SAPP or the mixture of STPP and SAPP at proper concentrations increased weight gain, moisture content and cooking yield as well as reduced the cooking loss. The lower cooking loss and higher cooking yield of the shrimp treated with phosphates indicated that the shrimp muscle had a higher water holding capacity even after cooking. Water molecules might be bound tightly with phosphate or proteins via ionic interaction. Froning and Sackett (1985) reported that use of salt in combination with phosphates had synergistic effect on tumbling turkey breast muscle to reduce expressible moisture and cooking losses. The cooking loss is a combination of liquid and soluble matters lost from the meat during cooking (Heymann *et al*.,1990). The water is probably lost due to heat induced denaturation of proteins during cooking of the meat, which causes less water to be entrapped within the protein structures held by

capillary forces (Aaslyng *et al*.,2003). Aaslyng *et al*. (2003) suggested that a higher cooking loss was found in the sample with the low pH, whereas there was no difference in water holding capacity when pH was medium and high. It could be inferred that shrimps soaked in phosphate solution containing SAPP at a higher level had the lower weight gain, moisture content, cooking yield, but increased cooking loss.

1.2 Effects of different single and mixed phosphates on protein patterns of soaking solution of Pacific white shrimp

Protein patterns of soaking solutions are shown in Figure 7. No MHC band was found in solution containing 3.5% SAPP and solution comprising 0.875% TSPP or 0.875% SHMP in the presence of 2.625% SAPP. It was suggested that SAPP might cause the coagulation instead of solubilization of muscle proteins caused by the pH lowering of this compound. Thus, SAPP might decrease the dissociation of myofibrillar protein and fiber swelling, as well as lower penetration of water or salt and phosphate solution into the shrimp muscle. MHC band intensity was greatest in solution containing 1.75% TSPP and 1.75% SAPP as well as solution with 2.625% STPP and 0.875% SAPP.

The result indicated that SHMP showed the lowest protein extraction ability for both MHC and proteins with MW of 65 and 67 kDa. SHMP might not be able to modify the charges of proteins and could not penetrate into muscle as effectively as other phosphates due to the larger molecular weight. As a result, the proteins were not leached out to a great extent into soaking solution. This was concomitant with lowest efficacy of SHMP for weight gain improvement and the reduction of cooking loss. On the other hand, TSPP and STPP in the presence of SAPP at appropriate level could enhance the extraction of proteins, particulary MHC.

(A)

(B)

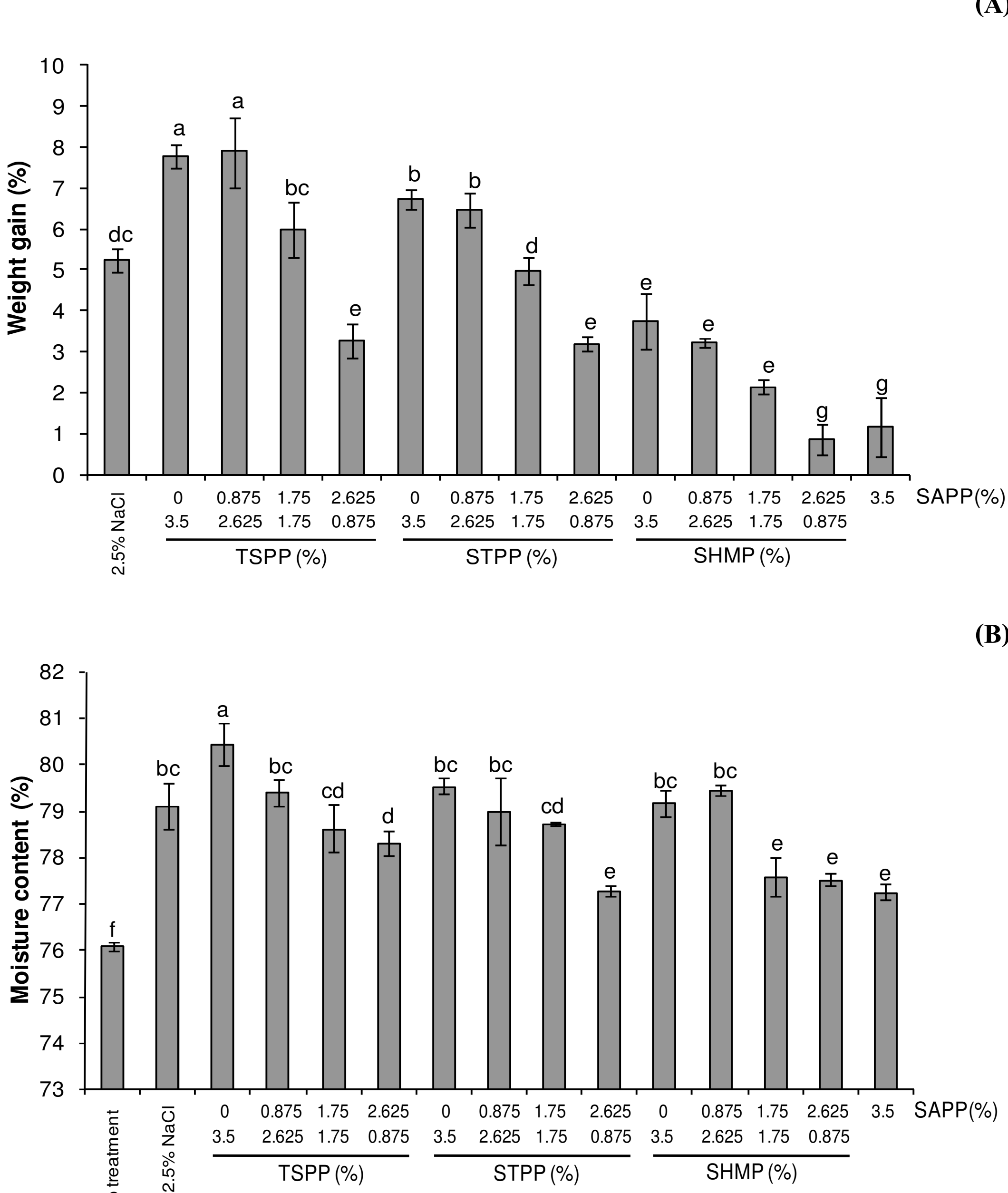


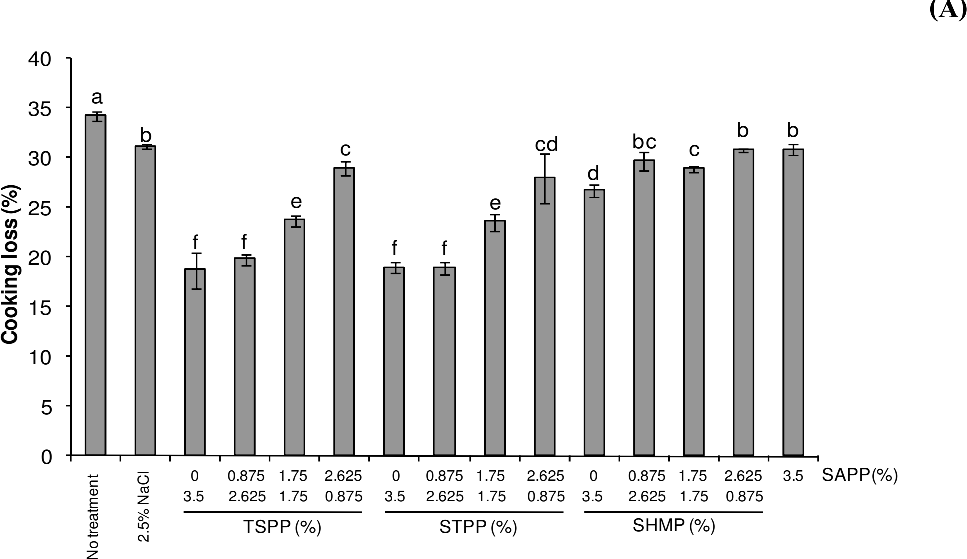
Figure 5. Weight gain (A) and moisture content (B) of Pacific white shrimp soaked in 2.5% NaCl containing single phosphate and different mixed phosphates. Bars represent the standard deviation from ten determinations. Different letters on the bar indicate the significant differences ($p<0.05$).

However, when SAPP at high concentration was used in combination with TSPP or STPP, the lower ability in protein extraction or swelling was obtained as indicated by the disappearance of MHC band. TSPP and STPP might facilitate protein extraction and dissociation myofibrillar protein due to the ionic effect and pH alteration (Bendall, 1954). The increase in MHC band intensity in soaking solutions was in agreement with the higher cooking yields and lower cooking loss found in the shrimp treated with 3.5% TSPP or 3.5% STPP or TSPP in combination with the lower concentration of SAPP (0.875%).

1.3 Effects of different single and mixed phosphates on the chemical composition of Pacific white shrimp

Phosphate contents of shrimp meat treated with different soaking solutions expressed as P_2O_5 are shown in Figure 8A. After treatment with different single and mixed phosphates, shrimp meat had the varying phosphate contents, depending on formulation. All treated shrimps contained higher phosphate content than shrimps with no treatment and shrimp treated in 2.5% NaCl solution ($p < 0.05$). Highest phosphate contents were found in shrimp treated with 2.5% NaCl containing 3.5% STPP or 3.5% SAPP or 1.75% STPP + 1.75% SAPP or 2.625% STPP + 0.875% SAPP ($p<0.05$). The results suggested that different phosphates with different size, shape or form of phosphates might penetrate into the shrimp muscle differently. Among all treatments, shrimps treated with 2.5% NaCl containing 3.5% SHMP or SHMP in combination with SAPP contained the lower phosphate content, compared with those treated with TSPP and STPP, regardless of SAPP combination. Larger size of SHMP might be associated with the lower penetration into muscle. However, phosphate contents in shrimps with all treatments were less than the standard value (5000 ppm) (Official Journal of the European Communities, 1995).

(A)



(B)

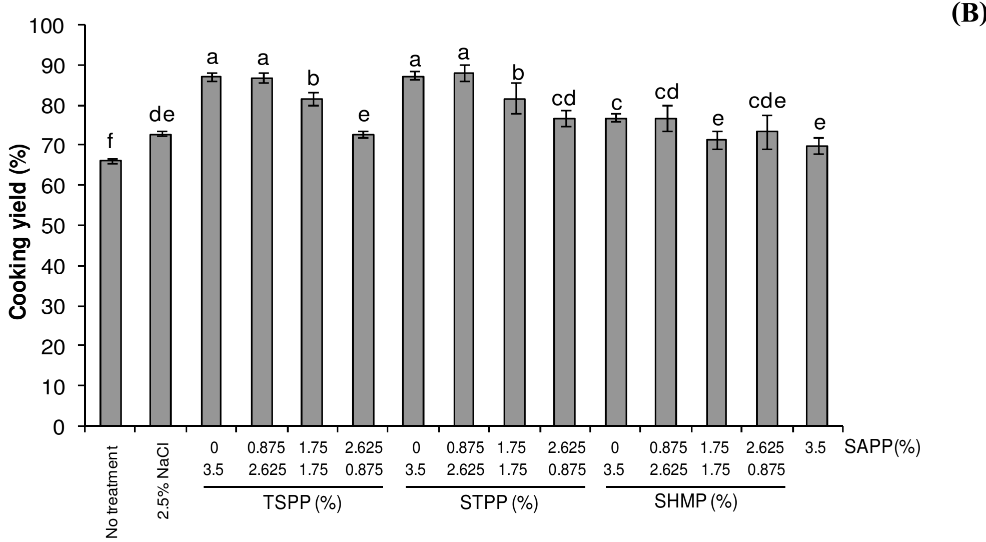


Figure 6. Cooking loss (A) and cooking yield (B) of Pacific white shrimp soaked in 2.5% NaCl containing single phosphate and different mixed phosphates. Bars represent the standard deviation from ten determinations. Different letters on the bar indicate the significant differences ($p<0.05$).

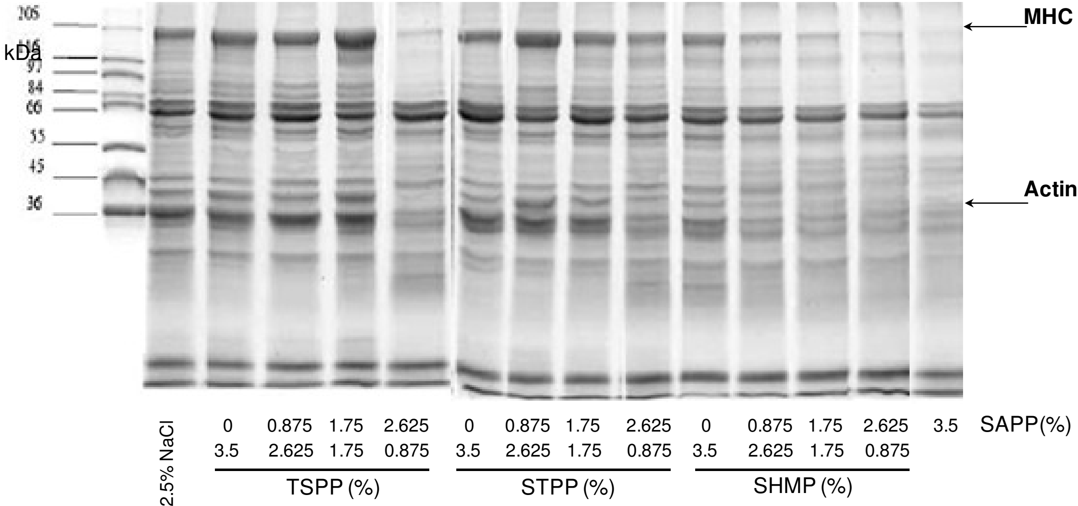
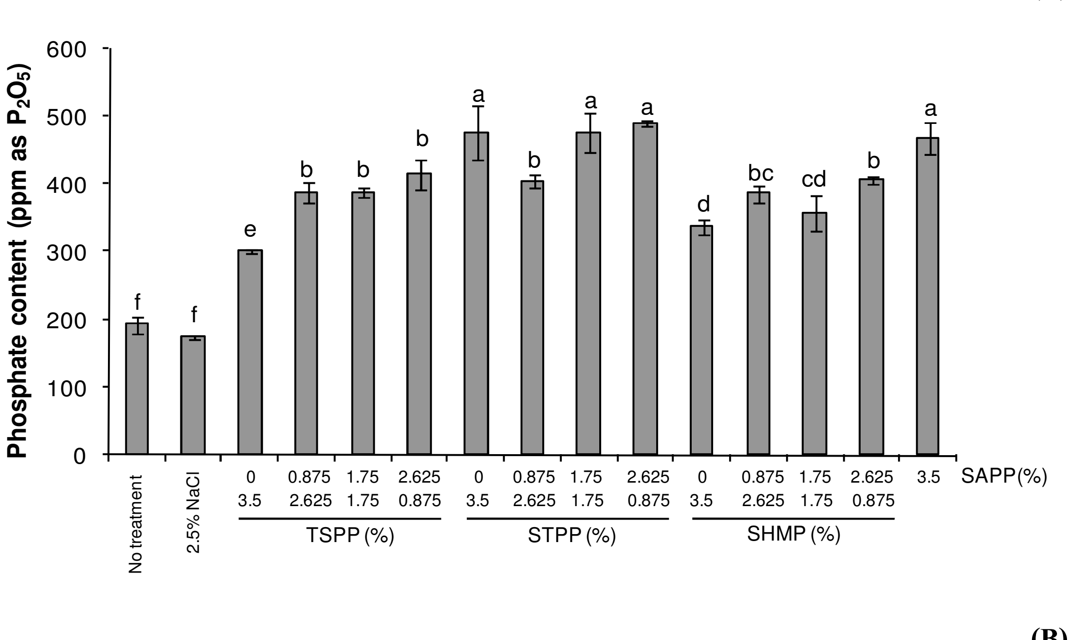


Figure 7. Protein patterns of 2.5% NaCl containing different single and mixed phosphates after soaking with Pacific white shrimp.

MHC: myosin heavy chain.

After soaking in 2.5% NaCl containing different mixed phosphates, higher salt content was noticeable in shrimp ($p<0.05$) (Figure 8B), compared with shrimp soaked in 2.5% NaCl. Nevertheless, no differences in salt content in all shrimps treated with single and mixed phosphates used were observed. Salt generally shows synergistic effect on shrimp quality improvement of phosphates. 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, 1972), and the sodium ions form an ion cloud around the filaments (Offer and Knight, 1983).

(A)



(B)

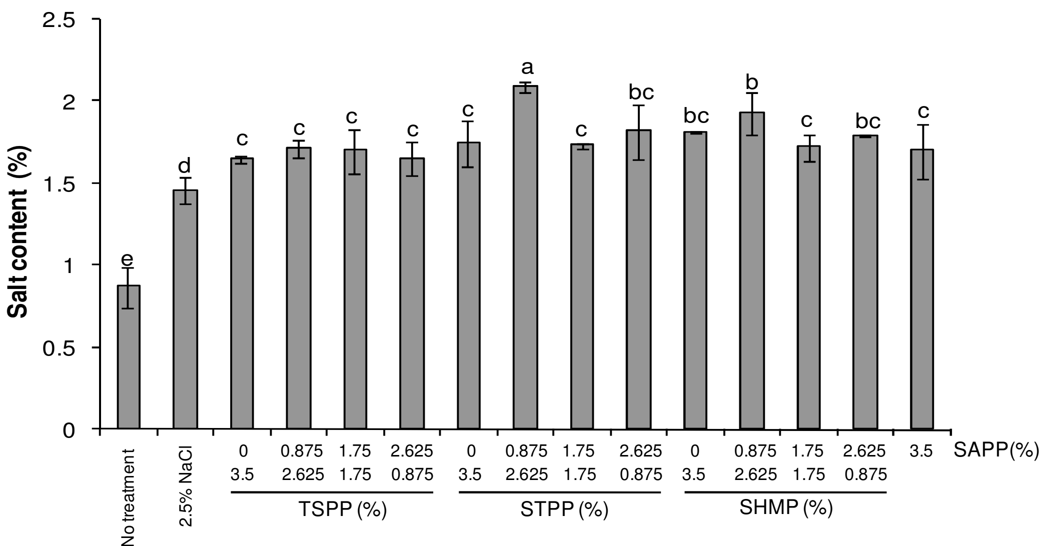


Figure 8. Phosphate (A) and salt contents (B) of Pacific white shrimp soaked in 2.5% NaCl containing single phosphate and different mixed phosphates. Bars represent the standard deviation from ten determinations. Different letters on the bar indicate the significant differences ($p<0.05$).

Therefore, 2.5% NaCl containing 3.5% TSPP or 2.625% TSPP + 0.875% SAPP or 3.5% STPP or 2.625% STPP + 0.875% SAPP were used to soak Pacific white shrimps to reduce translucence of cooked shrimps, while still maintained weight gain, cooking loss and cooking yield. Those formulations were used for further study.

2. Effect of freshness and deveining on the properties of Pacific white shrimp

2.1 Effects of phosphates and mixed phosphates on the physical properties of fresh and ice-stored shrimps with and without deveining

Cooked fresh and ice-stored shrimps without phosphate treatment had the similar opacity score, regardless of deveining. After being treated with 2.5% NaCl, all samples became more translucent as evidenced by the lower values (Figure 9). Translucence markedly increased with the treatment of phosphates as indicated by the decrease in opacity score. In general, cooked ice-stored shrimps treated with all phosphates or mixed phosphates were more translucent than fresh shrimps soaked in the same mixed phosphate solution. Deveining had no impact on translucence, regardless of shrimp freshness. For the fresh shrimp, the solution containing both TSPP and SAPP yielded the less translucent shrimp, when compared with the solution without SAPP ($p<0.05$). Nevertheless, the translucence of fresh shrimp treated with STPP in combination with SAPP was similar to that of sample treated with STPP ($p>0.05$). From the result, the use of SAPP in combination with TSPP or STPP had no impact on the translucence of ice-stored shrimps ($p>0.05$). When SAPP was used in combination with TSPP and STPP, the pH of solution (7.0-7.2) was lower than that of TSPP (10.0) and STPP (8.6). This might lower the repulsion force associated with very alkaline pH of phosphate solution. As a consequence, solubilization of muscle proteins could be decreased. It was postulated that the solubilized muscle proteins might undergo aggregation to form the ordered network, which most likely yielded the gel-like structure

with greater translucence. To reduce the translucence caused by phosphate treatments, SAPP might be used in combination with other phosphates.

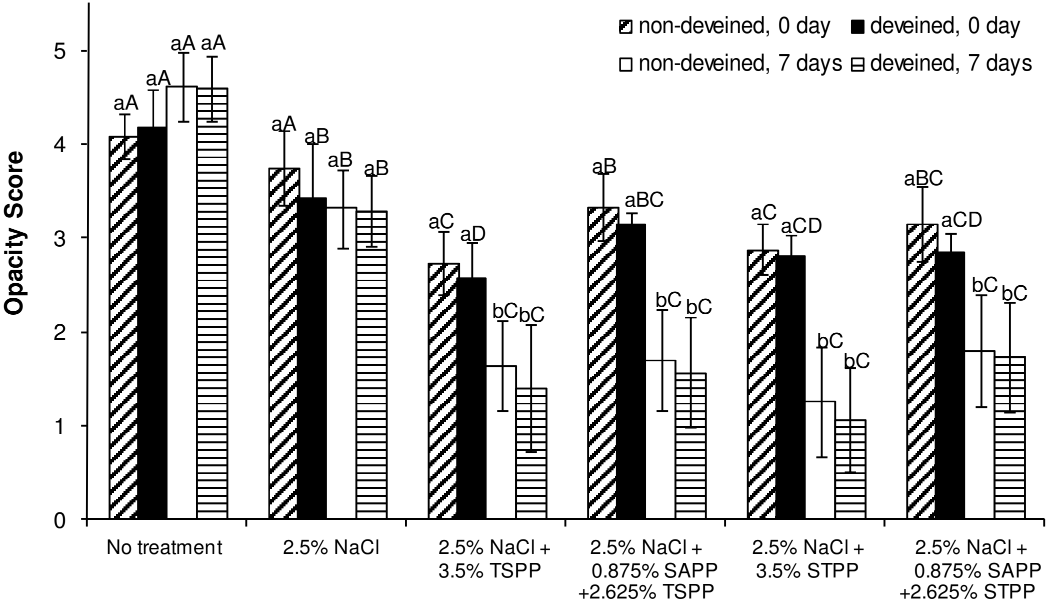


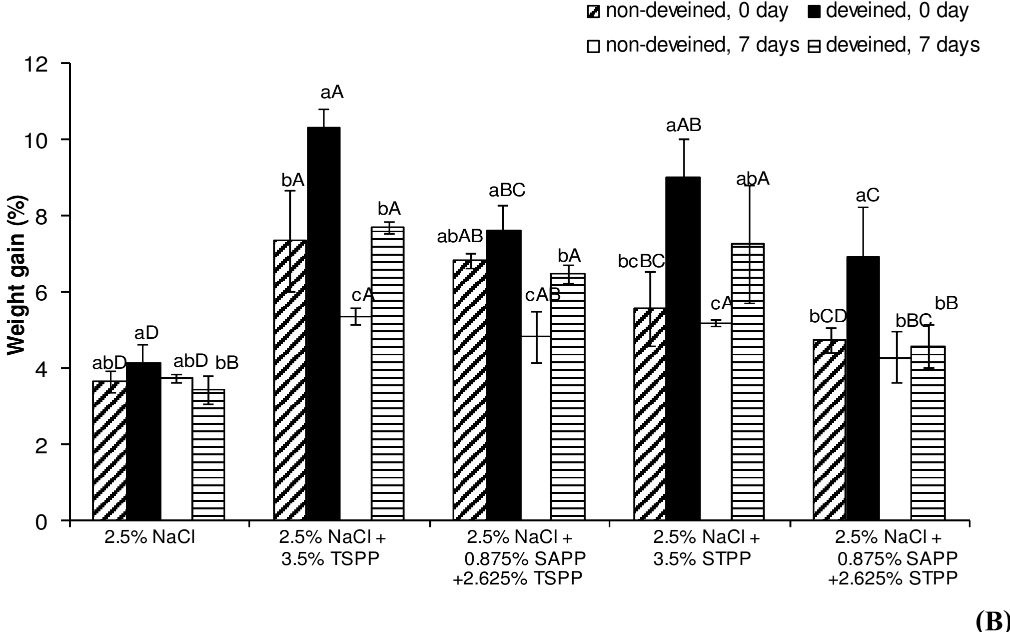
Figure 9. Opacity score of fresh and ice-stored Pacific white shrimp without and with deveining after soaking in 2.5% NaCl in the absence and the presence of different phosphates. The different lower case letters within the same treatment indicate significant difference ($p < 0.05$). The different upper case letters within the same sample indicate significant difference ($p < 0.05$). Bars represent the standard deviation from ten determinations.

(1: Very transparent ; 3: Moderately transparent ; 5: Turbid, dull)

Weight gains of shrimps soaked in 2.5% NaCl or 2.5% NaCl in combination with different phosphates are shown in Figure 10. Treatment with TSPP or STPP yielded the fresh deveined shrimps with the highest weight gain ($p<0.05$). The higher weight gain was observed in whole fresh shrimp treated with TSPP, compared with those treated with STPP ($p<0.05$). For ice-stored shrimp, no differences in weight gain were found between those treated with TSPP and STPP, irrespective of deveining ($p>0.05$). In the presence of SAPP, the lower weight gain was generally observed. Xiong *et al*. (2000) reported that pyrophosphate and tripolyphosphate were able to promote protein extraction, leading to the improved hydration properties of chicken muscle. Overall, phosphates influenced the ultrastructure of myofibrils and extraction of their constituents in the order: PP ~ TPP > HMP > P ~ nonphosphate control (Xiong *et al*., 2000).

From the result, the deveined shrimp possessed the greater weight gain, regardless of freshness or phosphates used. Deveining might allow phosphates to penetrate or adsorb into shrimp muscle easily. This could enhance water absorption in the shrimp muscle. After phosphate treatments, weight gain was lower in shrimps stored in ice for 7 days, compared with fresh shrimps (Figure 10A). This indicated that freshness, which related with muscle integrity, was another factor governing the efficacy of phosphate in increasing the yield of treated shrimps. Moisture content of ice-stored shrimp was higher than that of fresh shrimp ($p<0.05$) (Figure 10B). During storage in ice, some ice was molten and shrimps were immersed. As a consequence, the water was uptaken into shrimp muscle to a high extent as indicated by the increased moisture content. Deveining mostly had no impact on moisture content of shrimp meat ($p>0.05$). However, ice-stored shrimp with deveining had higher moisture content after treatment with 3.5%TSPP and 2.5% NaCl, compared with non-deveined shrimps ($p<0.05$). Deveining also resulted in an increase in moisture content of fresh shrimp treated with 3.5% STPP together with 2.5% NaCl ($p<0.05$). Deveined shrimp had a larger surface area than did non-deveined shrimp. As a result, phosphates as well as water were able to penetrate much easily into shrimp muscle.

(A)



(B)

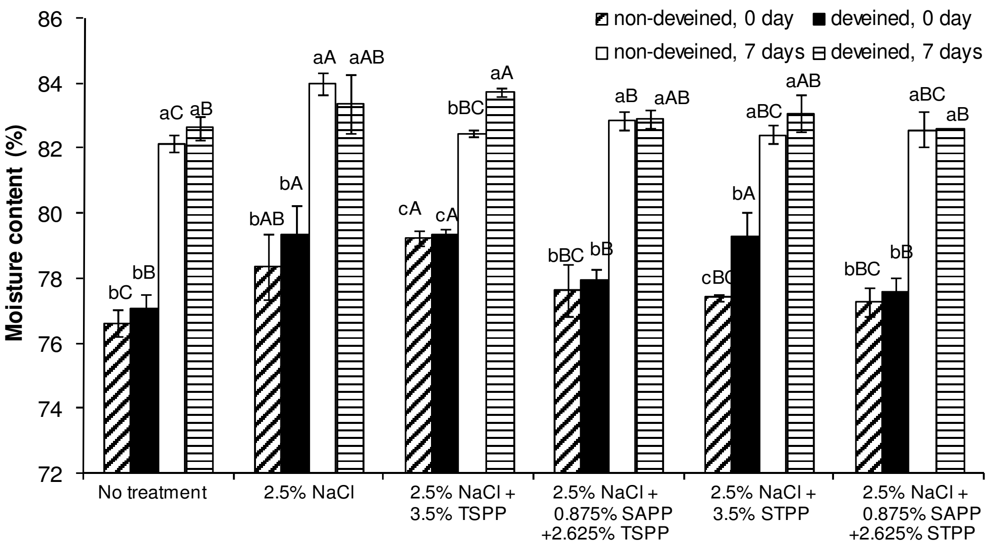


Figure 10. Weight gain (A) and moisture content (B) of fresh and ice-stored Pacific white shrimp without and with deveining after soaking in 2.5% NaCl in the absence and the presence of different phosphates. The different lower case letters within the same treatment indicate significant difference ($p < 0.05$). The different upper case letters within the same sample indicate significant difference ($p < 0.05$). Bars represent the standard deviation from five determinations.

Soaking shrimps, either fresh or ice-stored, in phosphate solutions resulted in the increased cooking yield and lowered cooking loss, compared with the samples without phosphate treatment ($p<0.05$) (Figure 11). In general, lower cooking yield was obtained in ice-stored shrimp, compared with fresh shrimp ($p<0.05$). For the sample treated with 2.5% NaCl, the increase in cooking yield was noticeable with ice-stored shrimp ($p<0.05$), but it had no effect on the cooking yield of the fresh shrimp ($p>0.05$). Slight increase in cooking yield was found in fresh deveined sample treated with 2.5% NaCl, compared with non-deviened fresh shrimp ($p<0.05$). With the treatment of phosphate in combination with 2.5% NaCl, cooking yield was much increased for both deveined and non-deveined shrimps, particularly for ice-stored shrimp, compared with samples treated with NaCl alone or without any treatment ($p<0.05$). Deveining process slightly increased the cooking yield. This might be associated with the higher phosphates adsorbed in the shrimp muscle. From the result, fresh shrimp without and with 2.5% NaCl treatment, had the lower cooking loss than ice-stored shrimps ($p<0.05$). However, cooking loss was lowered after the treatment of 2.5% NaCl together with phosphates. Deveining generally resulted in the lower cooking loss of shrimps. Deveining could allow the phosphate or NaCl to penetrate into the shrimp muscle more easily. From the result, the use of SAPP in combination of STPP or TSPP rendered the shrimps with slightly lower cooking yield but higher cooking loss, compared with the use of STPP or TSPP alone. The results were coincidental with the lower weight gain when SAPP was used in combination with STPP or TSPP. From the result, shrimps soaked in the mixture of TSPP and SAPP had higher cooking yield but lower cooking loss than those soaked in the mixture of STPP and SAPP solution ($p<0.05$) (Figure 11A and 11B). The lower cooking loss and higher cooking yield of the shrimp treated with phosphates indicated that the shrimp muscle had a higher water holding capacity even after cooking. Water molecules might be bound tightly with phosphate or proteins via ionic interaction. Froning and Sackett (1985) reported that use of salt in combination with phosphates had synergistic effect on tumbling turkey breast muscle to

reduce cooking loss and expressible moisture. Xiong and Kupski (1999) found that salt would produce a synergism with phosphate to dissociate actomyosin in chicken filets.

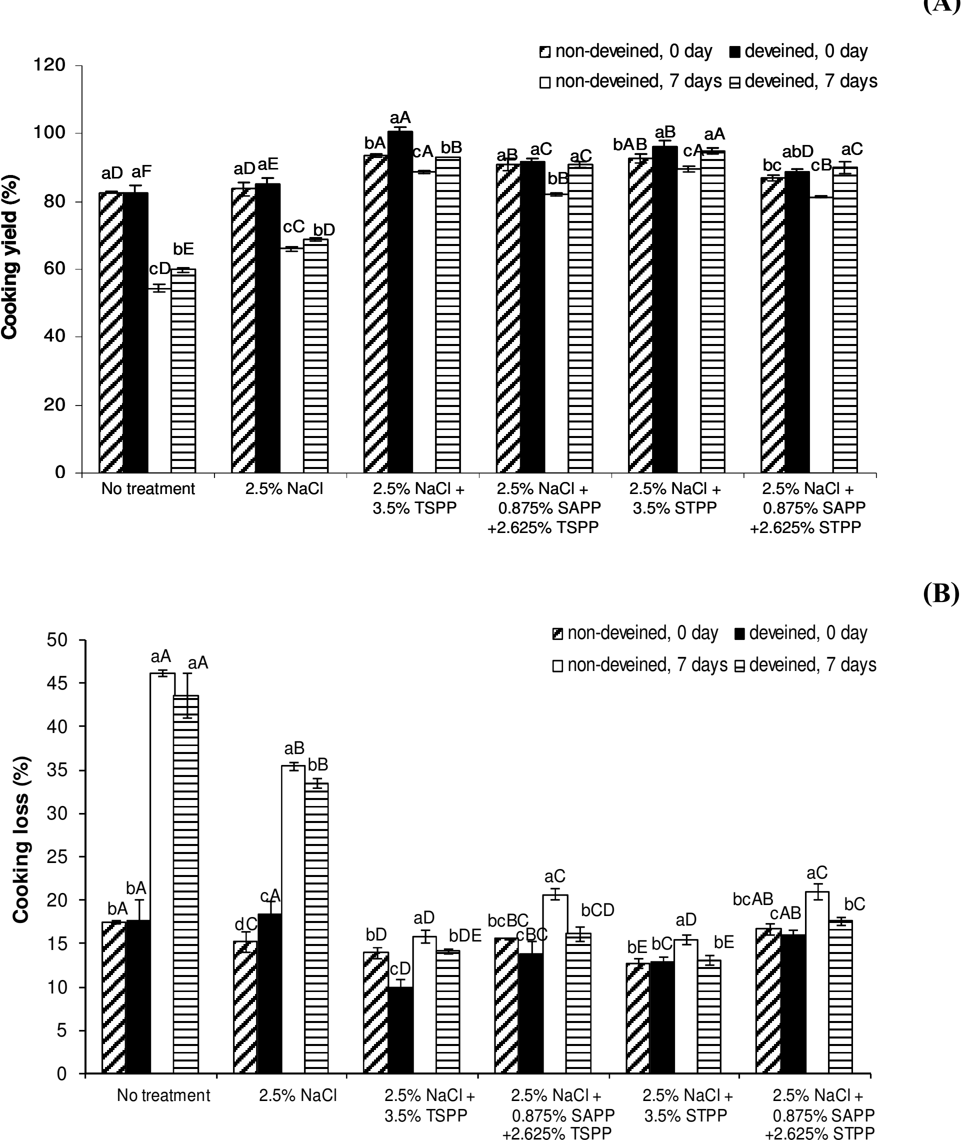


Figure 11. Cooking yield (A) and cooking loss (B) of fresh and ice-stored Pacific white shrimp without and with deveining after soaking in 2.5% NaCl in the absence and the presence of different phosphates. The different lower case letters within the same treatment indicate significant difference ($p < 0.05$). The different upper case letters within the same sample indicate significant difference ($p < 0.05$). Bars represent the standard deviation from five determinations.

2.2 Effects of phosphates and mixed phosphates on the chemical composition of fresh and ice-stored shrimps with and without deveining

Phosphate content (dry basis) of fresh shrimp and ice-stored shrimp without and with deveining after treatment with different solutions are shown in Figure 12. For the samples treated with phosphates, it was noted that the higher phosphate content was found in ice-stored shrimp, particularly deveined samples ($p<0.05$). During iced storage, endogenous and bacterial enzymes might involve in the degradation of shrimp tissues and structure (Martinez *et al*., 2001). Endogenous proteolytic enzymes including calpain and lysosomal proteases are certainly related to changes in myofibrillar proteins or collagen, which cause the loosening of the myofibrillar structure (Etherington, 1984; Peterson *et al*., 1988). Phosphate content of deveined ice-stored shrimp was higher than non-deveined counterpart ($p<0.05$), except for sample treated with TSPP together with SAPP, in which deveining had no effect on phosphate content. For fresh shrimp, deveined shrimps treated with TSPP or STPP in combination with SAPP showed the higher phosphate content than non-deveined counterpart ($p<0.05$). Deveined shrimps tended to have a slightly higher cooking yield, but lower cooking loss (Figure 3). This might be associated with the greater penetration of phosphate into the meat. However, phosphate contents in shrimps were less than the standard value (5,000 ppm) (Official Journal of the European Communities, 1995).

After soaking in 2.5% NaCl, either without or with phosphates, the increase in salt content was noticeable in shrimps, regardless of freshness and deveining ($p<0.05$) (Figure 13). In general, NaCl content was higher in ice-stored shrimp, compared with fresh counterpart. The impact of deveining on the salt content varied from samples to samples. NaCl has been used in combination with phosphate in order to obtain the synergistic effect on quality improvement. Shrimps, both fresh and ice stored, soaked in 2.5% NaCl containing mixed phosphates (0.875% SAPP and 2.625% TSPP) had a decrease in cooking loss, an increase in

cooking yield and lower translucence. Nevertheless, efficacy of mixed phosphates in quality improvement was lower for ice-stored shrimp.

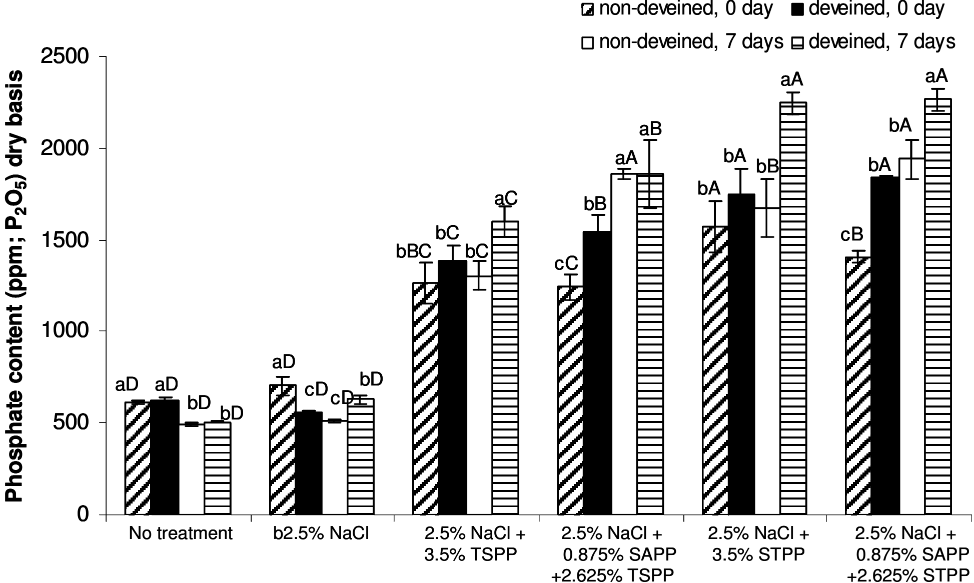


Figure 12. Phosphate content of fresh and ice-stored Pacific white shrimp without and with deveining after soaking in 2.5% NaCl in the absence and the presence of different phosphates. The different lower case letters within the same treatment indicate significant difference ($p < 0.05$). The different upper case letters within the same sample indicate significant difference ($p < 0.05$). Bars represent the standard deviation from five determinations.

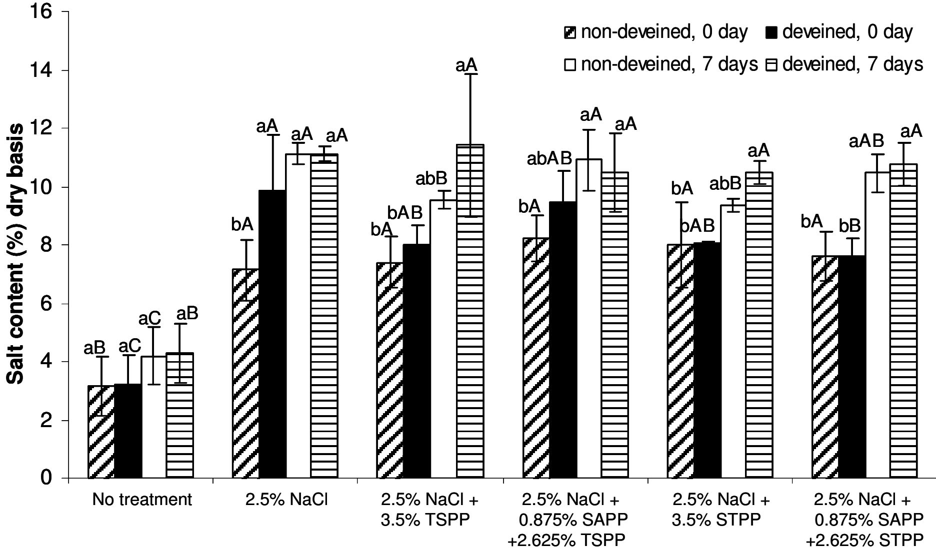


Figure 13. Salt content of fresh and ice-stored Pacific white shrimp without and with deveining after soaking in 2.5% NaCl in the absence and the presence of different phosphates. The different lower case letters within the same treatment indicate significant difference ($p < 0.05$). The different upper case letters within the same sample indicate significant difference ($p < 0.05$). Bars represent the standard deviation from five determinations.

2.3 Effect of phosphates and mixed phosphates on protein patterns of soaking solution of fresh and ice-stored shrimps with and without deveining

Protein patterns of different solutions obtained after soaking with shrimps for 2 h are shown in Figure 14. For fresh shrimp, all solutions contained protein bands with MW of 88 and 77 kDa with similar band intensity. Actin was also found in all solutions. However, band intensity of myosin heavy chain (MHC) in all phosphate solutions was greater than that found in 2.5% NaCl. For deveined fresh shrimps, a slightly larger band of MHC was noticeable. The result suggested that more proteins, particularly MHC, were solubilized and leached out for deveined samples. The increases in MHC band intensity correlated with the increases in weight gain and

cooking yield (Figure 14). For ice-stored shrimps, the protein patterns of different soaking solution were observed, compared with those found in fresh shrimps. Much lower band intensity of MHC and proteins with MW of 88 and 77 kDa was found in all solutions. The decrease in band intensity of those proteins might be associated with degradation of protein during iced storage.

Nevertheless, no changes in actin were observed. Actin was reported as the most resistant to degradation caused by either endogenous or microbial proteinase (Benjakul *et al*., 1997). Proteolytic degradation of other cytosolic and cytoskeletal proteins present in the muscle caused by microbial growth and structural disintegration also occurred during ice storage of fish (*Priacanthus tayenus* and *P. macracanthus*) (Benjakul *et al*., 2002). No marked differences in protein patterns were obtained between all solutions used for soaking the deveined and non-deveined samples. From the result, MHC band intensity was greater in the solutions containing TSPP or STPP, regardless of SAPP addition. TSPP and STPP might facilitate protein extraction and dissociation myofibrillar protein due to the ionic effect and pH alteration. Use of higher NaCl concentrations (0.1-1.0 M) increased the extraction of myofibrillar proteins from beef tissue and the inclusion of 10 mM TSPP to NaCl solutions enhanced the extraction of myofibrillar proteins (Xiong and Kupski, 1999). Increased myofibrillar proteins extraction was associated with increased beef myofibril swelling and increased beef muscle WHC (Paterson *et al*., 1988).

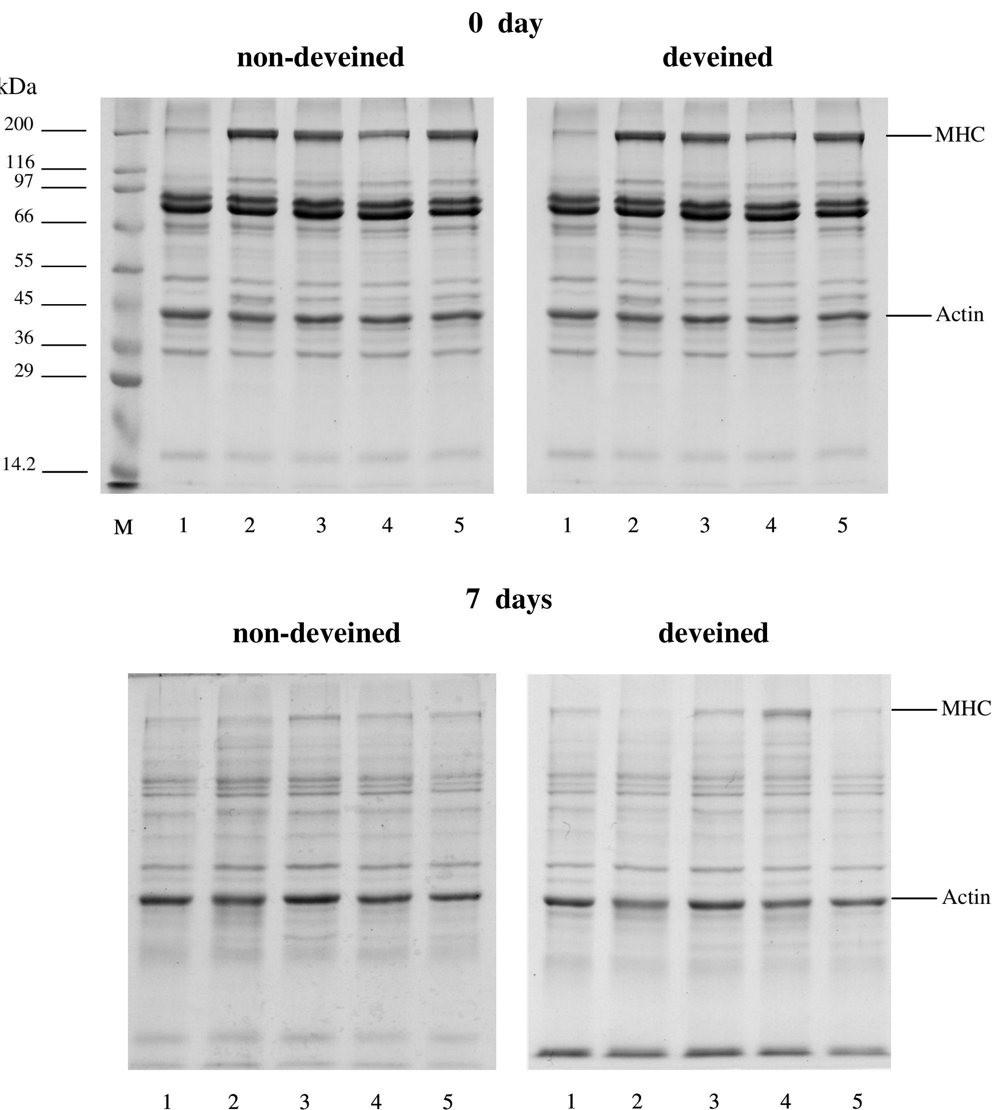


Figure 14. Protein patterns of various soaking solutions of fresh and ice-stored Pacific white shrimp without and with deveining. M: Standard marker; Lanes 1: 2.5% NaCl; Lane 2: 3.5% TSPP + 2.5% NaCl; Lane 3: 0.875% SAPP + 2.625% TSPP +2.5% NaCl; Lane 4: 3.5% STPP + 2.5% NaCl; Lane 5: 0.875% SAPP + 2.625% STPP +2.5% NaCl; MHC: myosin heavy chain

2.4 Effects of mixed phosphates on microstructure of fresh and ice-stored shrimps with and without deveining

Microstructures of Pacific white shrimp muscle treated with and without mixed phosphates are illustrated in Figures 15 and 16, respectively. Fresh shrimp without phosphate treatment had the well organized structure of the myofibrils. After 7 days of ice storage, the myofibrils were less attached with the loss of Z-disks. After being soaked in mixed phosphates, myofibrils became larger in size. However, myofibrils were less attached as indicated by gaping occurred. The shrinkage of sarcomere was obvious in cooked shrimps. It was suggested that heating process caused the shrinkage of muscle of shrimp. Heat processing enhanced the firmness and degree of shrinkage of *Penaeus japonicus* (Mizuta *et al*., 1999). After cooking, both fresh shrimp and ice-stored shrimp had more compact myofibrils arrangement with the shrinkage of sarcomere, compared with raw samples. For fresh shrimps, similar myofibril arrangement was observed between samples with and without phosphate treatment. Interestingly, disintegration of M-line was clearly observed in ice-stored shrimps treated with phosphates after cooking. During ice-chilling of whole freshwater prawn, the muscle fibers at the anterior-most sections were degraded gradually (Nip and Moy, 1988). Degradation of shrimp tissue starting from the perimysium, endomysium, the Z-line and the H-zones with concurrent degradation of the connective fibers and the sarcoplasm due to action of hepatopancreatic enzymes (Baranowski *et al*., 1984; Nip *et al*., 1985). From the result, the post mortem degradation might facilitate the penetration of phosphates into the muscle, in which proteins at 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).

For the transverse sections (Figure 16), similar microstructures of white shrimp were found. Dense structure was noticeable in fresh shrimps, while sponge-like structure was found in ice-stored shrimp. Cooked meats had more compact myofibril arrangements, compared with raw samples. Treated shrimps with mixed phosphate had loosen structure than those without phosphate treatment. For phosphate treated shrimp, the less attachment was found for fresh shrimp. Conversely, increased compact structure was observed in ice-stored shrimp after phosphate treatment. 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.

2.5 Effects of mixed phosphates on thermal property of fresh and ice-stored shrimp with and without deveining

Thermal transition of shrimps muscle proteins of shrimps with and without phosphate treatment using DSC are shown in Table 2. 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. 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. Whole cod muscle showed two maximal transitions on DSC thermogram with T_{max} at about 45 and 75 °C (Hasting *et al*., 1985) and whole muscle of fresh hake also showed two endothermic transitions with T_{max} values of 46 and 75 °C (Beas *et al*., 1990). The differences in T_{max} among the fish species seems to be correlated with the habitat temperature of the fish (Akahane *et al*., 1985; Hasting *et al*., 1985; Davies *et al*., 1988).

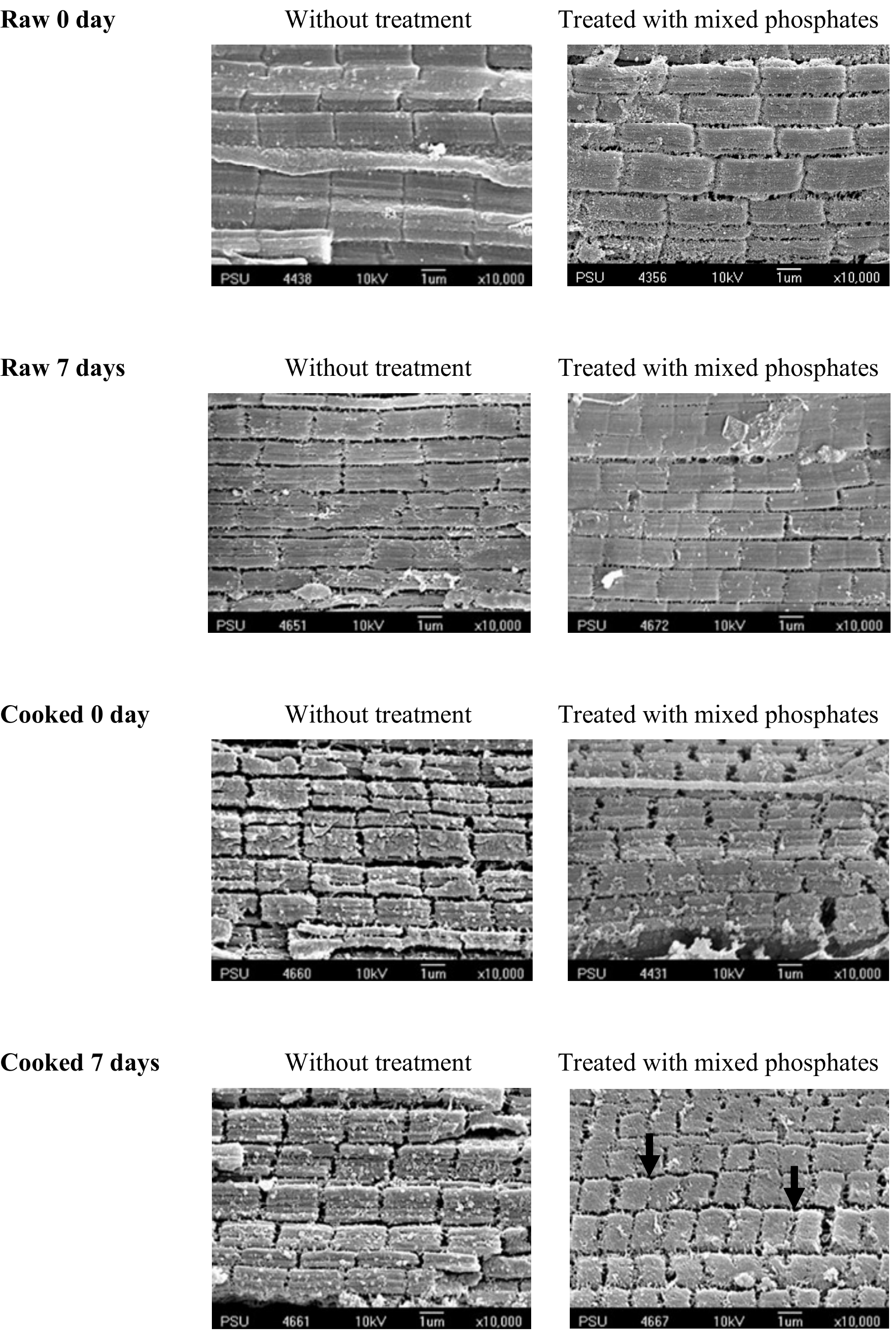


Figure 15. SEM micrographs of longitudinal section of raw and cooked fresh and ice-stored Pacific white shrimp meats without and with phosphate treatment.
Arrow indicates M-line

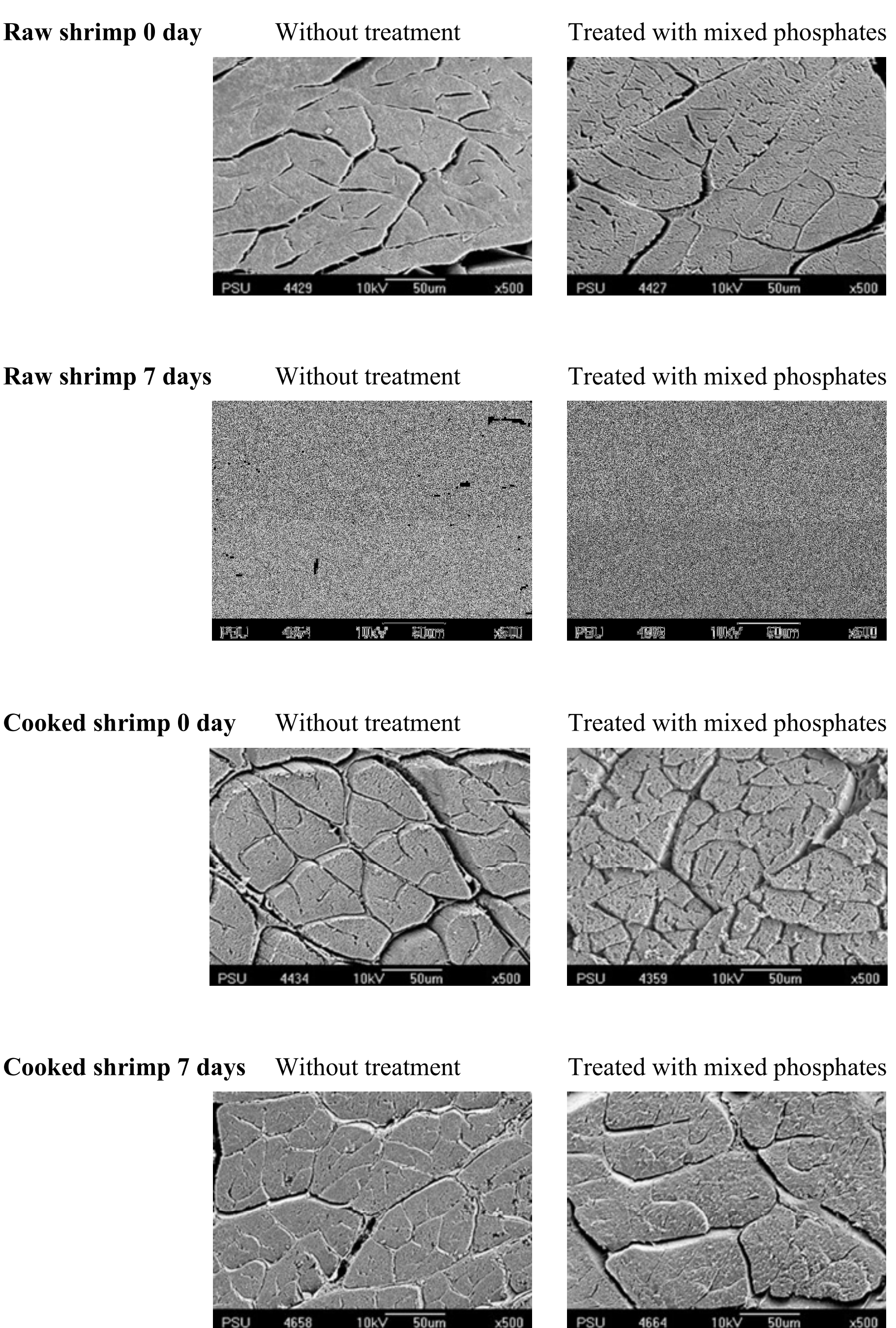


Figure 16. SEM micrographs of transverse section of raw and cooked fresh and ice-stored Pacific white shrimp meats without and with phosphate treatment.

After 7 days of storage in ice, T_{max} of both peaks shifted to the lower values. Additionally, ΔH was also decreased. This suggested that both myosin and actin underwent denaturation to some extent during the iced storage. After being treated with mixed phosphate, T_{max} of both peaks (myosin and actin) of fresh shrimp shifted to the lower temperature. A lower enthalpy was observed for actin peaks after phosphate treatment. Torigai and Konno (1996) reported a promotive effect of pyrophosphate on the dissociation of actin from myosin. As a result, free actin was easily denatured by salt. Wu *et al.* (1985) found that only the first peak shifted from 43 to 38 °C after addition of 1 % salt and the the second peak and third peak shifted to lower temperatures after addition of 2-3% salt. The addition of salt (3%) in tilapia or hake surimi caused a decrease in denaturation enthalpy and the shift of T_{max} to the lower temperature (Beas *et al*.,1991). The lower enthalpy of mince plus salt was probably due to the sensitizing effect of the Cl anion upon myofibrillar protein denaturation (Beas *et al*., 1991). Robe and Xiong (1992) found that addition of 0.25 % tripolyphosphate reduced or eliminated the first transition (T_{max}= 47 °C) and enhanced the second transition (T_{max} = 57 °C) of salt soluble proteins. The disappearance or reduction of thermal transition caused by the addition of tripolyphosphate probably resulted from a decrease thermal stability in the protein domains (Kijowski and Mast, 1988). Tripolyphosphate seemed to be more effective than NaCl in modifying the electrostatic forces in proteins, thereby altering salt soluble proteins aggregation pattern (Robe and Xiong, 1992). Therefore, salt and pyrophosphate decreased the heat stability of Pacific white shrimp muscle proteins, leading to the denaturation at lower temperature with less energy input. From this result, it can be inferred that the destabilizing effect of salt and pyrophosphate on the shrimp proteins affect the properties of shrimp proteins after heating or cooking. Similar results were obtained in ice-stored shrimp, in which T_{max} shifted to the lower after phosphate treatment. However, the increase in ΔH was noticeable in ice-stored shrimp treated with phosphates. Endogenous

proteolytic enzymes including calpain and lysosomal proteases might partially degrade MHC, leading to the case of denaturation, particularly after phosphate treatment.

Table 6. T_{max} and enthalpy of muscle protein of fresh and ice-stored Pacific white shrimps with and without phosphate treatment

Samples	T_{max}I (°C)*	ΔH (J/g)	T_{max} II (° C)	ΔH (J/g)
Fresh shrimp (0 day)				
Shrimp	50.1*±0.2^{a}**	1.65±0.13^{a}	71.3±0.5^{a}	0.25±0.05^{b}
Shrimp treated with mixed phosphates	49.1±0.1^{b}	1.52±0.17^{ab}	69.2±0.9^{b}	0.12±0.03^{c}
Storage shrimp (7 day)				
Shrimp	49.8±0.3^{b}	0.76±0.05^{c}	68.8±0.3^{b}	0.26±0.04^{b}
Shrimp treated with mixed phosphates	48.2±1.0^{c}	1.20±0.05^{b}	67.0±0.8^{c}	0.41±0.01^{a}

* Mean±SD from triplicate determinations.

** The different superscripts in the same column indicate the significant differences ($p<0.05$).

3. Combination effect of mixed phosphates and ProfixO on quality improvement of Pacific white shrimp

3.1 Effect of ProfixO at different concentrations in combination with and without mixed phosphates on the physical properties of fresh and ice-stored shrimps

The effect of ProfixO at different concentrations in combination without and with mixed phosphates (0.875% SAPP + 2.625% TSPP) on the opacity score of cooked fresh and ice-stored Pacific white shrimp is shown in Figure 17. After being treated with 2.5% NaCl, all samples, both fresh and ice-stored, became more translucent as evidenced by the lower value,

compared with the control (no treatment) ($p<0.05$). Opacity score markedly decreased with increasing ProfixO concentrations for both fresh and ice-stored samples. However, the decreasing rate were more pronounced for shrimps treated with ProfixO in the absence of mixed phosphates compared, with those treated with only ProfixO. In general, no differences in opacity score were observed in shrimps treated with different levels of ProfixO in the presence of mixed phosphates ($p>0.05$). At the same level of ProfixO either in combination with or without mixed phosphates, opacity score of cooked shrimp was not different between fresh and ice-stored shrimps ($p>0.05$) except shrimps soaked in 2.5% NaCl (0% ProfixO without mixed phosphate) in which ice-stored shrimp had higher opacity score than fresh shrimp. When ProfixO was used in combination with mixed phosphates, the pHs of solution (7.2-7.8) was lower than those of mixed phosphates (9.5-9.6). At 5% ProfixO, ice-stored shrimp had the higher opacity score when soaked in combination with mixed phosphates, compared with those treated with ProfixO without mixed phosphates incorporated. Therefore, the use of mixed phosphates in combination with ProfixO tended to increase the opacity score to some extent, in comparison with the used of only ProfixO.

Weight gain and moisture content of shrimp soaked in 2.5% NaCl or 2.5% NaCl in combination with ProfixO at different levels in the absence or in the presence of mixed phosphates (0.875% SAPP + 2.625% TSPP) are shown in Figure 18A and 18B, respectively. Without mixed phosphates, weight gain of fresh and ice-stored shrimp increased with increasing ProfixO levels, up to 3% ($p<0.05$). The decrease in weight gain was observed when ProfixO at a level of 5% was used ($p<0.05$). In the presence of mixed phosphates, the highest weight gain was obtained when the shrimps, both fresh and ice-store, were treated with 1% ProfixO. The higher weight gain was obtained in fresh shrimp treated with ProfixO in combination with/or without mixed phosphates, compared with that of ice-stored counterpart ($p<0.05$). This indicated that freshness, which related with muscle integrity, was another factor governing the efficacy of ProfixO in increasing the weight gain of shrimps. In the presence of mixed phosphates, similar

weight gain was noticeable when ProfixO at 1 and 3% were used. As ProfixO was greater than 3%, weight gain of both shrimps decreased (p<0.05).

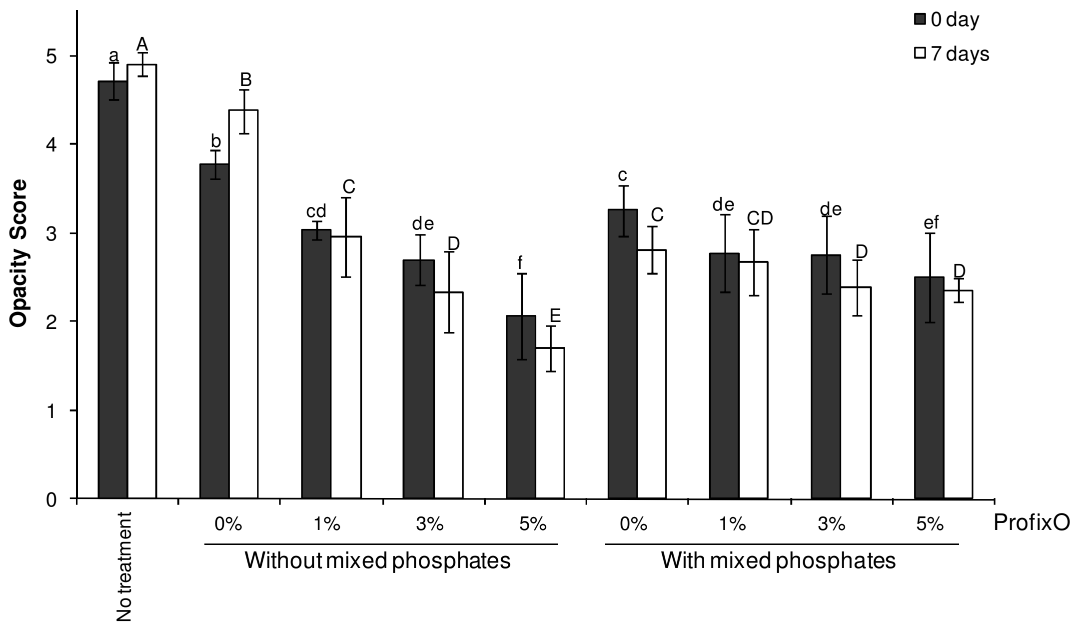
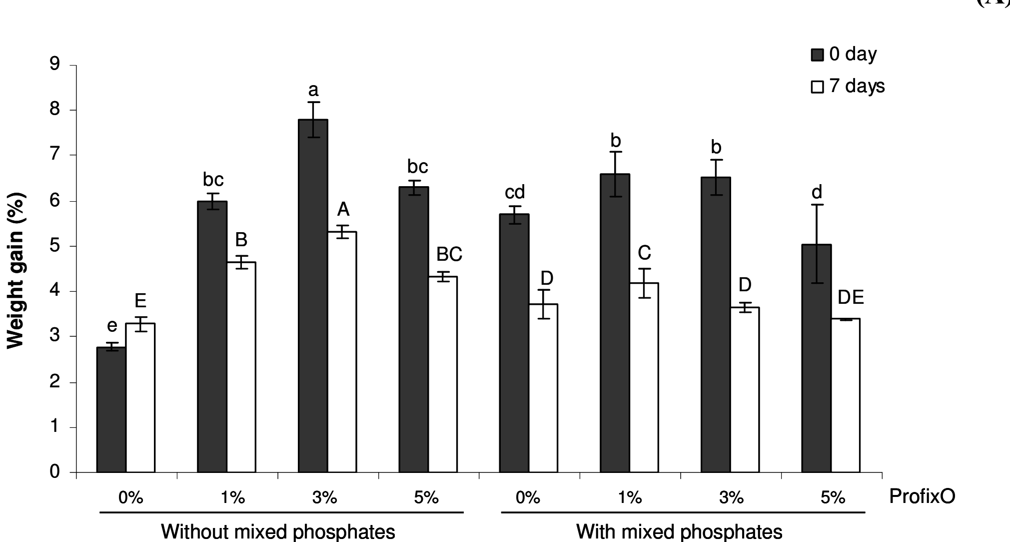


Figure 17. Opacity score of fresh and ice-stored Pacific white shrimps soaked in 2.5% NaCl containing ProfixO at different concentrations in the absence and the presence of mixed phosphates (0.875% SAPP + 2.625% TSPP). Different lower case letters or upper case letters within the same storage time indicate significant differences (p<0.05). Bars represent the standard deviation from ten determinations.

(1: Very translucent ; 3: Moderately translucent ; 5: Turbid, dull)

Moisture content of fresh and ice-stored shrimps treated with ProfixO at the different levels either in combination with or without mixed phosphates is depicted in Figure 14B. For all treatments, higher moisture content was found in samples stored in ice, compared with fresh counterpart ($p<0.05$). During storage in ice, some ice was molten and shrimps were immersed. As a consequence, the water was uptaken into shrimp muscle to a high extent as indicated by the increased moisture content ($p<0.05$). Moisture content of ice-stored shrimp treated with ProfixO in the absence of mixed phosphates was higher than that of samples treated with ProfixO in the presence of mixed phosphates at all level of ProfixO used ($p<0.05$). The result suggest that the pH of ProfixO without mixed phosphates solution were higher (pH 9.51-9.57) than those of ProfixO with mixed phosphate solution (pH 7.19-7.76). This result confirmed that the higher pH of soaking solution results in improved water holding/binding of muscle via solubilization of myofibrillar proteins (Park, 2000). For fresh shrimp, moisture content of shrimp increased when treated with ProfixO at levels of 1 and 3%, whereas the decrease in moisture content was observed when ProfixO at 5% was used. Nevertheless, no differences in moisture content of shrimp were observed when shrimps were treated with 1% ProfixO in combination with mixed phosphates. On the other hand, decreases in moisture contents were noticeable when shrimp were treated with ProfixO at concentration greater than 1% (3% and 5%). Without ProfixO, weight gain and moisture content of shrimp soaked in 2.5% NaCl in combination with mixed phosphates were higher than those of shrimps soaked in 2.5% NaCl without mixed phosphates. When considering the effect of shrimp quality on weight gain, it was noted that ice-stored shrimp showed the lower weight gain compared with fresh counterpart, regardless of solutions used.

(A)



(B)

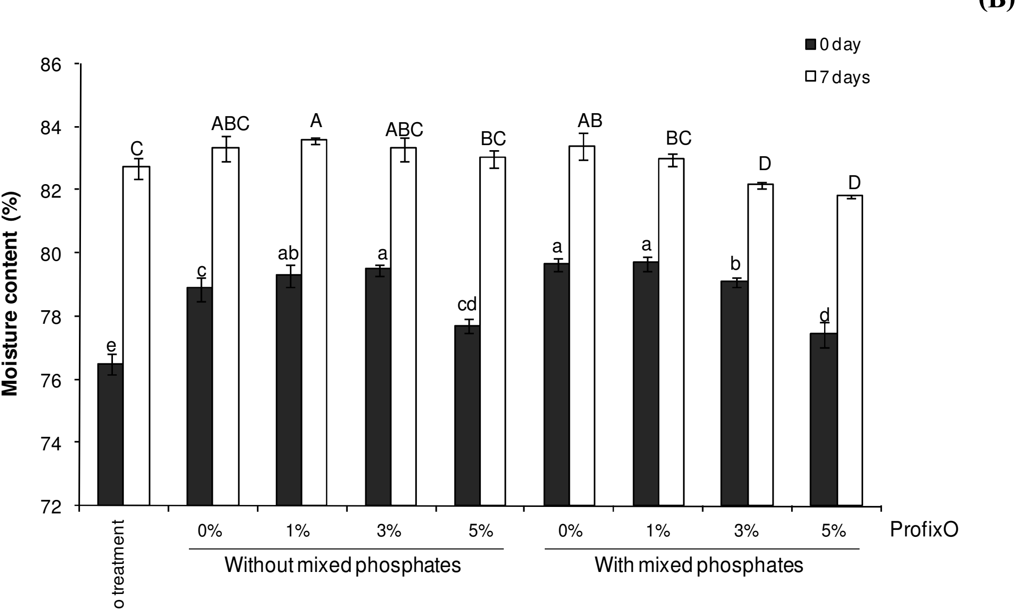


Figure 18. Weight gain (A) and moisture content (B) of fresh and ice-stored Pacific white shrimps soaked in 2.5% NaCl containing ProfixO at different concentrations in the absence and the presence of mixed phosphates (0.875% SAPP + 2.625% TSPP). Different lower case letters or upper case letters within the same storage time indicate significant differences ($p<0.05$). Bars represent the standard deviation from five determinations.

Cooking yield of fresh and ice-stored shrimps soaked in ProfixO at various concentrations in combination with and without mixed phosphates is shown in Figure 19A. After cooking, the yield of fresh shrimps increased continuously as ProfixO increased up to 3%, regardless of the combination with mixed phosphates. However, the increases in cooking yield were observed in ice-stored shrimp as ProfixO concentrations increased up to 5%. For all treatments, except the samples treated with 2.5% NaCl (0% ProfixO without mixed phosphates), fresh shrimps showed the higher weight gain than did ice-stored counterpart.

Cooking loss of shrimp treated with ProfixO at different levels in combination with and without mixed phosphates is shown in Figure 19B. Shrimps soaked in ProfixO at all levels in combination with mixed phosphate had lower cooking loss than those soaked in ProfixO without mixed phosphates for both fresh and ice-stored shrimps ($p<0.05$). The result suggested the synergistic effect between phosphates and ProfixO in holding the water of shrimp muscle during cooking. In general, the lowered cooking loss was coincidental with the higher cooking yield. When comparing cooking loss between fresh and ice-stored shrimps subjected to different treatments, it was found that a greater cooking loss was noticeable in ice-stored shrimp than fresh counterpart. Disintegration of muscle fiber arrangement as the shrimps were stored for an extended time might contribute to the lower ability of muscle to bind or hold the water. Therefore phosphate was an important aid to reduce cooking loss and increase cooking yield of cooked shrimps, particularly when used in combination with ProfixO at an appropriate level. Additionally, freshness was another factor governing the efficiency of phosphate and/or ProfixO in increasing the yield of shrimps.

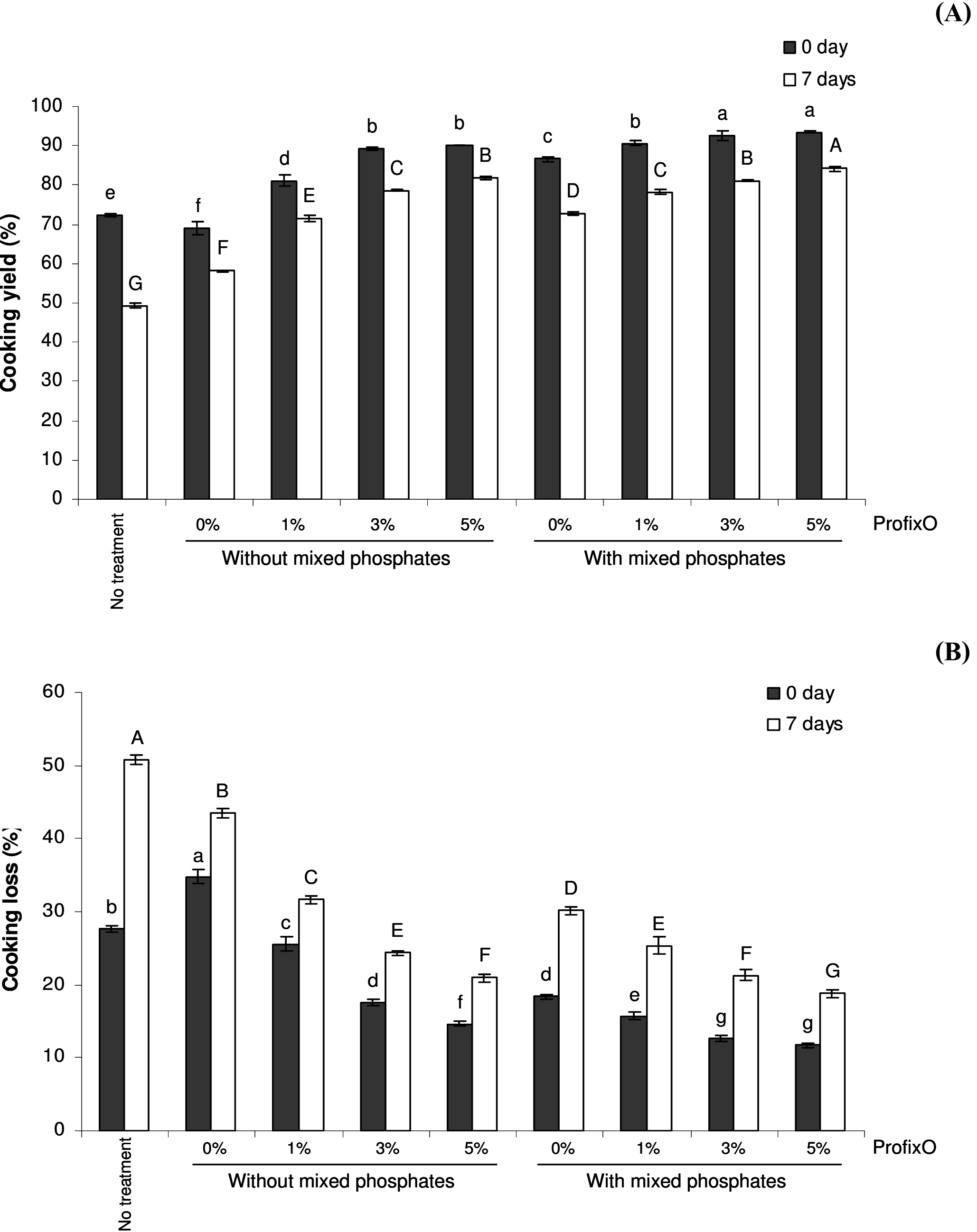


Figure 19. Cooking yield (A) and cooking loss (B) of fresh and ice-stored Pacific white shrimps soaked in 2.5% NaCl containing ProfixO at different concentrations in the absence and the presence of mixed phosphates (0.875% SAPP + 2.625% TSPP). Different lower case letters or upper case letters within the same storage time indicate significant differences ($p<0.05$). Bars represent the standard deviation from five determinations.

3.2 Effect of ProfixO at different concentrations in combination with and without mixed phosphates on the chemical composition of fresh and ice-stored shrimps

Phosphate and salt contents of fresh and ice-stored shrimp treated with 2.5% NaCl or 2.5% NaCl in combination with ProfixO at different levels in the absence or in the presence of mixed phosphates (0.875% SAPP + 2.625% TSPP) are shown in Figure 20A and 20B, respectively. Phosphate content was higher in shrimp soaked in the solution containing mixed phosphates, regardless of ProfixO addition. For fresh shrimps, no differences in phosphate content was found in samples treated with ProfixO at the levels of 1-5% ($p>0.05$). For ice-stored shrimp, the decrease in phosphate content was noticeable with increasing ProfixO level ($p<0.05$). This might be because ProfixO at higher level could migrate into the muscle in ice-stored shrimp a greater extent, in comparison with fresh counterpart. As a consequence, less phosphate could penetrate into muscle occupied with ProfixO. Phosphate contents in shrimp were less than the standard value (5000 ppm) (Official Journal of the European Communities, 1995).

NaCl content tended to increase when ProfixO was used in combination with 2.5% NaCl. For ice-stored shrimp, salt content was increased with increasing ProfixO ($p<0.05$). However, no differences in salt content were found in the same sample when ProfixO was used in combination with mixed phosphates. ProfixO had no impact on salt content of fresh shrimp, irrespective of levels used. It was noted that fresh shrimps contained lower phosphate and NaCl content than did ice-stored shrimp in all treatments studied. Higher destruction of muscle in shrimp kept for extended time most likely facilitated the penetration or migration of NaCl into shrimp muscle.

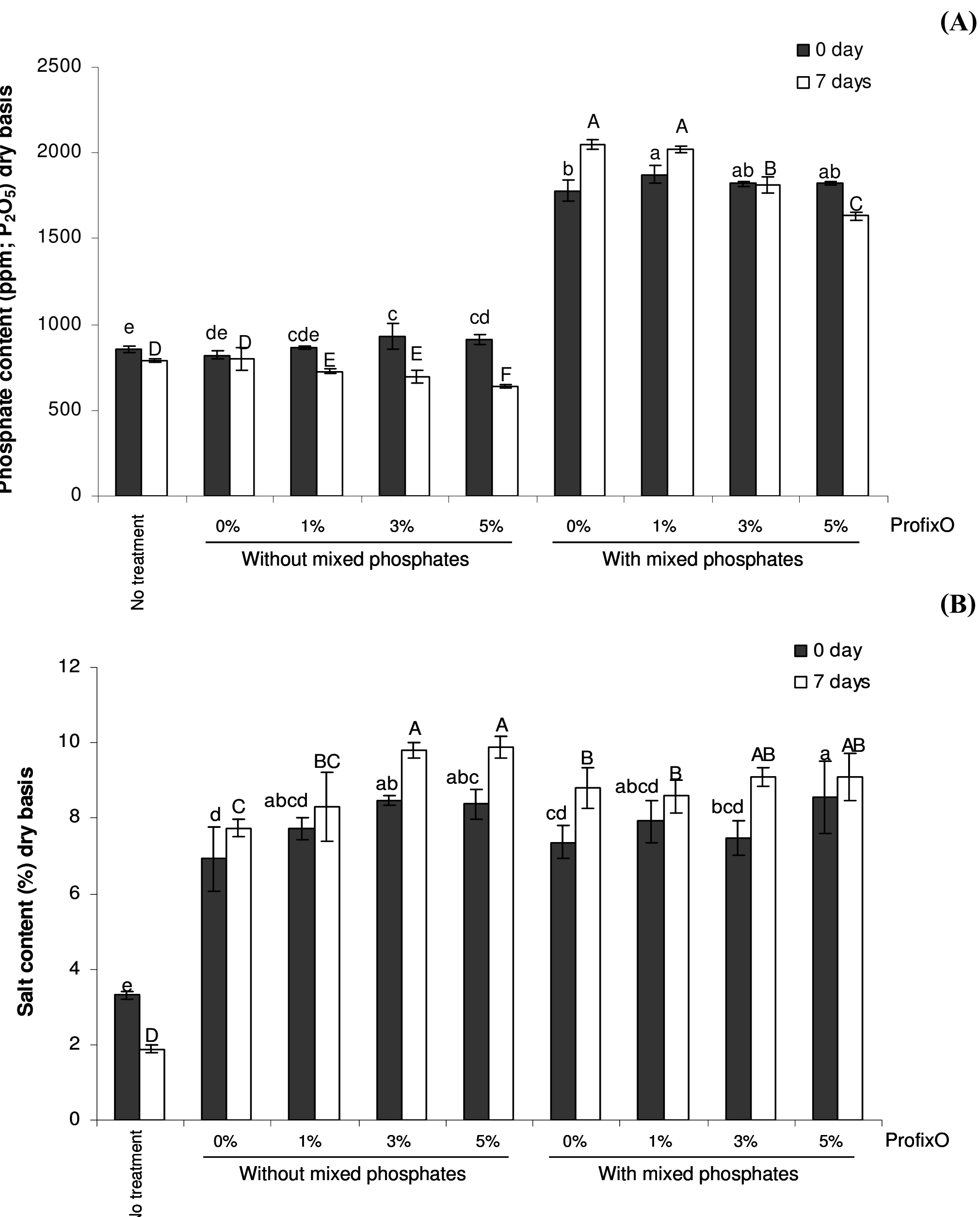


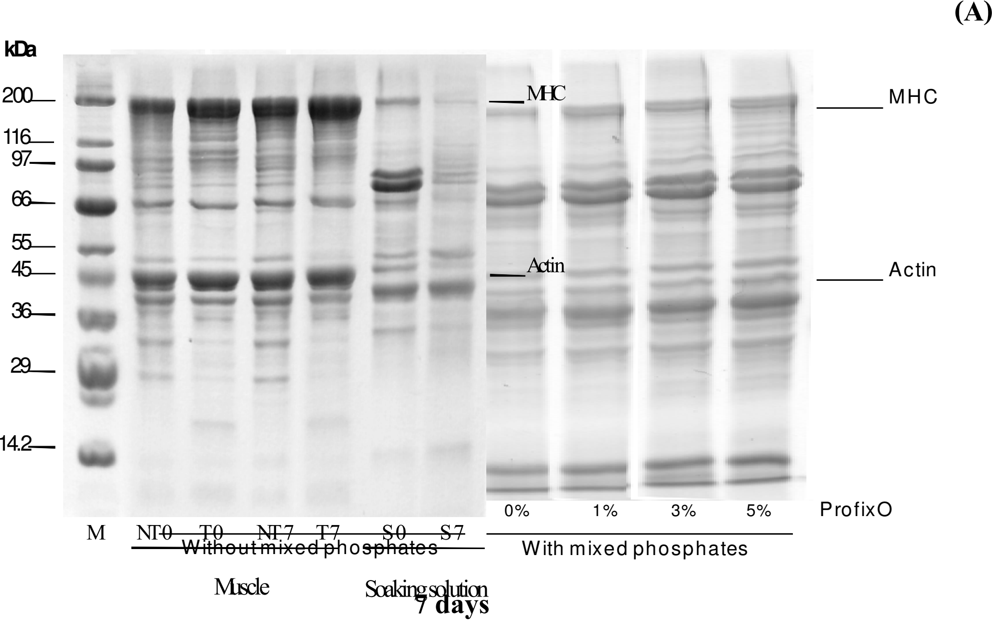
Figure 20. Phosphate (A) and salt (B) contents of fresh and ice-stored Pacific white shrimps soaked in 2.5% NaCl containing ProfixO at different concentrations in the absence and the presence of mixed phosphates (0.875% SAPP + 2.625% TSPP). Different lower case letters or upper case letters within the same storage time indicate significant differences ($p<0.05$). Bars represent the standard deviation from five determinations.

3.3 Effect of ProfixO at different concentrations in combination with and without mixed phosphates on protein patterns of soaking solutions of fresh and ice-stored shrimps

Protein patterns of soaking solutions (2.5% NaCl) containing ProfixO at different levels with and without mixed phosphates of fresh and ice-stored shrimps are shown in Figure 21. For fresh shrimp, all solution contained protein bands with MW of 88 and 77 kDa as the major proteins, followed by actin. Similar protein patterns were observed among all samples. MHC band intensity tended to increase with increasing ProfixO levels, regardless of mixed phosphates. However, no changes in actin were observed in all soaking solutions. The result suggested that proteins, especially MHC, were more solubilized as the higher ProfixO levels were used. The increases in band intensity of MHC were in agreement with the increases in weight gain and moisture content when the level of ProfixO did not exceed 3%. Repulsive forces between the proteins were more intense and protein-protein interaction became weaker, resulting in the leaching of proteins, particularly MHC into the soaking solution (Offer and Trinick, 1983; Honikel, 1989). For ice-stored shrimps, much lower band intensity of MHC and proteins with MW of 88 and 77 kDa was found in all solutions, when compared with those found in fresh counterpart. Both protein bands with MW of about 88 and 77 kDa diminished during storage. The decreases in band intensity of those proteins were possibly caused by proteolytic degradation mediated by both endogenous and microbial proteases. According to Martinez *et al* (2001), degradation of muscle protein occurred during iced storage. However, there were no appreciable changes in SDS-PAGE patterns of water and salt soluble proteins from *Penaeus japonicus* and *Penaeus monodon* after 4 days of iced storage. In contrast, salt soluble proteins from *Pandalus borealis* including myosin, proteins with MW of about 67 and 50 kDa disappeared after 1 day of storage (Martinez *et al*, 2001).

0 day

(A)



7 days

(B)

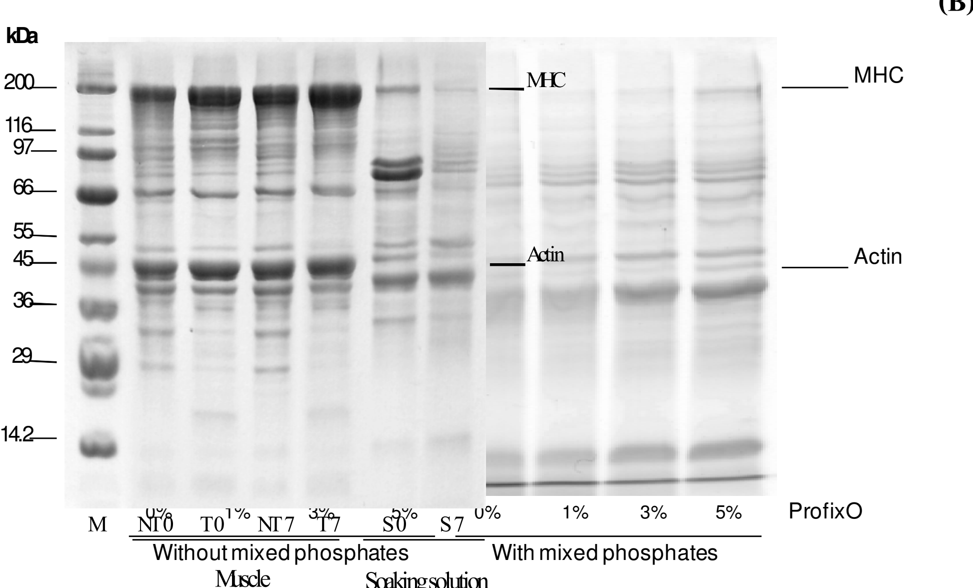


Figure 21. Protein patterns of 2.5% NaCl containing ProfixO at different concentrations in the absence and the presence of mixed phosphates (0.875% SAPP + 2.625% TSPP) after soaking with fresh and ice-stored Pacific white shrimps.

MHC: myosin heavy chain

At the same level of ProfixO, MHC band intensity was greater in the solution without mixed phosphates. The lower pH of soaking solution added with mixed phosphates most likely lowered the repulsive force between proteins, resulting in the lower extraction of proteins. This was evidenced by the lower band intensity of proteins appearing on the SDS-PAGE. ProfixO might facilitate protein extraction and dissociation myofibrillar protein due to the ionic effect and pH alteration.

Therefore, 2.5% NaCl containing 3% ProfixO and mixed phosphates were chosen for soaking Pacific white shrimp to reduce translucence of cooked shrimps, while still maintained weight gain and cooking yield. This formulation was used for further study.

3.4 Effect of ProfixO and mixed phosphates on thermal properties of fresh and ice-stored shrimps

Thermal transition of fresh and ice-stored Pacific white shrimp muscle proteins soaked in 2.5% NaCl containing 3% ProfixO and mixed phosphates (0.875% SAPP + 2.625% TSPP) treatment using DSC is shown in Table 3. DSC analysis has been used to determine the thermal transition or unfolding temperature of protein and also to quantify the enthalpy of conformational transition (John and Shastri, 1998). DSC thermograms of fresh shrimp meat showed 2 major endothermic peaks, with T_{max} values of 46.33 °C and 62.33 °C. Those peaks corresponded to myosin and actin peaks, respectively. For ice-stored shrimp, DSC thermogram showed only peak with T_{max} values of 45.61 °C. T_{max} and enthalpy of myosin peak shifted from 46.33 °C to 45.61 °C and 1.44 J/g to 0.09 J/g, respectively ($p<0.05$). Decreased of T_{max} and enthalpy was probably due to autolysis of shrimp muscle during storage in ice. Degraded MHC might facilitate the thermal denaturation of MHC. No transition peak of actin was noticeable in ice-stored shrimp, suggesting the complete denaturation of actin during iced storage. The result revealed that actin of ice-stored shrimp soaked with 2.5% NaCl in combination with 3% ProfixO

and of mixed phosphates was unstable to thermal denaturation. After being treated with mixed phosphates in combination with 3% ProfixO, T_{max} of myosin and actin peaks of fresh shrimp shifted to the lower temperature with lower ΔH, compared with shrimp with no treatment and shrimp soaked with mixed phosphates (in the absence of 3% ProfixO) ($p<0.05$). The result revealed that the destabilizing effect of additives used on the shrimp proteins affect the properties of shrimp proteins, particularly after heating or cooking.

Table 7. T_{max} and enthalpy of muscle proteins in fresh and ice-stored shrimps soaked in 2.5% NaCl containing mixed phosphates without and with 3% ProfixO

Samples	T_{max}I (°C)	ΔH (J/g)	T_{max}II (°C)	ΔH (J/g)
Fresh shrimp (0 day)				
Shrimp without treatment	50.06*±0.25^{a}**	1.65±0.13^{a}	71.28±0.48^{a}	0.25±0.05^{b}
Shrimp soaked with mixed phosphates for 2 h	49.08±0.08^{b}	1.52±0.17^{ab}	69.25±0.92^{b}	0.12±0.03^{c}
Shrimp soaked with ProfixO and mixed phosphates for 2 h	46.33±0.17^{d}	1.44±0.01^{b}	62.33±0.33^{d}	0.09±0.02^{c}
Storage shrimp (7 days)				
Shrimp without treatment	49.78±0.35^{ab}	0.76±0.05^{d}	68.83±0.35^{b}	0.26±0.04^{b}
Shrimp soaked with mixed phosphates for 2 h	48.17±1.01^{c}	1.20±0.05^{c}	67.00±0.83^{c}	0.41±0.01^{a}
Shrimp soaked with ProfixO and mixed phosphates for 2 h	45.61±0.35^{d}	0.07±0.01^{e}	ND***	ND

* Mean±SD from triplicate determinations.

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

*** Not detectable.

3.5 Effect of ProfixO and mixed phosphates on microstructure of fresh and ice-stored shrimps

Microstructures of Pacific white shrimp muscle treated with 2.5% NaCl containing 3% ProfixO and mixed phosphates are illustrated in Figure 22. For transverse section, similar structure was found between raw fresh and ice-stored shrimp. However, the coarser structure with a larger gap was observed in the ice-stored shrimp. The destruction of Z-line or M-line in ice-stored shrimp might facilitate the aggregation of protein and shrinkage. As a result, the proteins underwent coagulation to a greater extent. For longitudinal section, raw fresh shrimp had well organized structure of myofibrils with no destruction of M-line. After 7 days of storage, muscle detachment was more intense and slight increase in rupture of the Z-line was noticeable. After cooking, the shrinkage of sarcomere was found with the gap generated. Heat processing enhanced the firmness and degree of shrinkage of *Penaeus japonicus* (Mizuta *et al*., 1999). After cooking, both fresh shrimp and ice-stored shrimp had more compact myofibrils arrangement with the shrinkage of sarcomere, compared with raw samples. When comparing between cooked fresh and ice-store shrimp, it was found destruction of M-line was more pronounced in the latter. Disintegration of muscle was caused by endogenous proteolytic enzyme i.e. calpain and cathepsin, leading to the continuous degradation of the myofibrils during extended storage time (Geesink and Koohmaraie, 1999). Kong *et al* (2006) suggested that the proteolysis was likely the main cause for the disintegration of the Z-disks and I-bands and the rupture of sarcomere and fragmentation of the muscle fibers and myofibrils. Dutson *et al* (1974) reported the extensive loss of Z-lines in porcine muscle during postmortem. Olsson *et al* (2003) demonstrated that clear disruptions of the Z-discs occurred in Atlantic halibut muscle during postmortem storage. Several researchers have reported the association of Z-line disruption and the destroyed cytoskeletal proteins by the action of proteases, releasing a-actin, nebulin and titin (Busconi *et al*., 1989; Hernandez *et al* 2003; Luther and Squire, 2002; Olafsdottir *et al*., 1997; Pearson and Young, 1989). From the result, post mortem degradation might facilitate the penetration of

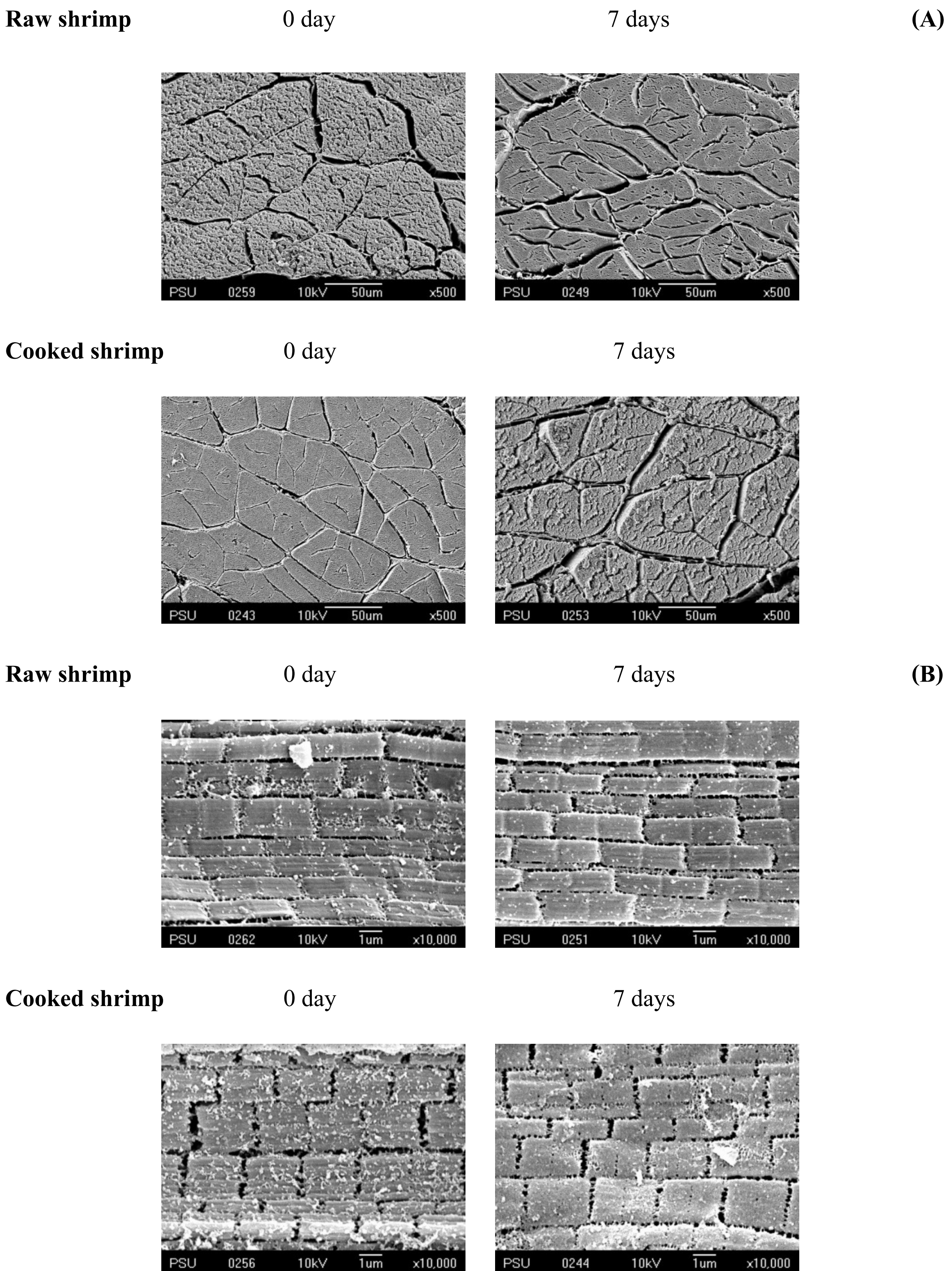


Figure 22. SEM micrographs of transverse section (A) and longitudinal section (B) of raw and cooked fresh and ice-stored Pacific white shrimp meats soaked with 2.5% NaCl containing 3% ProfixO and mixed phosphates.

ProfixO and mixed phosphates into the muscle, in which proteins at M-line might be solubilized or removed more effectively. Proteins associated with M-line are M-protein, myomesin and creatine kinase. (Pearson and Young, 1989).

4. Effect of mixed phosphates and ProfixO on quality improvement of Pacific white shrimp as affected by salt concentration and soaking time

4.1 Physical properties of fresh and ice-stored shrimps

Opacity score of cooked fresh and ice-stored Pacific white shrimp treated with mixed phosphates without and with 3% ProfixO in the presence of different salt concentrations (0, 1, 2.5% w/v) for various soaking times (60, 120, 240, 360 min) is shown in Figure 23. After being treated with mixed phosphates (2.625% TSPP + 0.875% SAPP) in the absence and in the presence of 3% ProfixO, both cooked fresh and ice-stored shrimps became more translucent as evidenced by the lower opacity score, compared with shrimp with no treatment ($p<0.05$). For fresh shrimp treated with mixed phosphates without 3% ProfixO (Figure 23A), translucence markedly increased with the increasing salt concentration and soaking time increased as indicated by the decrease in opacity score ($p<0.05$). Increasing salt concentration in soaking solution could induce the swelling of muscle fiber, in which phosphate as well as salt could solubilize proteins more effectively. Solubilized proteins could form ordered gel network with translucence in characteristic. In general, ice-stored shrimps were more translucent than fresh counterpart, regardless of salt concentration and soaking time. Additionally, salt concentration and soaking time had no marked impact on opacity score of ice-stored shrimp treated with mixed phosphates (Figure 23A). Therefore, freshness of shrimp had the influence on opacity score shrimp treated with mixed phosphates. In the presence of 3% ProfixO, opacity score of cooked fresh shrimp treated with mixed phosphates tended to decrease when soaking time increased.

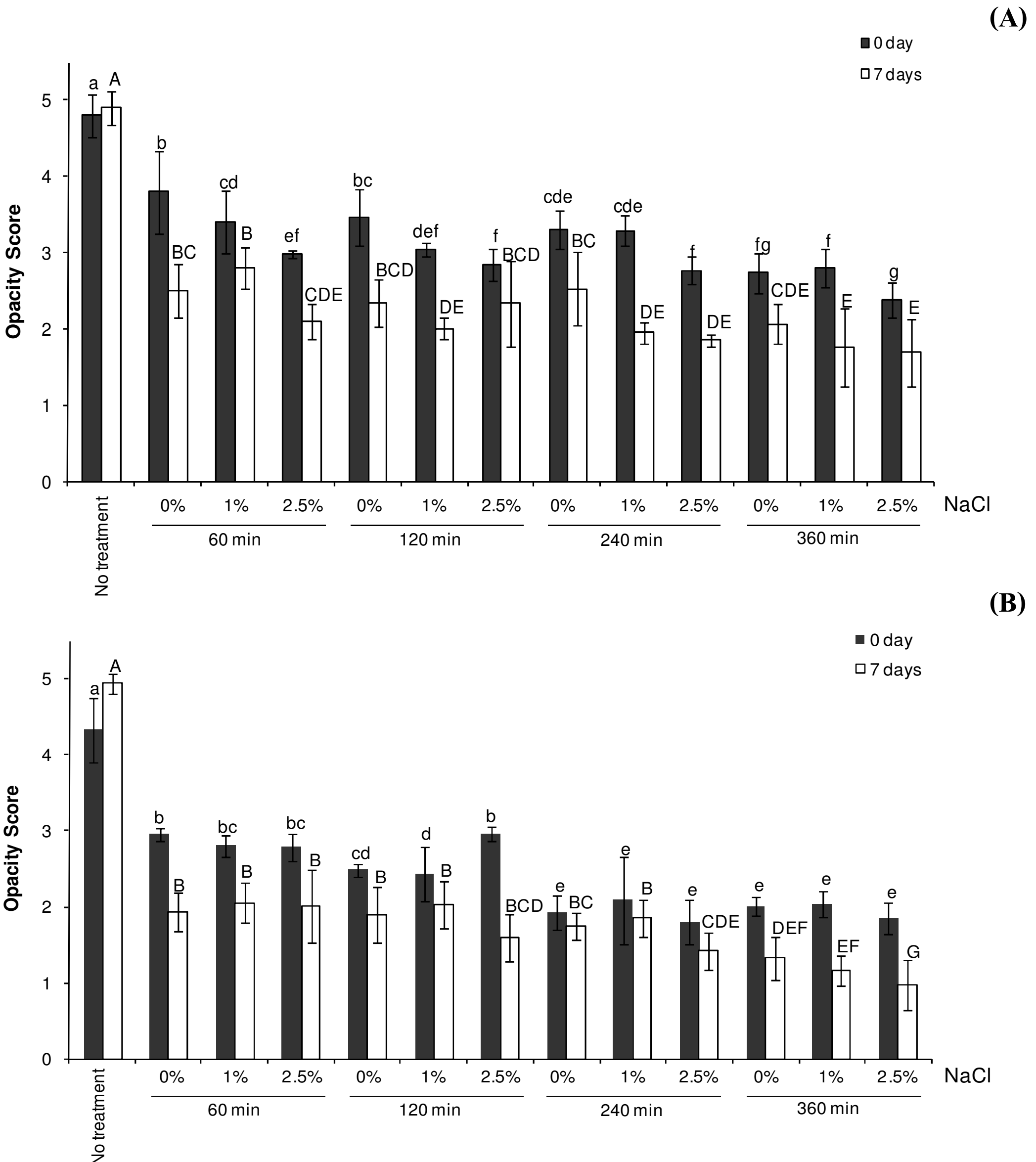


Figure 23. Opacity score of fresh and ice-stored Pacific white shrimps soaked in solution containing mixed phosphates (0.875% SAPP + 2.625% TSPP) without (A) and with 3% ProfixO (B) in the presence of salt at different concentrations for different soaking times. The different lower case letters or upper case letters within the same storage time indicate significant differences ($p<0.05$). Bars represent the standard deviation from ten determinations.

(1: Very translucent ; 3: Moderately translucent ; 5: Turbid, dull)

At the same soaking time, there were no differences in opacity score of cooked shrimp at all salt concentrations (0, 1 and 2.5%) ($p>0.05$). For ice-stored shrimp, salt concentrations also had no impact on opacity score, when soaking times were 60 and 120 min. For soaking times of 240 and 360 min, opacity score decreased as salt concentrations increased ($p<0.05$). The lowest opacity score was found in shrimp treated with 2.5% NaCl containing mixed phosphates and ProfixO for 360 min. Therefore, ProfixO showed the enhancing effect on translucence of resulting shrimp in concert with mixed phosphates, particularly for ice-stored shrimp. The lower integrity of muscle might facilitate the migration of ProfixO as well as mixed phosphates and more translucence could be observed.

Weight gain of shrimps treated with mixed phosphates without and with 3% ProfixO in the presence of salt at different concentrations for various soaking time is shown in Figure 24. For fresh and ice-stored shrimp treated with mixed phosphates, weight gain increased as salt concentration and soaking time increased ($p<0.05$) (Figure 24A). The similar result was observed for moisture content (Figure 25A), in which an increase in moisture content was noticeable with increasing salt concentrations and soaking time ($p<0.05$). For all treatments used, weight gain was lower in shrimps stored in ice for 7 days, whereas moisture content of ice-stored shrimp was higher than that of fresh shrimp ($p<0.05$). The result suggested that NaCl at high concentration showed the synergistic effect with mixed phosphates in improving weight gain of shrimp. Salt has been used in conjunction with phosphates to improve the yield of meat and seafood products (Trout and Schmidt, 1984). For fresh shrimp treated with mixed phosphates and 3% ProfixO, weight gain increased with increasing salt concentration and soaking time ($p<0.05$). However, no differences in weight gain were found in shrimp treated with mixed phosphates and ProfixO for 60 min when different salt concentrations were used ($p>0.05$). Weight gain of ice-stored shrimp increased with increasing soaking time ($p<0.05$). Nevertheless, at the same soaking time, salt concentration had no impact on weight gain of shrimp. For moisture content

(Figure 25B), fresh and ice-stored shrimps tended to have increasing moisture content as salt concentration increased.

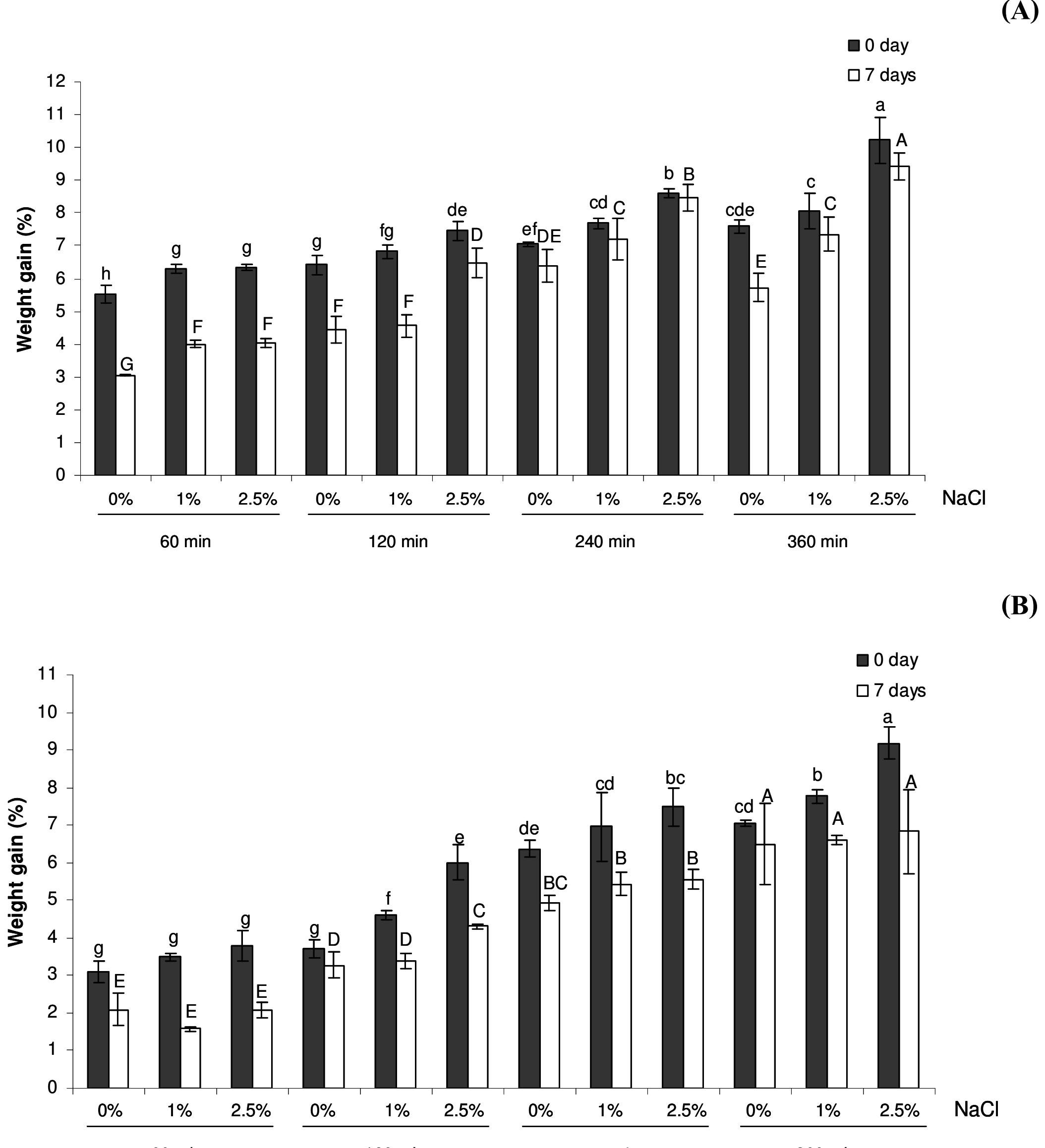


Figure 24. Weight gain of fresh and ice-stored Pacific white shrimps soaked in solution containing mixed phosphates (0.875% SAPP + 2.625% TSPP) without (A) and with 3% ProfixO (B) in the presence of salt at different concentrations for different soaking times. The different lower case letters or upper case letters within the same storage time indicate significant differences ($p<0.05$). Bars represent the standard deviation from five determinations.

Generally no differences in moisture content were found between samples treated with 1% and 2.5% salt ($p>0.05$). Increased weight gain and moisture content by the increasing in soaking time and salt concentration are achieved through muscle fiber expansion (swelling) caused by electrostatic repulsions, which allows move water to be immobilized in the myofibril lattices (Offer and Knight, 1988). From the result, muscle integrity was another factor governing the efficacy of mixed phosphates in increasing the yield of treated shrimps. During storage in ice, some ice was molten and shrimps were immersed. As a consequence, the water was uptaken into shrimp muscle to a high extent as indicated by the increased moisture content. However, lower weight gain was obtained for ice-stored shrimps, attributing to the low capacity of water holding in the weakened muscle structure. Xiong and Kupski (1999b) reported that water uptake by the chicken fillets was rapid in the initial 5 min, and was substantially slower from 15 to 30 min during tumbling marination. In the case of marinade absorption, retention of the absorbed marinade essentially reached a maximum after 15 min tumbling when salt was not a marinade ingredient. However, in the presence of 8% salt, both marinade absorption and retention continued to increase with tumbling time up to 30 min. Marinade absorption in the first 5 min was expected to occur in the muscle surface layers (Xiong and Kupski, 1999a). The combination of fiber disruption and marinade gradient between muscle surface and its inside tissue probably contributed to marinade absorption (Xiong and Kupski, 1999b).

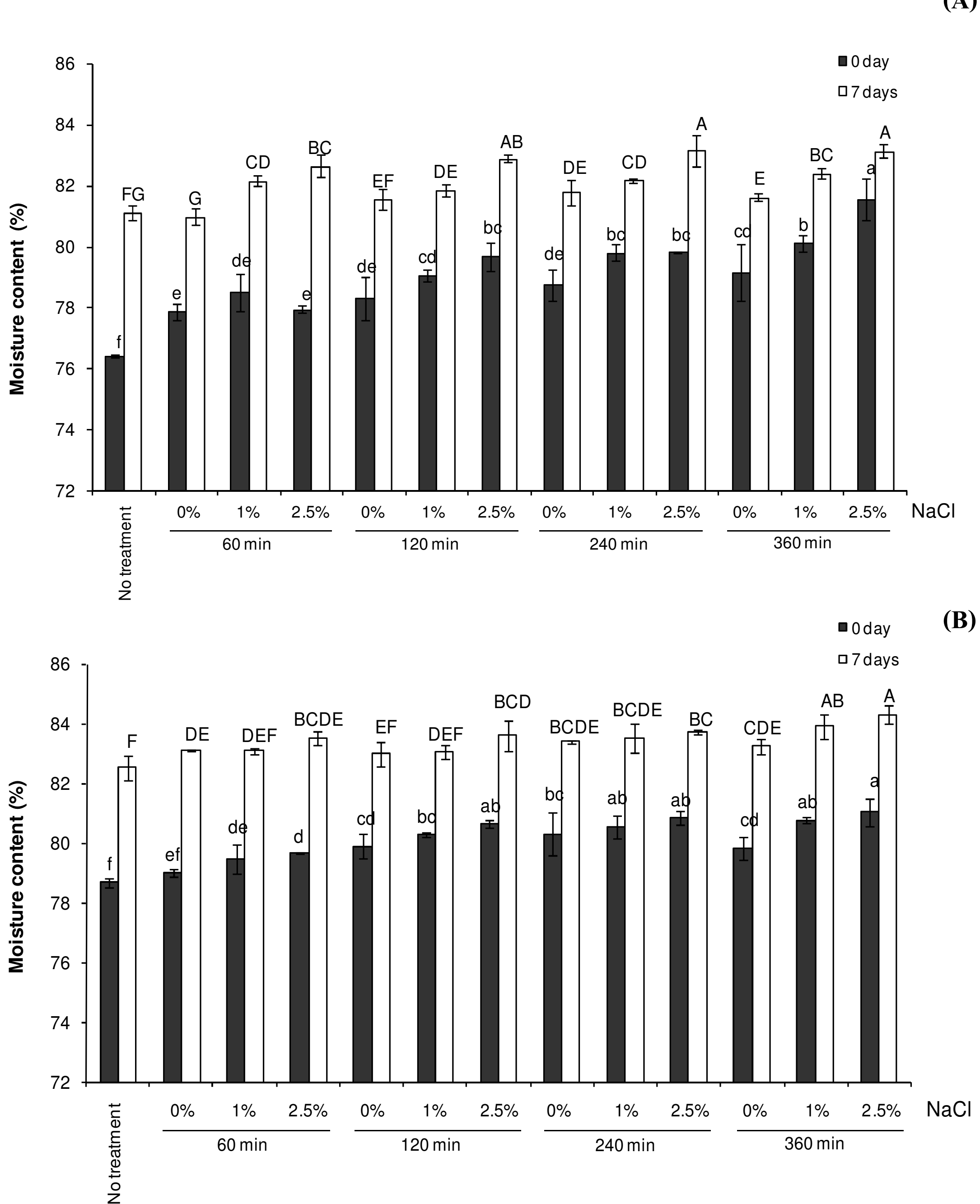


Figure 25. Moisture content of fresh and ice-stored Pacific white shrimps soaked in solution containing mixed phosphates (0.875% SAPP + 2.625% TSPP) without (A) and with 3% ProfixO (B) in the presence of salt at different concentrations for different soaking times. The different lower case letters or upper case letters within the same storage time indicate significant differences ($p<0.05$).Bars represent the standard deviation from triplicate determinations.

Cooking yield and cooking loss of fresh and ice-stored shrimps treated with mixed phosphates without and with 3% ProfixO in the presence of salt at different concentrations are shown in Figure 26 and 27, respectively. Both fresh and ice-stored shrimps treated with mixed phosphates had the increased cooking yield and lowered cooking loss as the soaking time increased ($p<0.05$). However, the decrease in cooking yield was noticeable in fresh and ice-stored shrimps soaked in mixed phosphates for 360 min as the salt concentration increased ($p<0.05$). Cooking yield of fresh and iced-stored shrimps soaked in mixed phosphates and 3% ProfixO generally increased with increasing salt concentration and soaking time ($p<0.05$). For soaking time of 360 min, weight gain of fresh shrimp decreased with increasing salt concentration ($p<0.05$). From the result, high salt concentration and longer soaking time led to an increase in absorption of phosphate, salt as well as ProfixO. Those compounds might contribute to the stabilization of protein structure and holding water in the muscle. For ice-stored shrimp soaked in mixed phosphates containing 3% ProfixO, salt concentration had no impact on cooking yield when soaking times of 60 and 120 min were used ($p>0.05$). Soaking time of 240 min resulted in the highest cooking yield. In the presence of ProfixO, no differences in cooking yield between fresh and ice-stored shrimp at all salt concentrations were found when soaking times of 60 and 120 min were used ($p>0.05$). Cooking loss of shrimp treated with mixed phosphates tended to decrease with increasing soaking time. However, the lowest cooking loss was found in fresh shrimp treated with mixed phosphate and ProfixO when soaking time was 240 min, irrespective of salt concentration. For ice-stored shrimp, the lowest cooking loss was found when soaking times of 240 and 360 min were used, regardless of salt concentration.

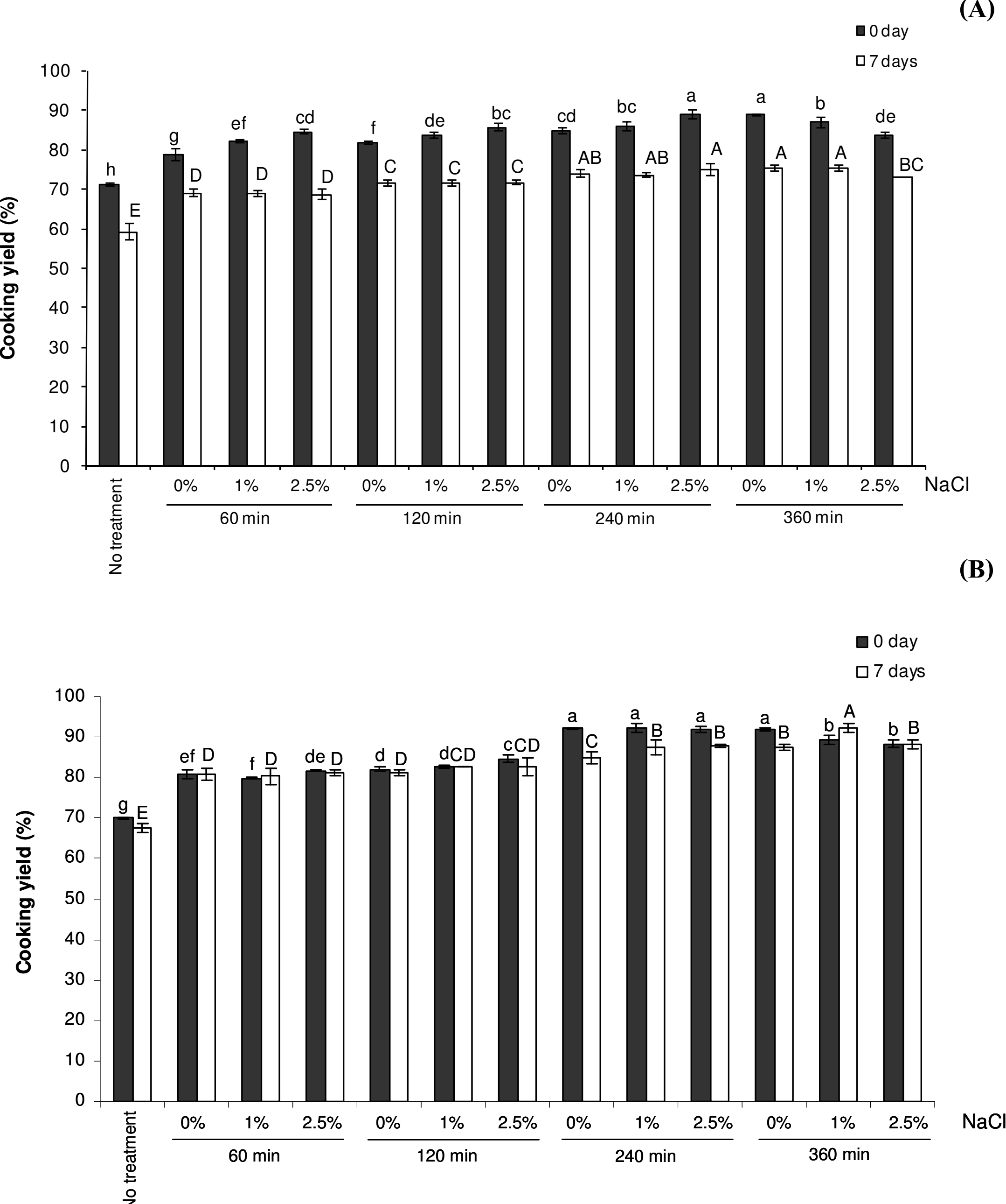


Figure 26. Cooking yield of fresh and ice-stored Pacific white shrimps soaked in solution containing mixed phosphates (0.875% SAPP + 2.625% TSPP) without (A) and with 3% ProfixO (B) in the presence of salt at different concentrations for different soaking times. The different lower case letters or upper case letters within the same storage time indicate significant differences ($p<0.05$). Bars represent the standard deviation from five determinations.

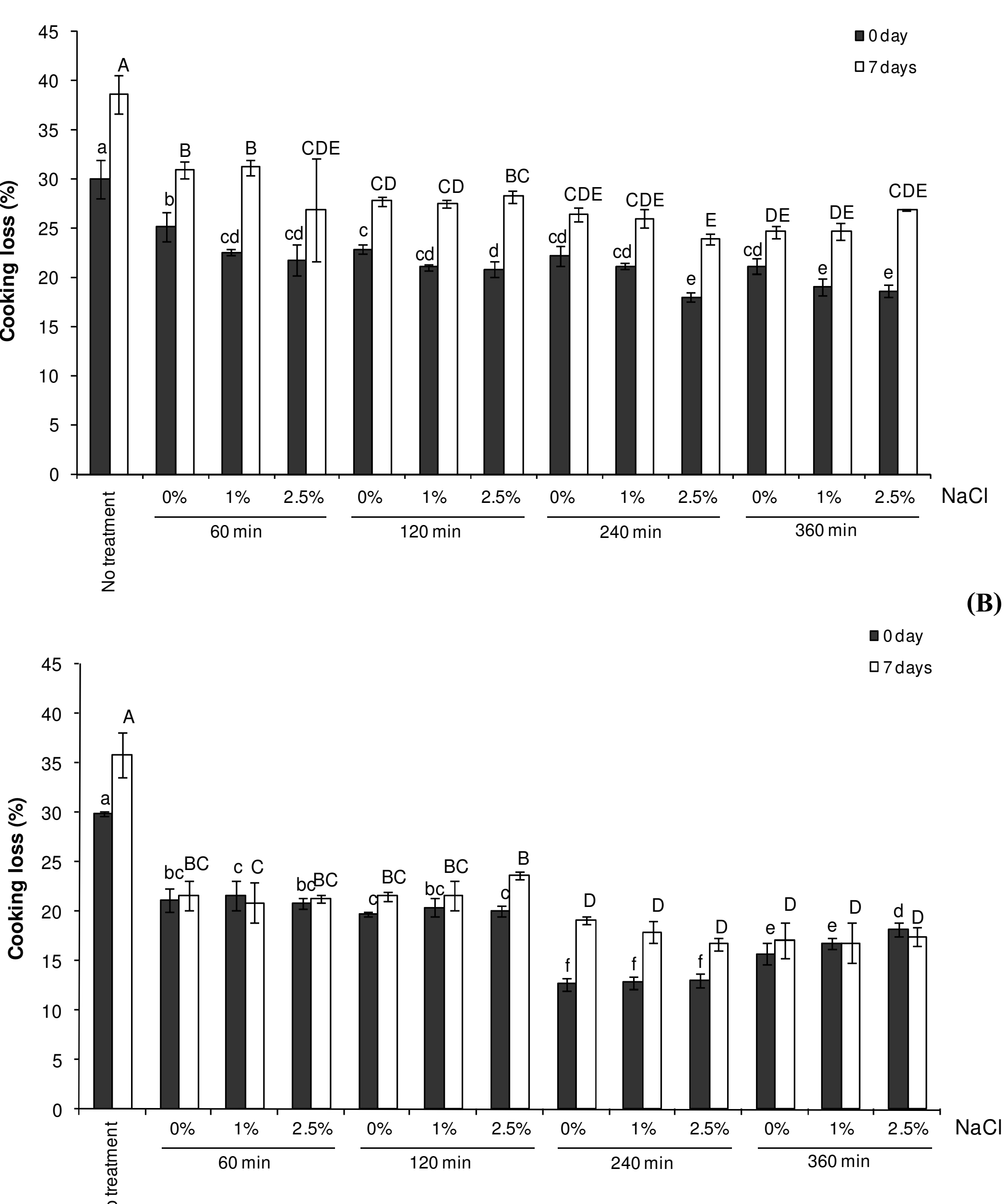


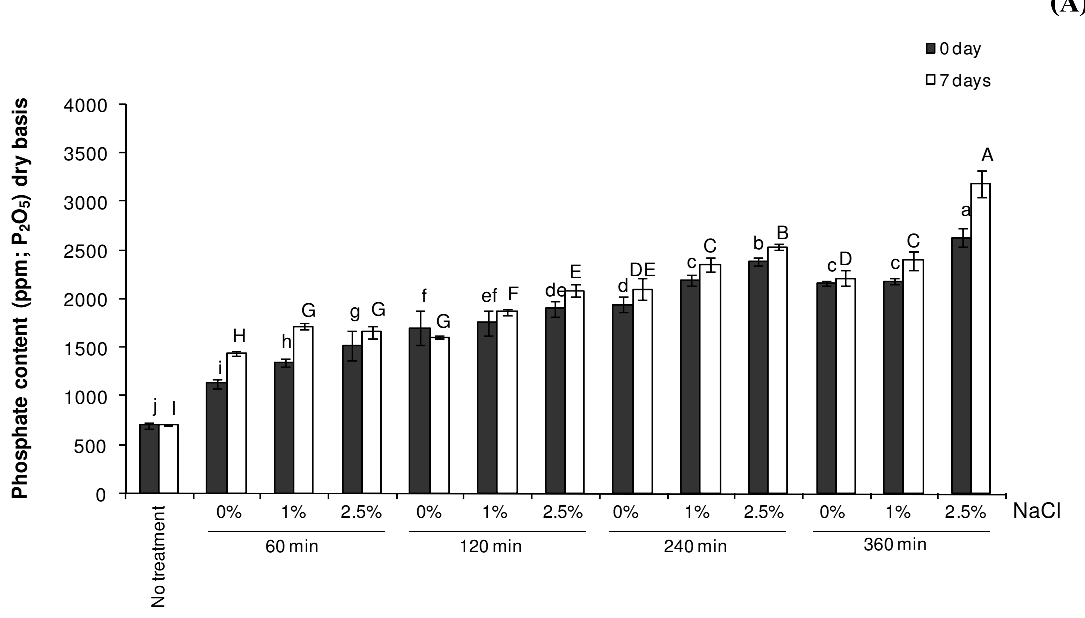
Figure 27. Cooking loss of fresh and ice-stored Pacific white shrimps soaked in solution containing mixed phosphates (0.875% SAPP + 2.625% TSPP) without (A) and with 3% ProfixO (B) in the presence of salt at different concentrations for different soaking times. The different lower case letters or upper case letters within the same storage time indicate significant differences ($p<0.05$). Bars represent the standard deviation from five determinations.

4.2 Chemical composition of fresh and ice-stored shrimps

Phosphate content and salt content (dry basis) of fresh and ice-stored shrimp treated with mixed phosphates in combination without and with 3% ProfixO in the presence of salt at different concentrations for various soaking times are shown in Figure 28 and 29, respectively. Phosphate content of fresh and ice-store shrimps increased with increasing salt concentration and soaking time. Ice-stored shrimp contained higher phosphate content than did fresh counterpart ($p<0.05$) for all treatments. For the same soaking time, phosphate content increased with increasing salt concentration. The similar result was observed when mixed phosphates in combination with ProfixO were used (Figure 28B). Nevertheless, for the same salt concentration and soaking time, higher phosphate content was found in shrimp treated with both mixed phosphates and ProfixO. Thus ProfixO might work synergistically in disrupting of muscle structure, in which more phosphates could migrate into muscle to a greater extent.

For salt content similar result was observed, when compared with phosphate content. At the same soaking time, salt content increased with increasing salt concentration used in soaking medium. With the same salt concentration used in soaking solution, salt content in shrimp muscle tended to increase when soaking time increased. Salt content was higher in ice-stored shrimp, compared with fresh counterpart. Increases in water binding and hydration in muscle fibers are generally attributed to enhanced electrostatic repulsions between myofibril filaments, causing the filamental lattices to expand for water entrapment (Xiong *et al*., 2000). Phosphate and salt contents in muscle at higher levels were most likely associated with the improved cooking yield and cooking loss of cooked fresh and ice-stored shrimp. Phosphate content and salt content have both been known to increase swelling of the muscle to take up and retain water (Offer and Knight, 1988; Fennema, 1990).

(A)



(B)

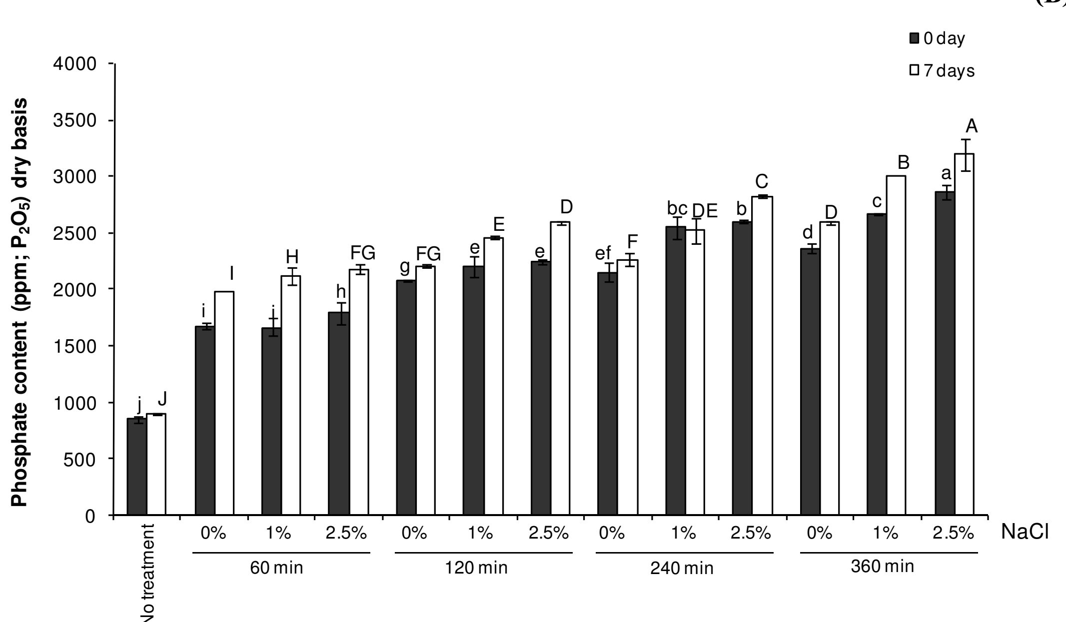


Figure 28 Phosphate content of fresh and ice-stored Pacific white shrimps soaked in solution containing mixed phosphates (0.875% SAPP + 2.625% TSPP) without (A) and with 3% ProfixO (B) in the presence of salt at different concentrations for different soaking times. The different lower case letters or upper case letters within the same storage time indicate significant differences ($p<0.05$). Bars represent the standard deviation from triplicate determinations.

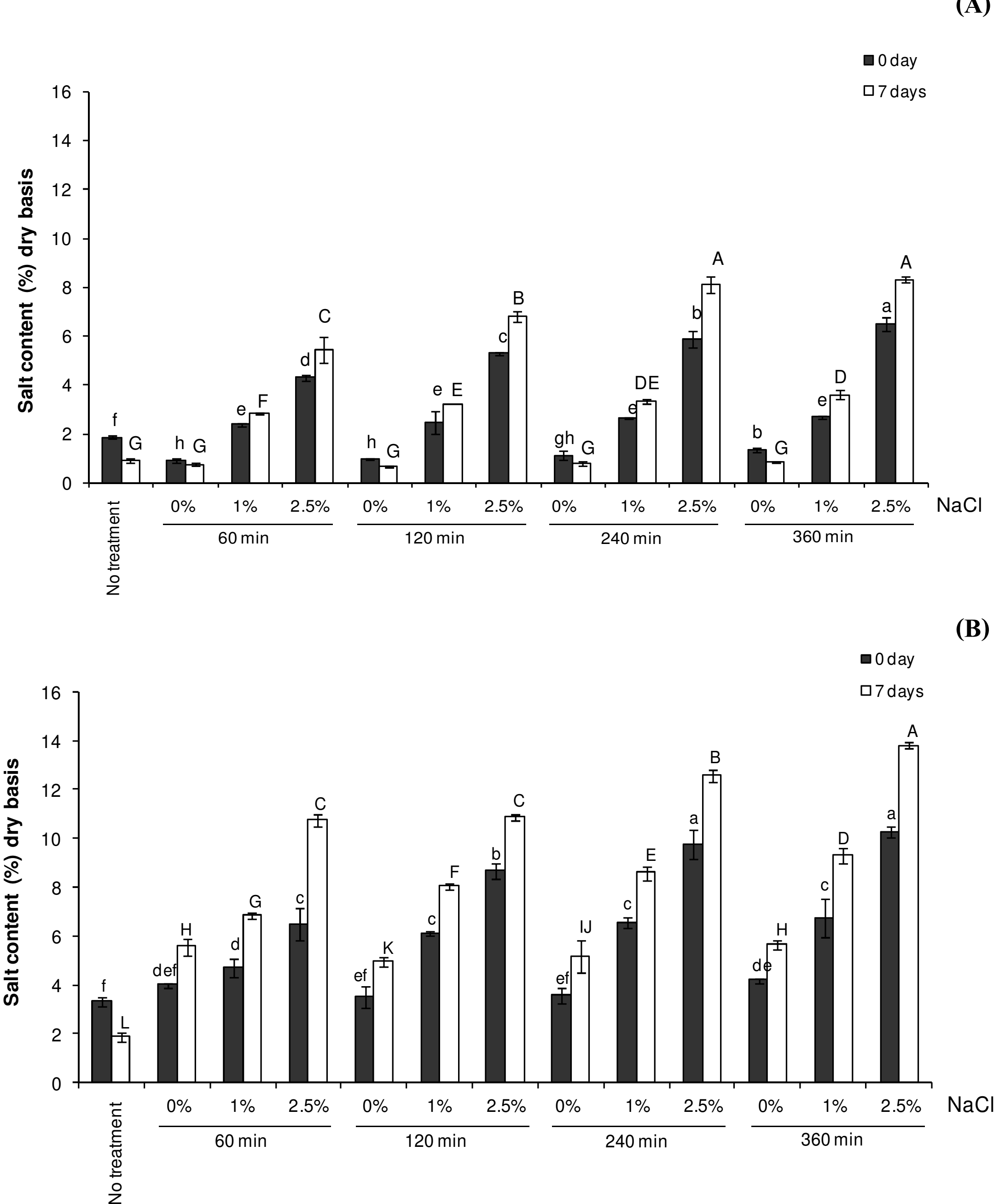


Figure 29 Salt content of fresh and ice-stored Pacific white shrimps soaked in solution containing mixed phosphates (0.875% SAPP + 2.625% TSPP) without (A) and with 3% ProfixO (B) in the presence of salt at different concentrations for different soaking times. The different lower case letters or upper case letters within the same storage time indicate significant differences ($p<0.05$). Bars represent the standard deviation from triplicate determinations.

Binding of phosphate anion to proteins and simultaneous cleavage of cross-linkages between actin and myosin result in increased electrostatic repulsion between peptide chains and swelling of the muscle system (Hamm, 1970). If exterior water is available, it then can be taken up in an immobilized state within the loosen protein network. Because the ionic strength has been increased, the interaction between proteins is perhaps reduced to a point where part of myofibrillar proteins form a colloidal solution (Hamm, 1970). However, phosphate contents in shrimp were less than the standard value (5000 ppm) (Official Journal of the European Communities, 1995).

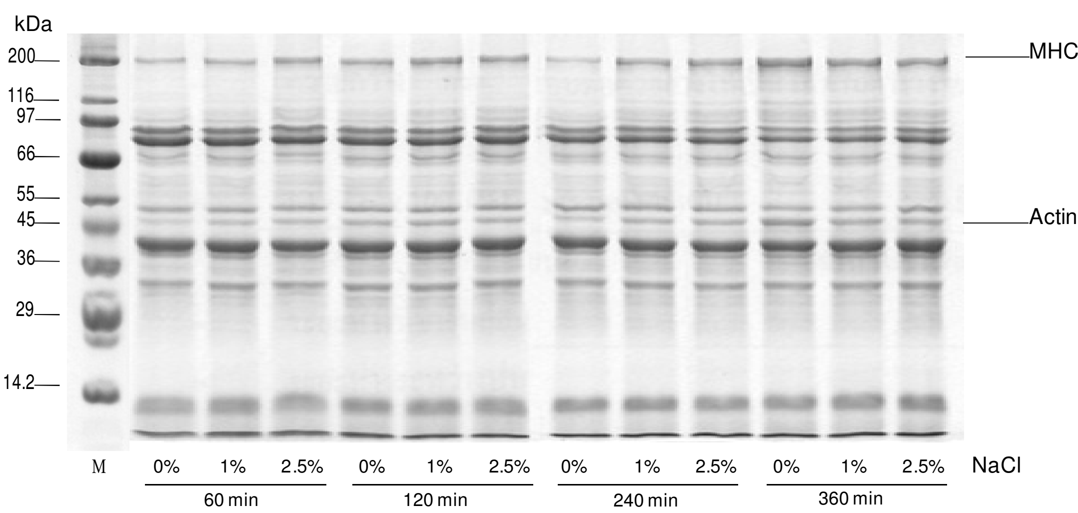
4.3 Protein pattern of soaking solution after treated with fresh and ice-stored Pacific white shrimps

Protein patterns of various soaking solutions containing mixed phosphates of fresh and ice-stored Pacific white shrimps as influenced by ProfixO, salt concentration and soaking time are shown in Figures 30 and 31, respectively. For all soaking solutions of fresh shrimps, protein with MW of 40 kDa was dominant, followed by protein with MW of 77 and 88 kDa. Nevertheless, no protein bands with of 77 and 88 kDa were detected in soaking solution of iced-stored shrimps. The decrease in band intensity of those proteins might be caused by the degradation of protein during iced storage, as described previously. Myosin heavy chain (MHC) and actin were also found in all soaking solutions. For soaking solution of fresh and ice-stored shrimps, MHC band intensity increased slightly when the levels of NaCl and soaking time increased. No marked differences in actin band intensity were observed in soaking solution with all NaCl levels and soaking time used. The result suggested that more proteins, particularly MHC, were solubilized and leached out to a greater extent as salt concentration used increased. Also sufficient time for phosphates as well as salt and ProfixO to migrate into muscle resulted in more solubilization of muscle proteins, particularly MHC. The increases in MHC band intensity

correlated with the increases in weight gain and cooking yield. Nevertheless, soaking time of 360 min caused a decrease in cooking yield and an increase in cooking loss of fresh and ice-stored shrimps. This was associated with the excessive solubilization of muscle proteins, in which proteins at high amount were leach out. Thawornchinsombut and Park (2005) reported that MHC intensity of soluble fish protein isolated from cryoprotected Pacific whiting mince significantly increased as ionic strength increased from 10 to 600 mM. Solubilization of muscle proteins under high ionic strength involves two events: the depolymerization of the thick filament backbone, and the subsequent dissociation of the myosin heads from the actin filaments (Parsons and Knight 1990). For shrimp, either fresh or ice-stored, treated with mixed phosphates in combination with ProfixO, similar results were observed in comparison with those of soaking solutions containing mixed phosphates (Figure 30). However, the release of MHC was more pronounced when mixed phosphates were used in combination with ProfixO. The greater ability to extract or solubilize proteins of combined compounds resulted in ability to lower cooking loss (Figure 27).

From the result, optimum condition to reduce translucence of cooked shrimps, while still maintained weight gain and cooking yield of fresh and ice-stored Pacific white shrimp were 1% NaCl + 0.875% SAPP + 2.625% TSPP without or with 3% ProfixO and soaking time of 240 min.

0 day



7 days

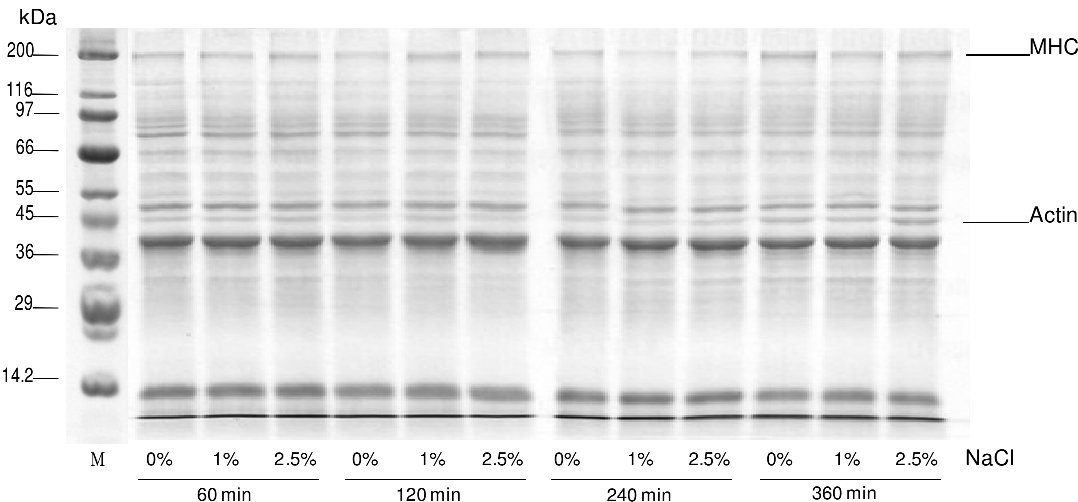
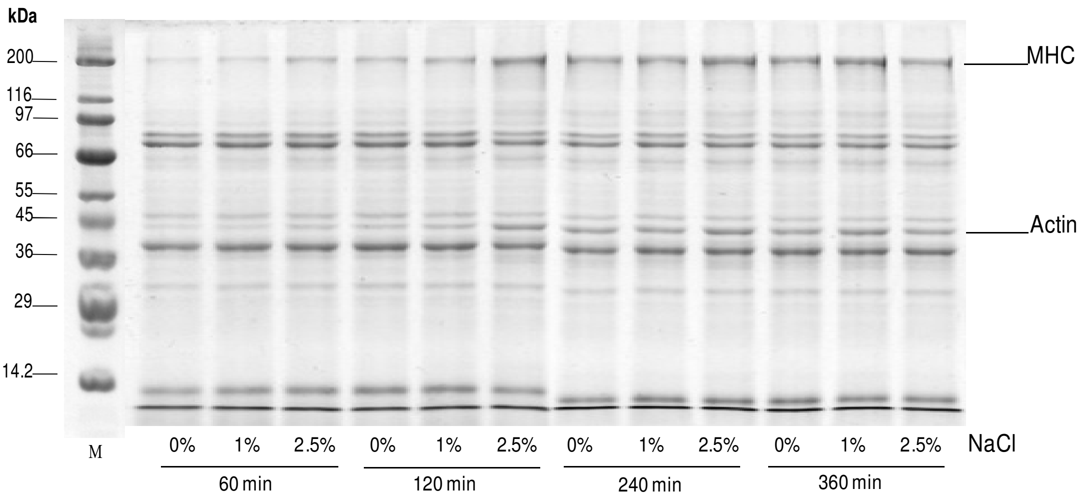


Figure 30. Protein patterns of various soaking solution containing mixed phosphates and ProfixO of fresh and ice-stored Pacific white shrimps as affected by salt concentrations and soaking times.

MHC: myosin heavy chain

0 day



7 days

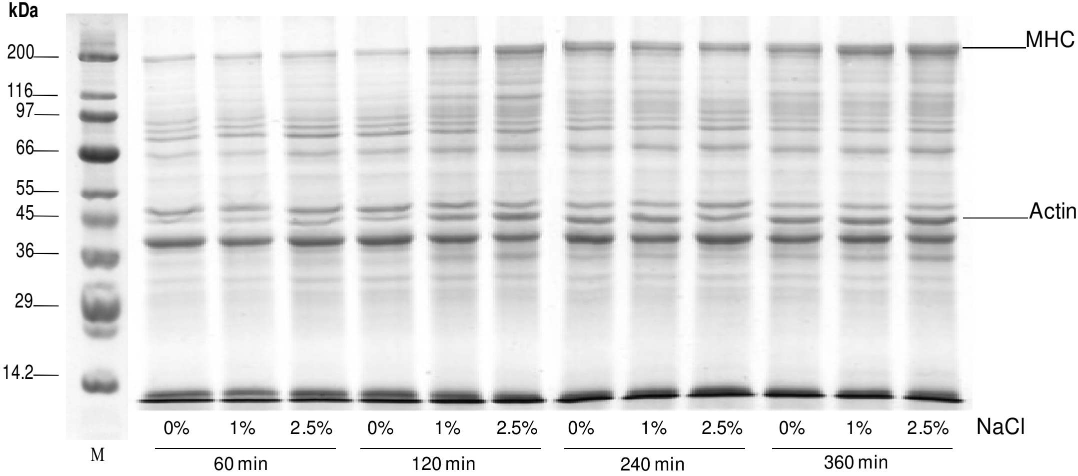


Figure 31. Protein patterns of various soaking solution containing mixed phosphates of fresh and ice-stored Pacific white shrimps as affected by salt concentrations and soaking times.

MHC: myosin heavy chain

4.4 Thermal properties of fresh and ice-stored shrimps treated with mixed phosphates

Thermal transition of fresh and ice-stored shrimps soaked in the solution containing 1% NaCl and mixed phosphates (0.875% SAPP + 2.625% TSPP) in combination with or without 3% ProfixO for 4 h is shown in Table 8. DSC thermogram of fresh and ice-stored shrimp showed two endothermic transition peak corresponding to myosin and actin peaks. Myosin peak of fresh shrimp soaked in the solution without and with 3% ProfiixO shifted to lower T_{max} of 49.28 and 48.33 °C, respectively. T_{max} of myosin peak of ice-stored shrimp soaked in the solution without and with 3% ProfixO shifted from 49.28 °C to 44.97 °C and 44.67 °C, respectively. It was indicated that MHC of shrimp underwent thermal denaturation easily after the treatment with mixed phosphates, especially in combination with ProfixO. It was noted that MHC of ice-stored shrimp was more susceptible to heat denaturation after the treatment of mixed phosphates, regardless of ProfixO coordination, as evidenced by the lower T_{max}. No transition peak of actin was noticeable in both fresh and ice-stored shrimps after the treatment with mixed phosphates in the presence and in the absence of ProfixO. The result revealed that actin of fresh and ice-stored shrimp soaked in 1% NaCl and mixed phosphates (0.875% SAPP + 2.625% TSPP) in combination with or without 3% ProfixO for 4 h was unstable to thermal denaturation.

Table 8. T_{max} and enthalpy of muscle proteins of fresh and ice-stored soaked with 1% NaCl containing mixed phosphates in the absence or presence of 3% ProfixO for 240 min

Samples	T_{max}I (°C)	ΔH (J/g)	T_{max} II (°C)	ΔH (J/g)
Fresh shrimp (0 day)				
Shrimp without treatment	50.06*±0.25[a]**	1.65±0.13[a]	71.28±0.48[a]	0.25±0.05[a]
Shrimp soaked with mixed phosphates for 4 hr	49.28±0.10[b]	1.28±0.22[b]	ND***	ND
Shrimp soaked with ProfixO and mixed phosphates for 4 hr	48.33±0.33[c]	1.35±0.05[b]	ND	ND
Storage shrimp (7 days)				
Shrimp without treatment	49.78±0.35[b]	0.76±0.05[c]	68.83±0.35[b]	0.26±0.04[a]
Shrimp soaked with mixed phosphates for 4 hr	44.97±0.05[d]	1.15±0.03[b]	ND	ND
Shrimp soaked with ProfixO and mixed phosphates for 4 hr	44.67±0.17[d]	1.17±0.10[b]	ND	ND

* Mean±SD from triplicate determinations.

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

*** Not detectable.

4.5 Microstructure of fresh and ice-stored shrimps

Microstructures of Pacific white shrimp muscle treated with 1% NaCl containing mixed phosphates without and with of 3% ProfixO are illustrated in Figures 18 and 19, respectively. For transverse section of raw sample, fresh and ice-stored shrimp had more compact structure when ProfixO was used in combination with mixed phosphates. Similar structures of fresh and ice-stored shrimp were found in cooked shrimps, regardless of freshness and ProfixO incorporated. Cooked samples of fresh and ice-stored shrimp generally had more compact fiber arrangements, compared with raw samples. 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. Nip and Moy (1988) reported the microstructure changes of boiled meat of prawn (*Macrobranchium rosenbergii*). The more compact fibers might be associated with the increased shear force values of cooked shrimp meats.

For longitudinal section of raw samples, fresh shrimps soaked in the solution in combination with or without 3% ProfixO had well organized structure of myofibrils. After 7 days of ice storage, slight destruction of Z-lines was noticeable. Myofibrils were less attached with the loss of Z-lines. Several researchers have reported the association of Z-line disruption and the destroyed cytoskeletal proteins by the action of proteases, releasing α-actin, nebuli and titin (Busconi *et al*., 1989; Hernandez-Herrero, *et al*., 2003; Luther and Squire, 2002; Olafsdottir *et al*., 1997; Pearson and Young, 1989). Ham and Cormack (1979) reported that the disruption of mitochondria causes the release of enzymes into the sarcoplasm, which initiate the breakdown of sarcoplasmic proteins. The mitochondrial degradation occurred in conjunction with the disruption of the myofibril structure (disruption of Z-lines, I-bands and M-lines). After soaking in the solution containing 3% ProfixO, myofibrils of both fresh and ice-stored shrimps became larger in size or more swelling. NaCl and mixed phosphates along with ProfixO induced swelling of the myofibril. After cooking, the shrinkage of sarcomere was more obvious in ice-stored shrimp, compared with fresh counterpart. Interestingly, disintegration of M-line and Z-line was clearly observed in fresh and ice-stored shrimps soaked in the solution without 3% ProfixO after cooking. In general, cooked ice-stored shrimp underwent more destruction of M-line and Z-line, compared with fresh shrimp. In the presence of 3% ProfixO, destruction of M-line was minimized. However, larger gap at Z-line was noticeable. Therefore, ProfixO might prevent the destruction or solubilization of M-line proteins, in which M-line was still maintained after heating. The structure of shrimp treated with mixed phosphates might contribute to water holding capacity of cooked samples.

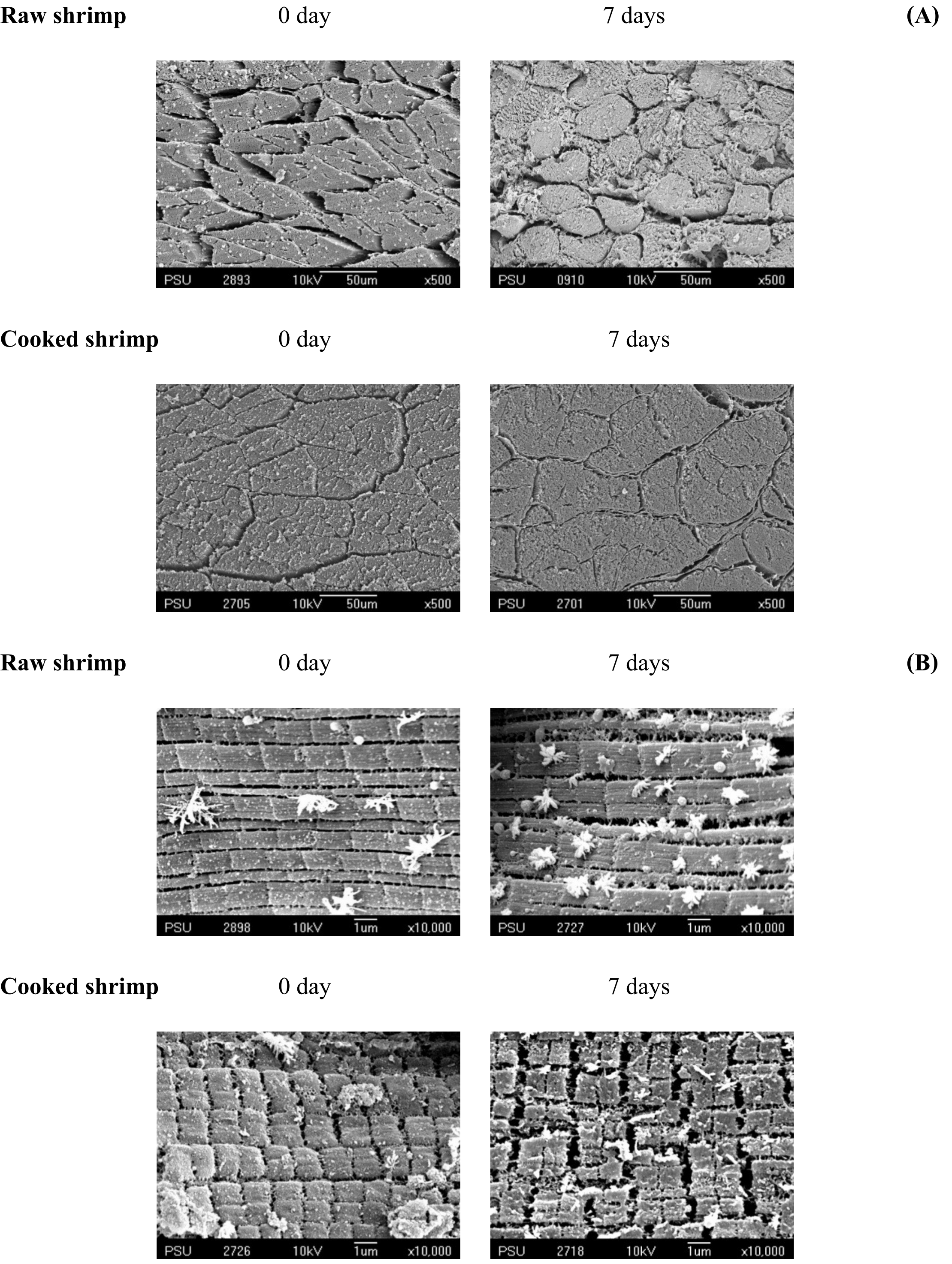


Figure 32. SEM micrographs of transverse section (A) and longitudinal section (B) of raw and cooked fresh and ice-stored Pacific white shrimp meats soaked in 1% NaCl containing mixed phosphates without 3% ProfixO.

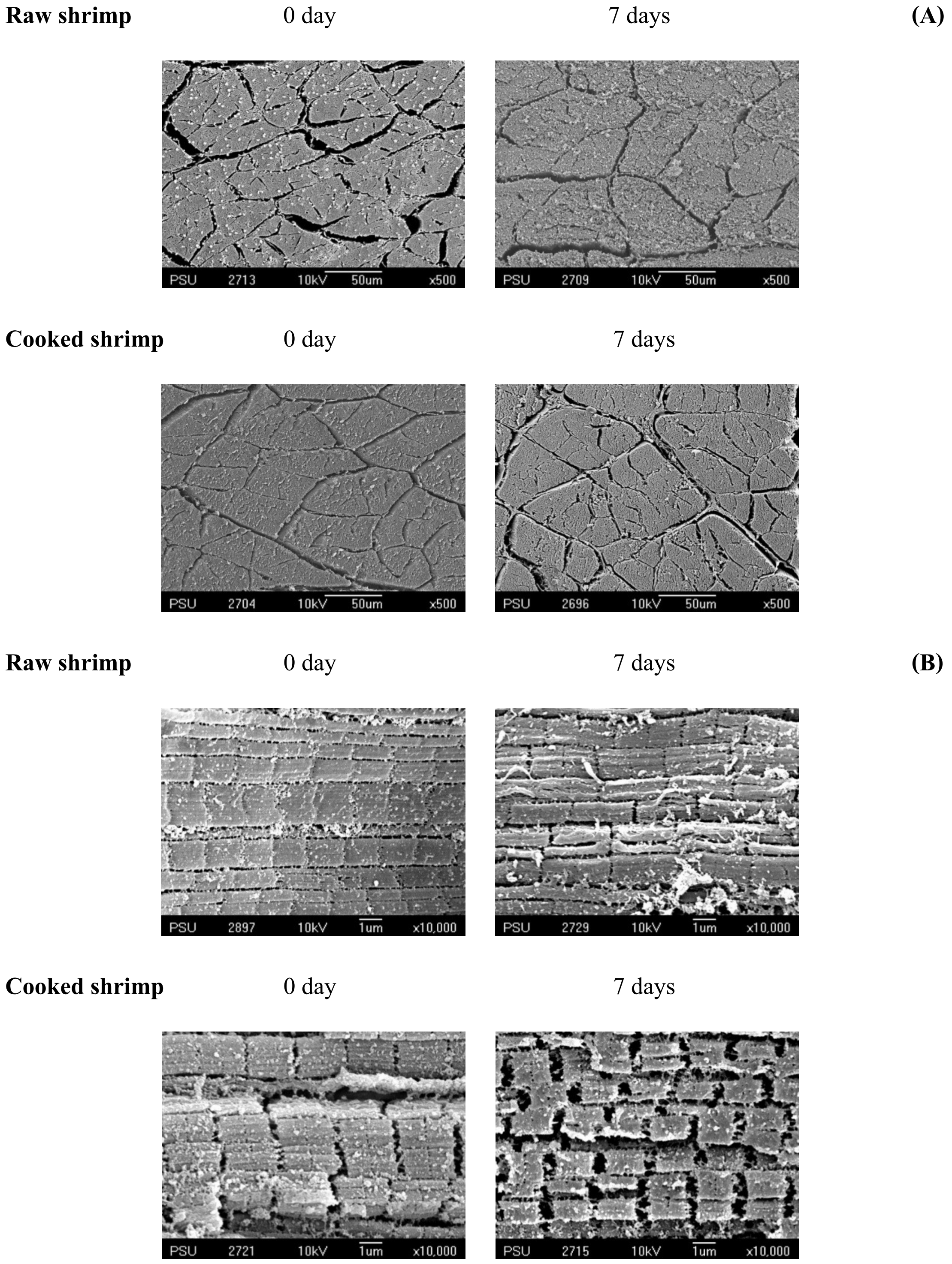


Figure 33. SEM micrographs of transverse section (A) and longitudinal section (B) of raw and cooked fresh and ice-stored Pacific white shrimp meats soaked in 1% NaCl containing mixed phosphates with 3% ProfixO.

4.6 Proximate composition

Proximate compositions of fresh and ice-stored Pacific white shrimps treated with 1% NaCl containing mixed phosphate (0.875% SAPP + 2.625% TSPP) in the absence and in the presence of 3% ProfixO solution for 240 min are shown in Table 9. Moisture content of treated samples was higher than that of shrimps without treatment ($p<0.05$). In general, ice-stored shrimp had higher moisture content than fresh shrimp ($p<0.05$). This indicated that loosen of muscle structure during storage in ice allowed molten ice to migrate more into the muscle. Shrimps treated with mixed phosphates and 3% ProfixO had the higher moisture content than those treated with only mixed phosphates ($p<0.05$). Xiong and Kupski (1999b) report that, the enhanced moisture retention in salt-treated chicken fillets ostensibly resulted from increased capillary forces, due to counteracting the osmotic pressure, that enabled water to be chemically bound or physically entrapped in the muscle. From the result, ProfixO would produce a synergism with salt and mixed phosphates to dissociate shrimp muscle, especially actomyosin. Increased moisture retention ability by the phosphates is achieved through muscle fiber expansion (swelling) caused by electrostatic repulsion, which allow more water to be immobilized in the myofibril lattices (Offer and Trinick, 1983). For, protein and fat contents the higher values were found in shrimps with no treatment, compared with shrimp treated with both solutions ($p<0.05$). The contents of protein and fat were conversely proportional to the increased moisture content after treatment. Additionally, proteins were extracted and leached into the soaking solution. However, no differences in protein content of fresh and ice-stored shrimps were observed when treated with the solution containing with both mixed phosphates and 3% ProfixO ($p>0.05$). ProfixO played a role in preventing the disintegration of M-line protein and more compact structure was obtained. As a result, lower protein amount was leached out, espectively from M-line region. During soaking, NaCl, phosphates and ProfixO were penetrated into shrimp muscle. As a consequence, higher ash content was found in shrimp muscle compared with shrimp without treatment ($p<0.05$). Shrimps treated with mixed phosphate and ProfixO contained the highest ash content, indicating that inorganic compounds in ProfixO could be absorbed in the shrimp muscle to a high extent.

Table 9. Proximate composition of fresh and ice-stored Pacific white shrimp soaked in 1% NaCl containing mixed phosphates in the absence or presence of ProfixO

Composition (% wet weight basis)	Shrimp (no treatment)		Shrimp treated with mixed phosphates		Shrimp treated with mixed phosphates and ProfixO	
	0 day	7 days	0 day	7 days	0 day	7 days
Moisture	77.24*±0.20[f]**	81.01±0.08[c]	79.14±0.20[e]	82.19±0.04[b]	79.99±0.14[d]	83.69±0.17[a]
Protein	19.76±0.02[a]	17.65±0.09[b]	17.17±0.04[c]	15.47±0.07[f]	16.42±0.03[d]	16.09±0.01[e]
lipid	1.68±0.02[a]	1.59±0.03[a]	1.59±0.06[a]	1.27±0.01[b]	1.21±0.15[b]	1.15±0.03[b]
Ash	1.18±0.08[c]	0.54±0.09[d]	1.76±0.01[b]	1.76±0.10[b]	2.29±0.09[a]	2.31±0.12[a]

* Mean±SD from triplicate determination

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

5. Effect of frozen storage on physical and physiochemical properties of Pacific white shrimp

5.1 Changes in drip loss and cooking loss of Pacific white shrimp during frozen storage

Drip loss and cooking loss of shrimp with no treatment (No treatment), shrimp treated with mixed phoshates (1% NaCl + 0.875% SAPP + 2.625% TSPP) (MP) and shrimp treated with mixed phosphates in combination with 1% ProfixO (MP+PO) stored at -20 °C for up to 12 weeks are shown in Figures 34A and 34B, respectively. Drip loss and cooking loss of shrimp muscle increased with increasing frozen storage time ($p<0.05$). The lowest drip loss was observed in MP+PO ($p<0.05$). The increasing rate of drip loss was highest in shrimp with no treatment, especially after 6 weeks of storage. 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, 1997b). Drip loss has been linked to partial denaturation of proteins taking place during freezing, which leads to decreased

WHC (Shenouda, 1980; Mackie, 1994). The result suggested that mixed phosphates in combination with ProfixO most likely increased water holding capacity of proteins. Those compounds might enhance electrostatic repulsion of protein molecules, in which water could be entrapped more in the muscle. Alternatively, mixed phosphates and ProfixO might function by modifying water structure, thereby retarding the formation and growth of ice crystals in the muscle.

Cooking loss of shrimp soaked in mixed phosphate in combination with ProfixO was lower than that of shrimp soaked with and without mixed phosphates alone ($p<0.05$). When comparing cooking loss between MP+PO and shrimp with no treatment, the former showed approximately 50% lower cooking loss than the latter. Slight increase in cooking loss was observed as the storage time increased ($p<0.05$). Woyewoda and Bligh (1986) suggested that phosphate may improve the cooking yield of frozen cod fillets by maintaining protein hydration during frozen storage. The treatment of shrimp with mixed phosphates in combination with ProfixO was effective in decreasing cooking loss after cooking. ProfixO might assist proteins in holding water, though heating was applied. The water-binding varies with the type of proteins (Karmas and Turk, 1976). The combination of capelin protein hydrolysate (CPH) and salt was shown to increase the cooking yield of pork. CPH at higher concentration led to higher cooking yield (Shahidi *et al.*, 1995). The addition of shark protein hydrolysate gave similar results. Proteinaceous components in ProfixO might function as water binder, which could imbibe water in muscle after cooking.

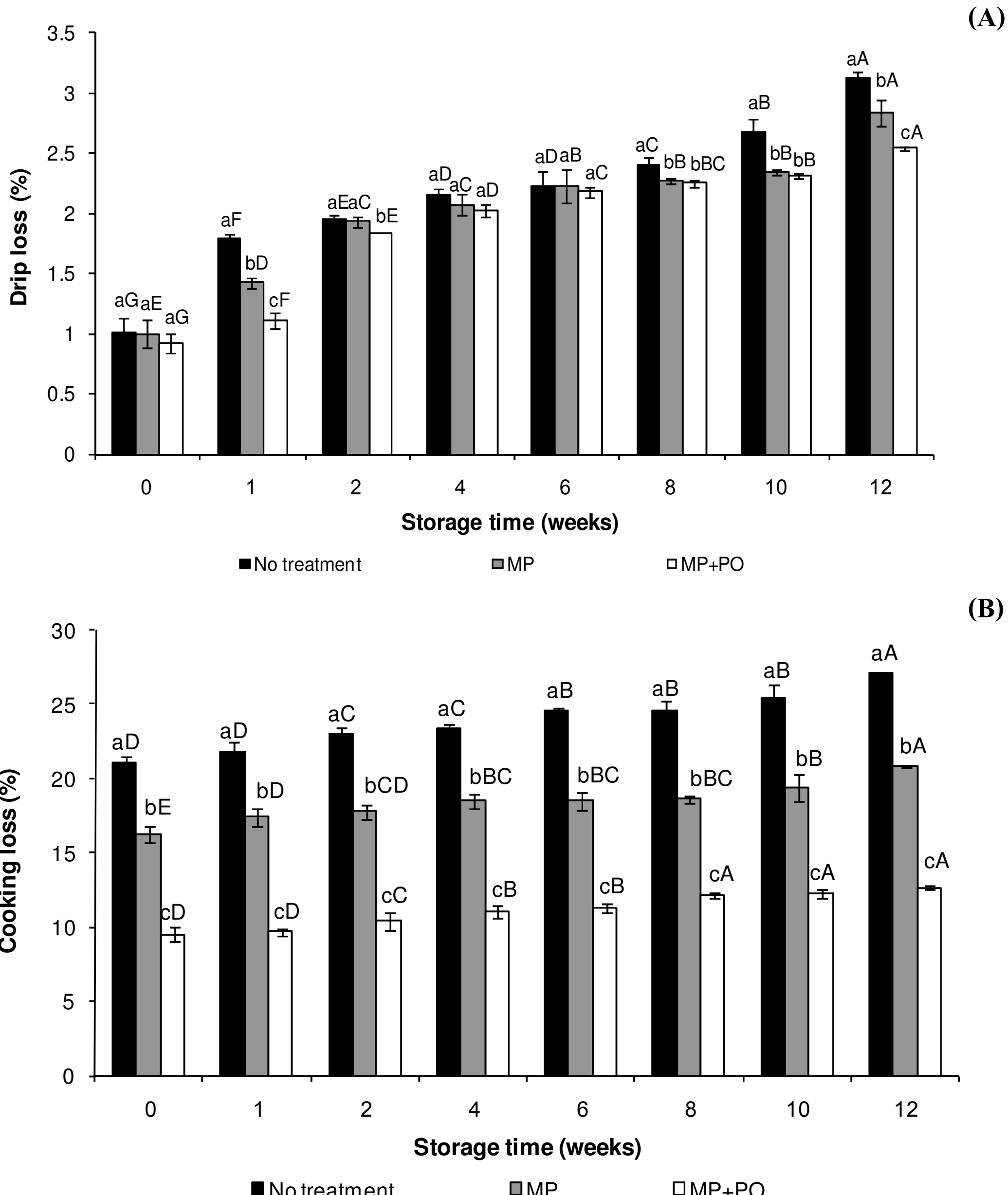


Figure 34. Changes in drip loss (A) and cooking loss (B) of Pacific white soaked in 1% NaCl containing mixed phosphates in combination without and with ProfixO during frozen storage. The different lower case letters within the same storage time indicate significant differences (p<0.05). The different upper case letters within the same treatment indicated significant differences (p<0.05). Bars represent the standard deviation from five determinations.

5.2 Changes in protein solubility of Pacific white shrimp during frozen storage

Solubility is a sensitive indicator and is widely used as an index for protein denaturation (Xiong, 1997b). Protein solubility in 0.6 M KCl of Pacific white shrimp including the shrimp with no treatment, MP and MP+PO is shown in Figure 35. During the storage, protein solubility of shrimps decreased continuously during prolonged storage ($p<0.05$). Formation of disulfide bonds and hydrophobic interaction during frozen storage was associated with the aggregate formed during frozen storage (Jiang *et al*., 1988). Jiang *et al*. (1988) found that solubility of milk fish actomyosin in 0.6M KCl decreased during frozen storage. Moreover, Raminez *et al*. (2000) found that protein solubility in 0.6M KCl of tilapia decreased sharply in the first 5 days of frozen storage at -20 °C, losing 20% of its initial solubility. Decrease in protein solubility by 73% was obtained at day 15. Those changes were accelerated in presence of salts whose the concentration was increased in unfrozen phase. Protein molecules may move closer together and aggregation subsequently occur (Yoshikawa et al., 1995). The lowest solubility was observed in shrimp with no treatment, especially when the storage time increased. After 12 weeks of frozen storage, solubility of shrimp with no treatment decreased to 39.08%, while solubility of MP and MP+PO decreased to 43.86 and 45.87%, respectively. Therefore, treatment of Pacific white shrimp using mixed phosphates either in combination with or without ProfixO could retard the loss in solubility of muscle during frozen storage. Mixed phosphates and ProfixO might reduce the denaturation of protein via binding with water as well as repulsive effect of protein molecules. As a consequence, the formation of ice crystals from free water could be lowered. The retarded losses in protein solubility of samples treated with mixed phosphates and ProfixO were in agreement with lower drip loss and cooking loss (Figure 34).

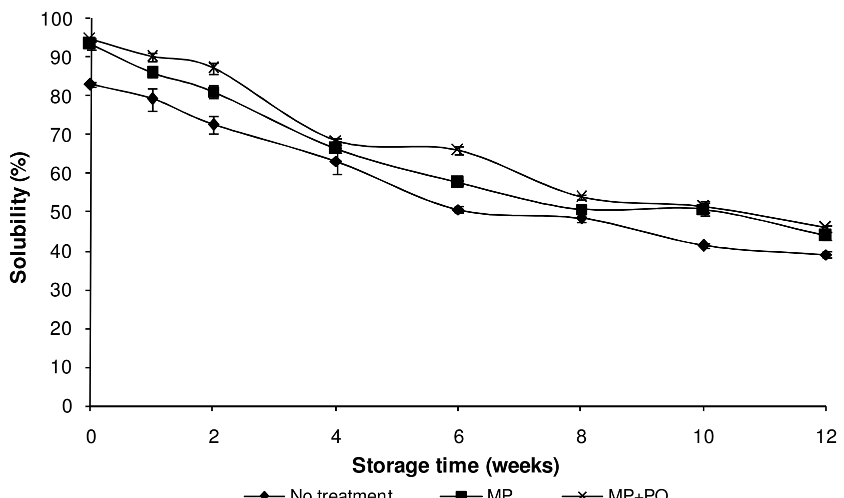


Figure 35. Changes in protein solubility of Pacific white shrimps soaked in 1% NaCl containing mixed phosphates in combination without and with ProfixO during frozen storage. Bars represent the standard deviation from triplicate determinations.

5.3 Changes in ATPase activity of Pacific white shrimp during frozen storage

ATPase activities of NAM extracted from Pacific white shrimp muscle during frozen storagse are depicted in Figure 36. At day 0, no differences in Ca^{2+}-ATPase activities of all samples were observed ($p>0.05$). Ca^{2+}-ATPase activities of NAM from all samples decreased throughout 12 weeks of frozen storage ($p<0.05$). The result suggested that muscle protein particularly myosin underwent denaturation during frozen storage. Ca^{2+}-ATPase activity of NAM extracted from MP+PO tended to show a slightly higher activity, while no differences were found between MP and shrimp with no treatment ($p>0.05$). Ca^{2+}-ATPase activity is a good indicator of the integrity of the myosin molecule (Roura and Crupkin 1995). Nambudiri and Gopakumar (1992) also found the decreased in ATPase activity of fresh water and brackish fish during frozen storage. A sharp decrease in activity for the tilapia surimi without cryoprotectants during extended frozen storage indicated the denaturation of myosin, especially in the head region. The decrease in ATPase activity was possibly associated with the oxidation of SH on myosin

globular head and tertiary structural changes, which were caused by ice crystals and increase in ionic strength of muscle system(Hamada *et al*., 1977; Jiang *et al*., 1988). Benjakul *et al*. (2003) reported the marked decrease in Ca^{2+}-ATPase activity in lizardfish, croaker, threadfin bream and bigeye snapper muscle after 24 weeks of frozen storage at -18 °C and the rearrangement of protein via protein–protein interaction also contribute to the loss in activity (Benjakul and Bauer, 2000).

Mg^{2+}-ATPase and Mg^{2+}-Ca^{2+}-ATPase activities of NAM extracted from all samples decreased during frozen storage (Figures 35B, 35C). Mg^{2+}-ATPase has been used to indicate the integrity of actin (Azuma and Conno, 1998) and Mg^{2+}-Ca^{2+}-ATPase activity can be used as the indicator for integrity of actin-myosin complex (Roura and Crupin, 1995; Benjakul *et al*., 1997). The result suggested that denaturation of actin and actin-myosin complex in NAM extracted from all samples took place during frozen storage. Mg^{2+}-ATPase activity was greater in MP+PO shrimp than were MP and shrimps with no treatment throughtout the storage ($p<0.05$), MP+PO and MP treatment could maintain actin to some degree during frozen storage. Mg^{2+}-ATPase activity of carp myofibril was found to increase in the first 5 days of frozen storage at -20 °C, followed by the gradual decrease up to 60 days (Azuma and Konno, 1998). Therefore the treatment with mixed phosphates together with Profix could retard the denaturation of actin during the extended frozen storage. The similar result was found for Mg^{2+}-Ca^{2+}-ATPase activity, in which mixed phosphates and ProfixO could maintain actomyosin more effectively.

For Mg^{2+}-EGTA-ATPase activity, it was found that the activity in all sample increased gradually. After 12 weeks of storage, the activity in shrimp with no treatment showed the highest activity, compared to that found in MP and MP+PO. Mg^{2+}-EGTA-ATPase is indicative of the integrity of troponin-tropomyosin complex (Benjakul *et al*., 1997). It was suggested that frozen storage induced the changes in troponin-tropomyosin in shrimp muscle. Jiang *et al*. (1988) also found the increase in Mg^{2+}-EGTA-ATPase activity of milkfish actomyosin during 8 weeks of frozen storage. Therefore, freezing and frozen storage resulted in the denaturation of myosin, actin and actin-myosin complex as shown by the decreases in Ca^{2+}-ATPase, Mg^{2+}-ATPase and Mg^{2+}-Ca^{2+}-ATPase activities, respectively, and denaturation of troponin-tropomyosin complex as indicated by the increase in Mg^{2+}-EGTA-ATPase activity.

However, the use of mixed phosphates in combination with or without ProfixO could lower the loss of ATPase activity of shrimp muscle during frozen storage.

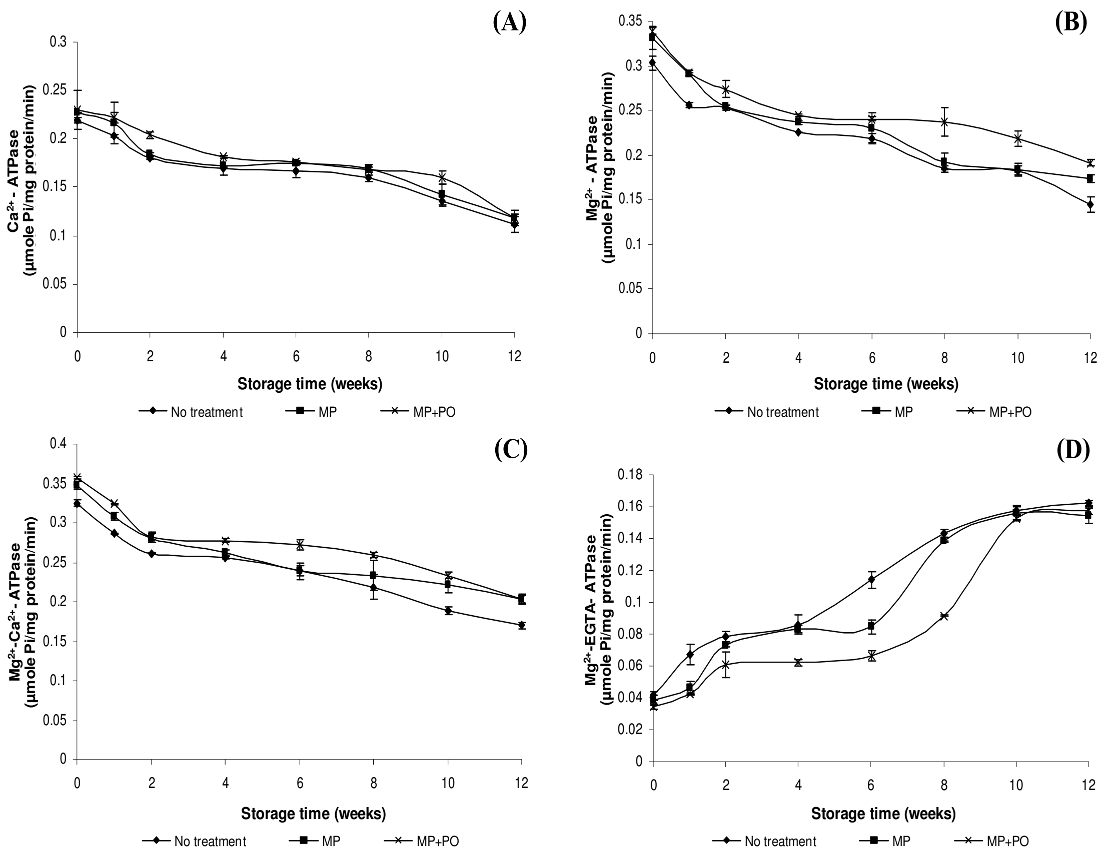


Figure 36. Changes in ATPase activities of natural actomyosin extracted from Pacific white shrimps soaked in 1% NaCl containing mixed phosphates in combination without and with ProfixO during frozen storage. Ca^{2+} -ATPase (A), Mg^{2+} -ATPase (B), Mg^{2+} -Ca^{2+} -ATPase (C) and Mg^{2+} -EGTA –ATPase (D). One unit activity was defined as that releasing 1 µmol Pi/mg protein/min. Bars represent the standard deviation from triplicate determinations.

The decrease in Ca^{2+}-sensitivity was observed in all samples as the storage time increased (Figure 37). During the first 10 weeks of storage, MP+PO showed the highest Ca^{2+}-sensitivity, followed by MP and shrimp with no treatment, respectively. The decrease in Ca^{2+}-sensitivity was in accordance with the increase in Mg^{2+}-EGTA-ATPase activity in all samples (Figure 36D). The result suggested that frozen storage induced the denaturation of tropomyosin in Pacific white shrimp muscle. Ca^{2+}-sensitivity of myofibrillar protein is attributed to the activity of native tropomyosin (Benjakul *et al*., 1997). The decrease in Ca^{2+}-sensitivity indicated the loss in Ca^{2+} regulation of troponin, which presumably underwent the conformation changes during frozen storage. Mixed phosphates together with ProfixO could retard the losses in Ca^{2+}-sensitivity.

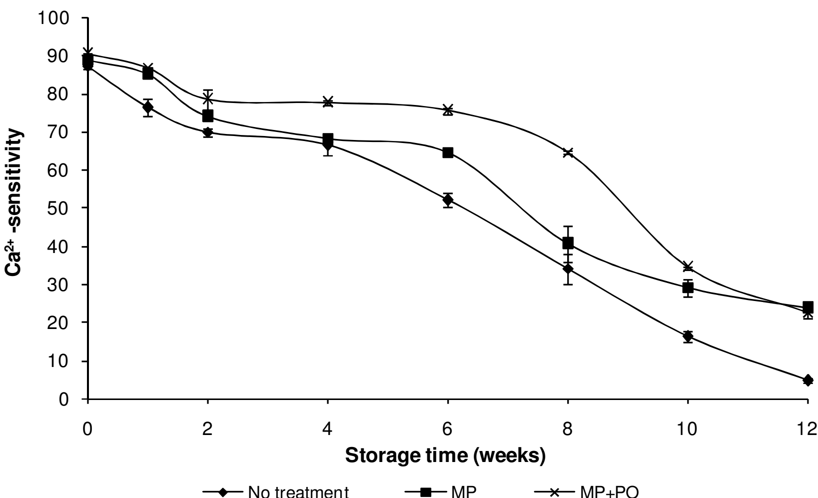


Figure 37. Changes in Ca^{2+}-sensitivity of natural actomyosin extracted from Pacific white shrimps soaked in 1% NaCl containing mixed phosphates in combination without and with ProfixO during frozen storage. Bars represent the standard deviation from triplicate determinations.

5.4 Changes in surface hydrophobicity of Pacific white shrimp during frozen storage

Increases in surface hydrophobicity (SoANS) of Pacific white shrimps were observed throughout the storage up to 12 weeks as shown in Figure 36. An increase in SoANS, is assumed to result from structural alterations, which, in some cases, would mean an irreversible denaturation (Nakai and Li-Chan, 1988). In general, surface hydrophobicity of NAM from shrimp with no treatment, MP and MP+PO shrimps increased markedly after 8 weeks of storage. After 12 weeks, surface hydrophobicity of NAM from MP and MP+PO shrimp was lower than shrimp with no treatment ($p<0.05$). During extended frozen storage, increase in surface hydrophobicity of protein during frozen storage can be attributed to the unfolding of proteins and the exposure of hydrophobic aliphatic amino acid (Badii and Howell, 2001). Del Mazo *et al.* (1994) and Cofardes (1994) also reported the increase in surface hydrophobicity during frozen storage of hake natural actomyosin. The increase in surface hydrophobicity was correlated with the reduction in ATPase activity (located at the myosin head) (Ang and Hultin, 1989). Hydrophobic interaction of protein molecules might take place, leading to the aggregation and loss in solubility (Figure 38). Benjakul *et al*. (2003) reported that the decrease in protein solubility was coincidental with the increase in surface hydrophobicity NAM from of croaker, threadfin bream and bigeye snapper during frozen storage at -18 °C for up to 24 weeks. When cryoprotectants were added, the slower increase in surface hydrophobicity of tilapia surimi was also found in accordance with slower decrease in protein solubility. It was suggested that these cryoprotectants could prevent the exposure of hydrophobic portions of proteins. Therefore the treatment of Pacific white shrimp with mixed phosphates, particularly in combination with ProfixO could decrease, the hydrophobic interaction of proteins, resulting in the remaining protein solubility.

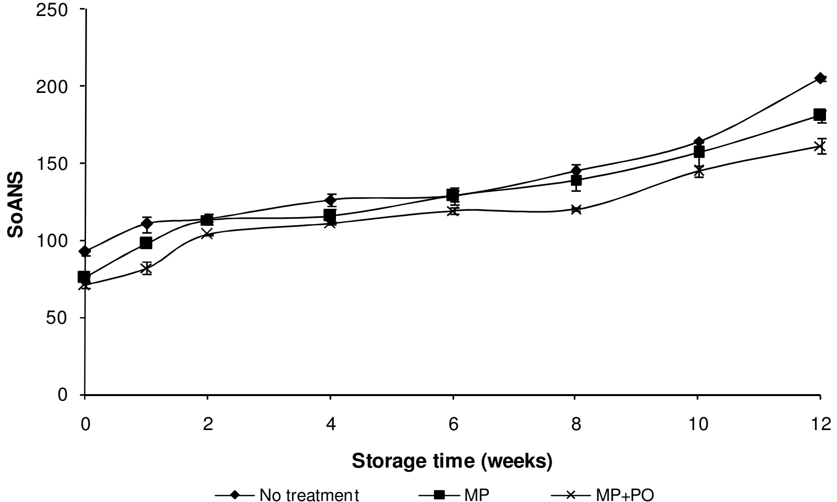


Figure 38. Changes in surface hydrophobicity of Pacific white shrimps soaked in 1% NaCl containing mixed phosphates in combination without and with ProfixO during frozen storage. Bars represent the standard deviation from triplicate determinations.

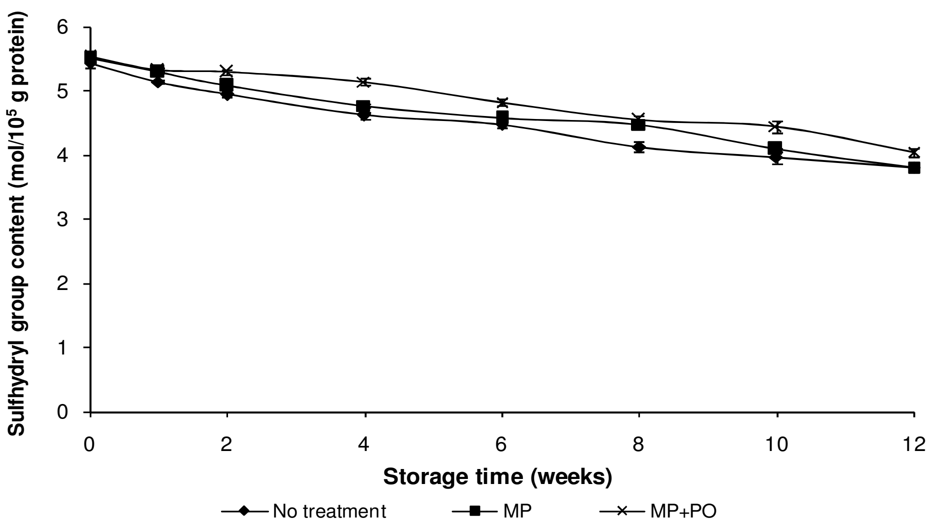
5.5 Changes in sulfhydryl group content and disulfide bond of Pacific white shrimp during frozen storage

Sulfhydryl (SH) group content of NAM extracted from all samples decreased gradually during storage up to 12 weeks ($p<0.05$) (Figure 39A). Among all samples, MP and MP+PO showed higher sulfhydryl group content, compared with shrimp with no treatment, throughout the storage of 12 weeks. After 12 weeks of frozen storage, the SH group contents of NAM from shrimp with no treatment, MP and MP+PO decreased to 3.80, 3.81 and 4.05 mole/10^5 g protein, respectively, and were decreased by 30.0, 30.8 and 26.6 % in comparison with their initial values, respectively. The differences in SH group content among samples during frozen storage were postulated to be due to the difference in susceptibility in sulfhydryl oxidation of myofibrillar proteins. Decrease in SH groups generally resulted from the formation of disulfide

bonds through oxidation of SH groups or disulfide interchanges (Hayakawa and Nakai, 1985). Mazo *et al*. (1999) also found that the sulfhydryl groups of NAM extracted from hake fillets decreased with increasing storage time at -20 and -30 °C. Ramirez *et al*. (2000) reported that reactive sulfhydryl groups decreased to 55% of the initial value after 5 days of frozen storage. Additionally, Jiang *et al.* (1988) reported that, during the first 2 weeks of frozen storage of milkfish actomyosin, the reactive sulfhydryl groups decreased. During prolonged storage at -20 °C, the tertiary structure of actomyosin was changed by the formation of disulfide, hydrogen and hydrophobic bonds. Consequently, the reactive sulfhydryl groups, masked in molecules, were gradually exposed and oxidized to disulfides. The rate of exposure and oxidation of reactive sulfhydryl groups might reach an equilibrium state during storage when the amount of the reactive sulfhydryl groups no longer changed (Jiang *et al*., 1988). The accelerated denaturation of myosin molecules, especially the conformational changes, in which the reactive sulfhydryl groups were exposed to oxidation, might result in the increased disulfide bond formation. Sultanbawa and Li-Chan (2001) found that the NAM and surimi from ling cod without cryoprotectants had increased amounts of disulfide bonds after freezing.

Disulfide bond formation in NAM was observed throughout the frozen storage of 12 weeks (Figure 39B). After 12 of frozen storage, disulfide bond content of NAM from shrimp with no treatment, MP and MP+PO decreased to 1.96, 1.91 and 1.85 mole/10^5 g protein, respectively. The increased disulfide bond content was generally coincidental with the decrease in sulfhydryl group content. It was noted that shrimp with no treatment contained the highest content of disulfide bond, followed by MP shrimp and MP+PO shrimp, respectively. Pacific white shrimps treated with mixed phosphates in combination with and without ProfixO tended to have the lower decrease in SH group content with the lower disulfide bond formation, compared with shrimp with no treatment.

(A)



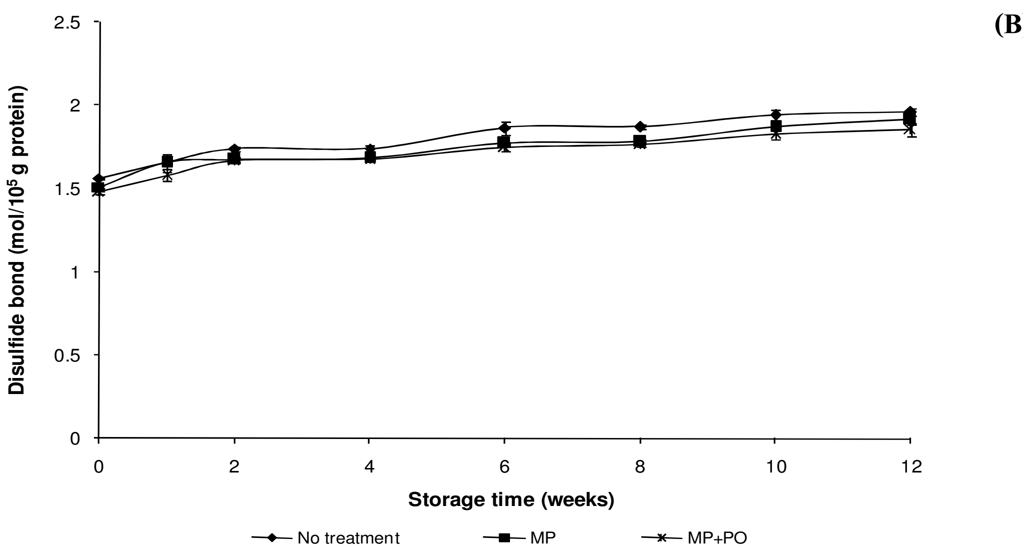


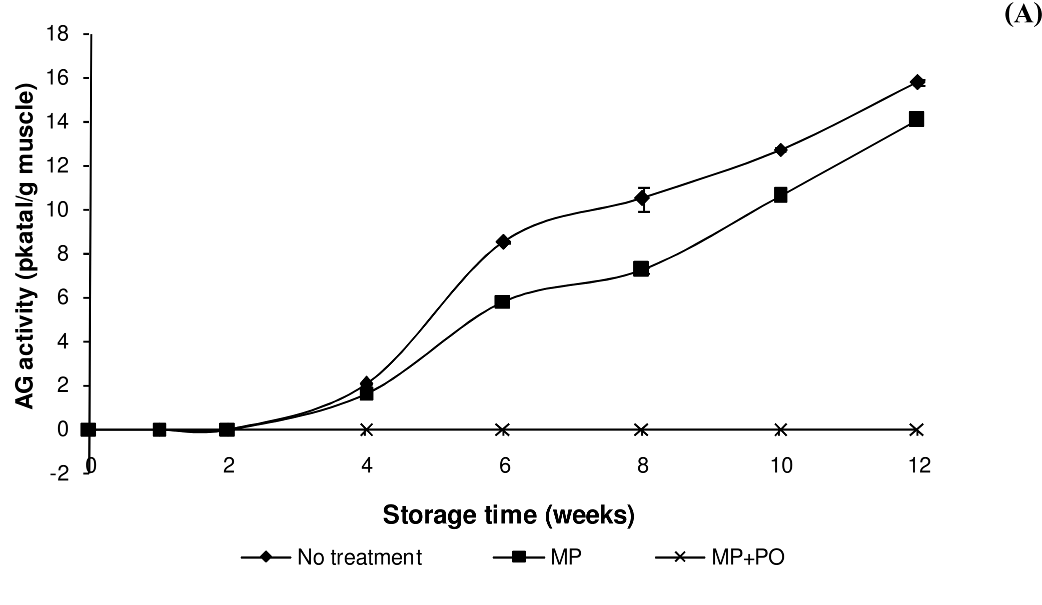
Figure 39. Changes in sulfhydryl group content (A) and disulfide bond (B) of natural actomyosin extracted from Pacific white shrimps soaked in 1% NaCl containing mixed phosphates in combination without and with ProfixO during frozen storage. Bars represent the standard deviation from triplicate determinations.

5.6 Changes in membrane integrity of Pacific white shrimp during frozen storage

Membrane integrity of Pacific white shrimp muscle was monitored during frozen storage up to 12 weeks using AG and NAG as the markers (Figure 40). AG and NAG have been used as the marker of freezing and thawing process of fish muscle (Benjakul and Bauer, 2000; Rehbein, 1979; Shimomura *et al.,* 1987). After 2 weeks of storage, both enzyme activities of shrimp with no treatment and MP increased as the frozen storage times increased ($p<0.05$) (Figures 40A and 40B). However, no AG and NAG activities were detectable in MP+PO since no exudates were collected for determination of enzyme activities. The differences in activities indicated the differences in stability of shrimp tissues during frozen storage. The formation and accretion of ice crystals, dehydration and increase in solute induced the changes in muscle tissue (Shenouda, 1980).

Cell damage of muscle was mainly attributed to ice crystal growth as well as the increased salt concentration in the unfrozen phase. Therefore, freezing can disrupt muscle cells, resulting in the release of mitrochondrial and lysosomal enzymes into sarcoplasm (Hamm, 1979). Thus, the integrity of muscle membrane of Pacific white shrimp could be retained during frozen storage with the treatment of mixed phosphates and ProfixO.

(A)



(B)

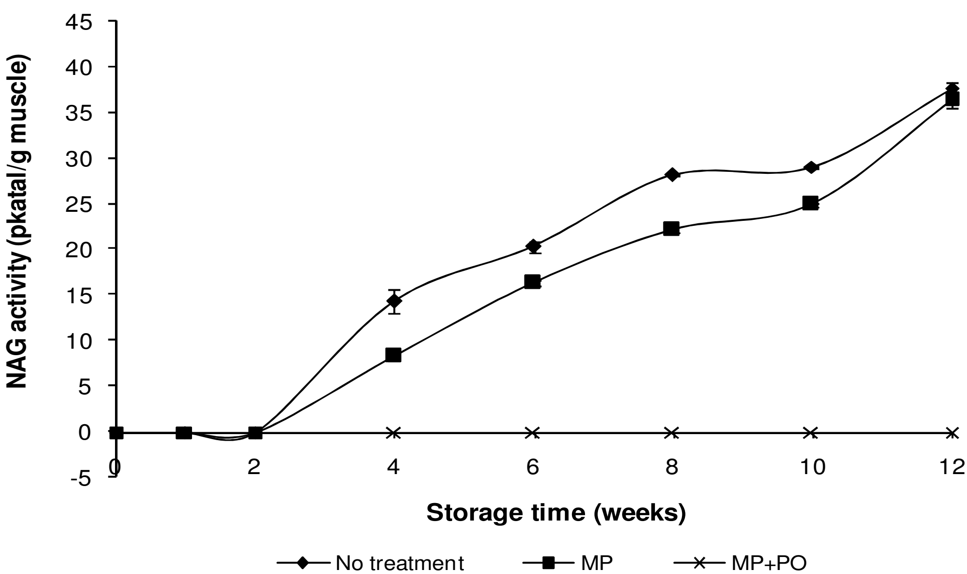


Figure 40. Changes in α-glucosidase (A) and β-N-acetyl-glucosaminidase (B) activity of Pacific white shrimp soaked in 1% NaCl containing mixed phosphates in combination without and with ProfixO. Bars represent the standard deviation from triplicate determinations.

6. Effect of freeze-thawing cycles on physical and physiochemical properties of Pacific white shrimp

6.1 Changes in drip loss and cooking loss of Pacific white shrimp after freeze-thawing

Drip loss and cooking loss of shrimp with no treatment, MP and MP+PO subjected to multiple freeze-thaw cycles are presented in Figures 41 A and 41B, respectively. Higher amounts of drip loss and cooking loss were observed when the freeze-thaw cycles increased ($p<0.05$). With the same freeze-thaw cycle, drip loss and cooking loss were much greater in shrimp with no treatment than were MP and MP+PO ($p<0.05$). Treatment of Pacific white shrimp with mixed phosphates decreased drip loss and cooking loss ($p<0.05$). The lowering effect was more pronounced when ProfixO was incorporated with mixed phosphates. The result suggested that mixed phosphates and ProfixO can prevent disintegration of muscle cell during freeze-thawing process as evidenced by lowered drip loss. Furthermore, during cooking of shrimp, those compounds might help in holding the water within the protein structures of shrimp muscle. Aaslyng *et al*. (2003) suggested that a higher cooking loss was registered for the samples with a low water holding capacity (high drip loss and thawing loss).

6.2 Changes in protein solubility of Pacific white shrimp after freeze-thawing

Protein solubility in 0.6 M KCl of Pacific white shrimp muscle subjected to multiple freeze-thaw cycles is depicted in Figure 42. Protein solubility of shrimps significantly ($p<0.05$) decreased when the freeze-thaw cycles increased ($p<0.05$). Among all samples, shrimp with no treatment had the greater decrease in protein solubility than did MP and MP+PO at all freeze-thaw cycles tested ($p<0.05$). After 5 freeze-thaw cycles, solubility was reduced to 70.1%

for shrimp with no treatment, while the solubility of 76.9%-77.8% was found in MP and MP+PO samples. ProfixO had no synergistic effect with mixed phosphates on maintaining protein solubility of Pacific white shrimp (p>0.05).

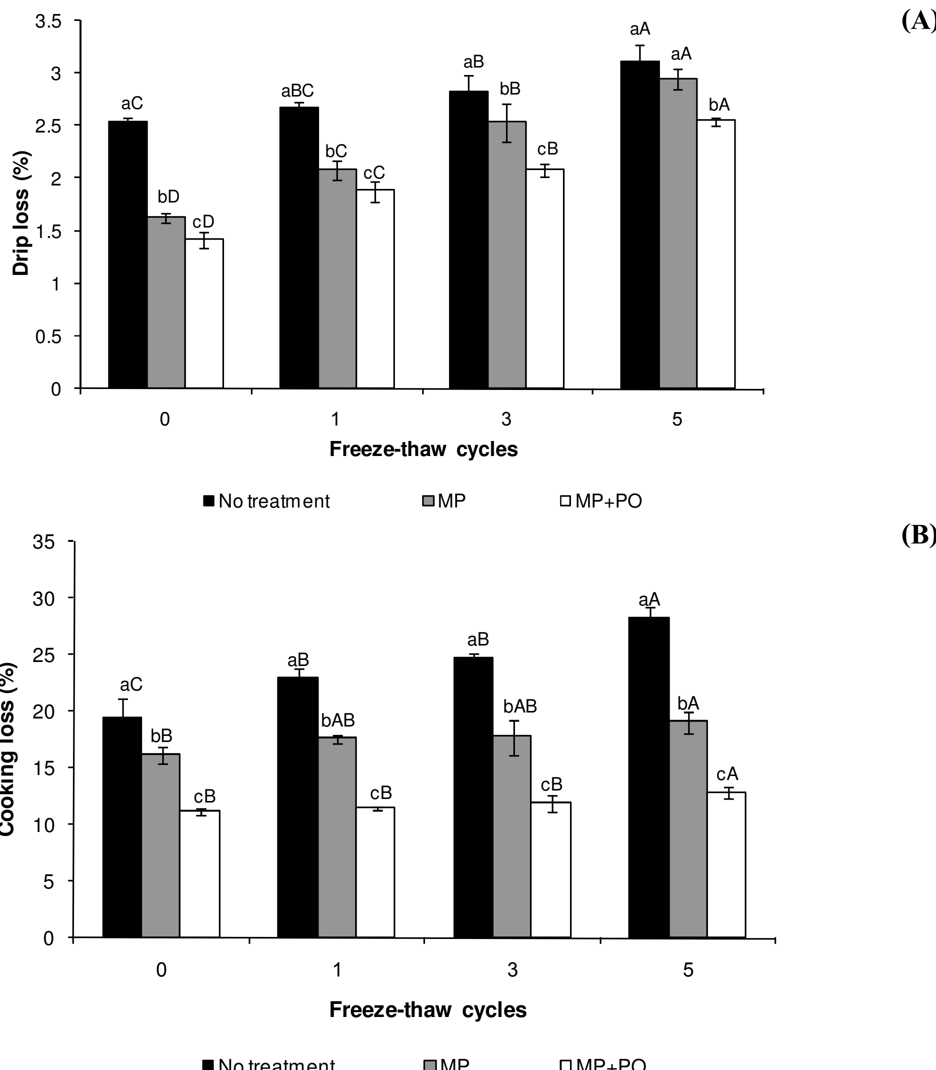


Figure 41. Changes in drip loss (A) and cooking loss (B) of Pacific white shrimp soaked in 1% NaCl containing mixed phosphates in combination without and with ProfixO and subjected to different-freeze thaw cycles. The different lower case letters within the same freeze-thaw cycle significant differences (p<0.05). The different upper case letters within the same treatment indicated significant differences (p<0.05). Bars represent the standard deviation from five determinations.

The loss of salt soluble protein suggested that protein denaturation was induced by the freeze-thawing process. The decrease in solubility of protein has been used as a marker of oxidative deterioration of muscle protein (Decker *et al.,* 1993; Srinivasan and Hultin, 1997; Xiong and Decker, 1995). Thermodynamically, a decrease in protein solubility is the result of a shift from a balance of protein intermolecular interaction and protein–water interaction, resulting in a situation, where protein intermolecular interaction is forced, while protein water interaction is weakened (Vojdani, 1996). As a result of loss of ordered tertiary structure, the cross-linkages are formed among proteins as evidenced by the decrease in solubility. Free radical attack is also a major cause of decreased protein solubility (Decker *et al.,* 1993).

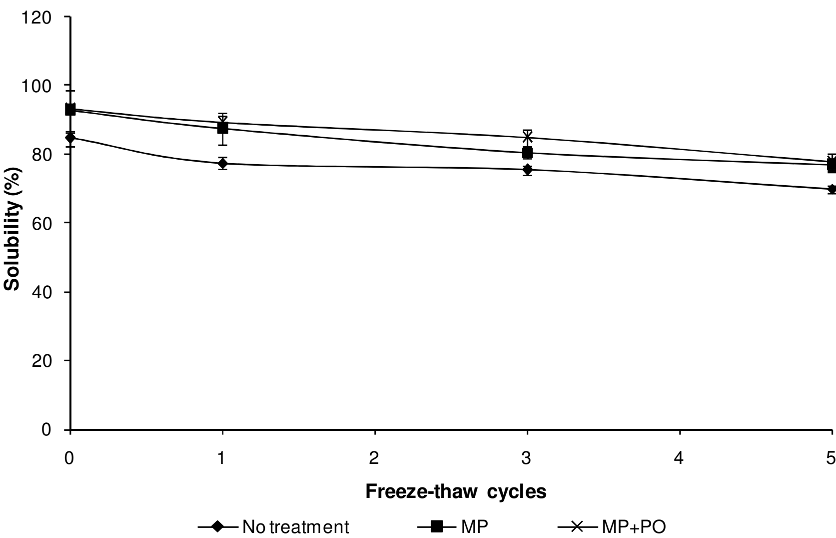


Figure 42. Changes in protein solubility of Pacific white shrimp soaked in 1% NaCl containing mixed phosphates in combination without and with ProfixO subjected to different freeze-thaw cycles. Bars represent the standard deviation from triplicate determinations.

6.3 Changes in ATPase activity of Pacific white shrimp after freeze-thawing

Ca^{2+} -ATPase, Mg^{2+} -ATPase and Mg^{2+} -Ca^{2+} -ATPase activities decreased as the freeze thaw cycles increased ($p<0.05$) (Figure 43). However, Mg^{2+} -EGTA-ATPase activity increased with increasing freeze-thaw cycles ($p<0.05$). In general, the greater changes in all ATPase activities were more intense in the shrimp with no treatment. The rate of changes was lowered when the shrimps were treated with mixed phosphates, especially when combined with ProfixO. From this trial, the decrease in Ca^{2+} -ATPase, Mg^{2+} -ATPase and Mg^{2+} -Ca^{2+} -ATPase activities after the freeze-thaw process indicated that myosin underwent denaturation and the actin-myosin complex was disrupted. The loss of ATPase activity was postulated to be due to the tertiary structural changes caused by ice crystals and the increase in ionic strength of the system (Benjakul and Bauer, 2000). The decrease in Ca^{2+}-ATPase activity was possibly due to the conformational changes of myosin globular head as well as the aggregation of this portion (Okada *et al.,* 1986). Rearrangement of proteins via protein-protein interaction induced by freeze-thawing process might contribute to the loss in ATPase activity (Benjakul and Bauer, 2000). The result suggested that muscle proteins, mainly myosin, underwent denaturaion to a lower extent when treated with mixed phosphates in combination with ProfixO. Additionally, the increases in Mg^{2+} -EGTA-ATPase activity was retarded after the treatment with mixed phosphates and ProfixO, suggesting the decreased changes in troponin-tropomyosin complexes. The lower decreases in Ca^{2+}-sensitivity were in accordance with the lower increases in Mg^{2+} -EGTA-ATPase activity (Figure 43D). Ca^{2+}-sensitivity had been used to indicate the denaturation of troponin C, which confers calcium regulation in contraction process of muscle. Thus, the used of mixed phosphates together with ProfixO most likely retarded the denaturation of troponin-tropomyosin complex as induced by repeated freeze-thawing.

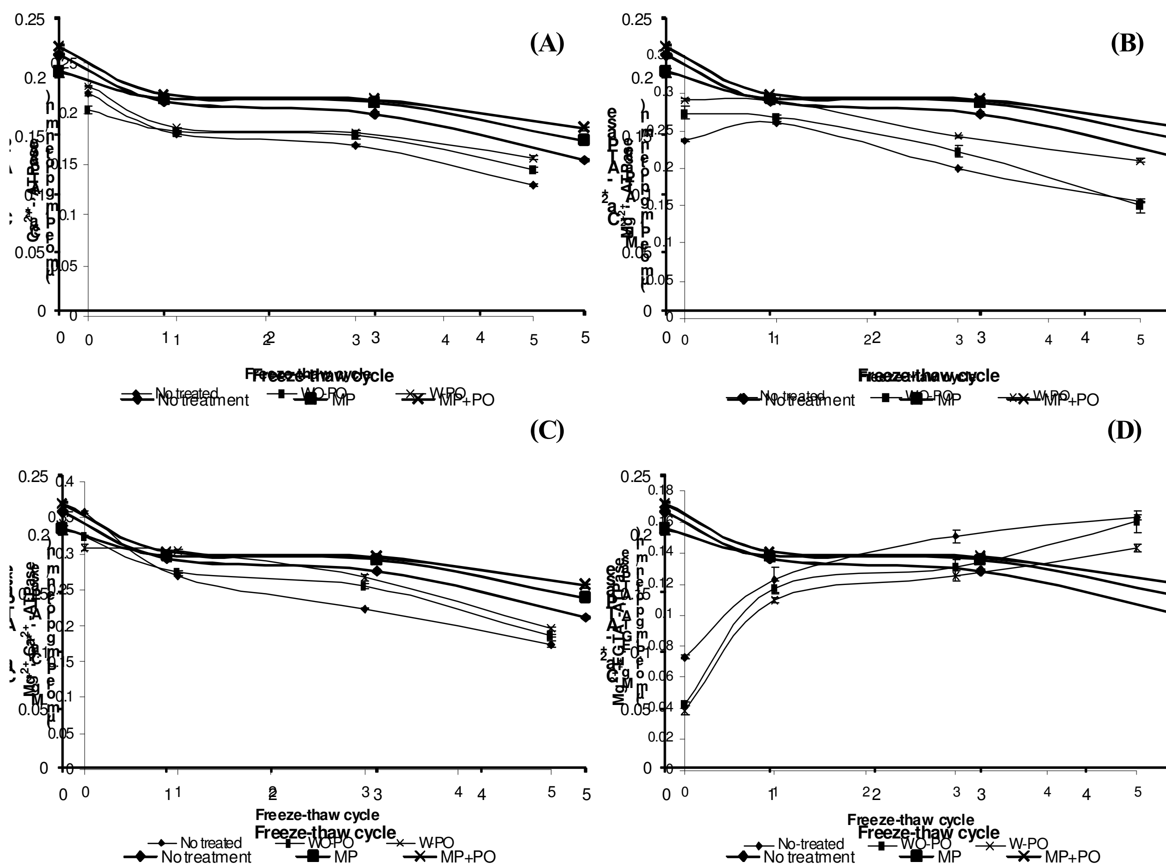


Figure 43. Changes in ATPase activities of natural actomyosin extracted from Pacific white shrimp soaked in 1% NaCl containing mixed phosphates in combination without and with ProfixO and subjected to different-freeze thaw cycles. Ca^{2+} -ATPase (A), Mg^{2+} -ATPase (B), Mg^{2+} -Ca^{2+} -ATPase (C) and Mg^{2+} -EGTA –ATPase (D). One unit activity was defined as that releasing 1 μmol Pi/mg protein/min. Bars represent the standard deviation from triplicate determinations.

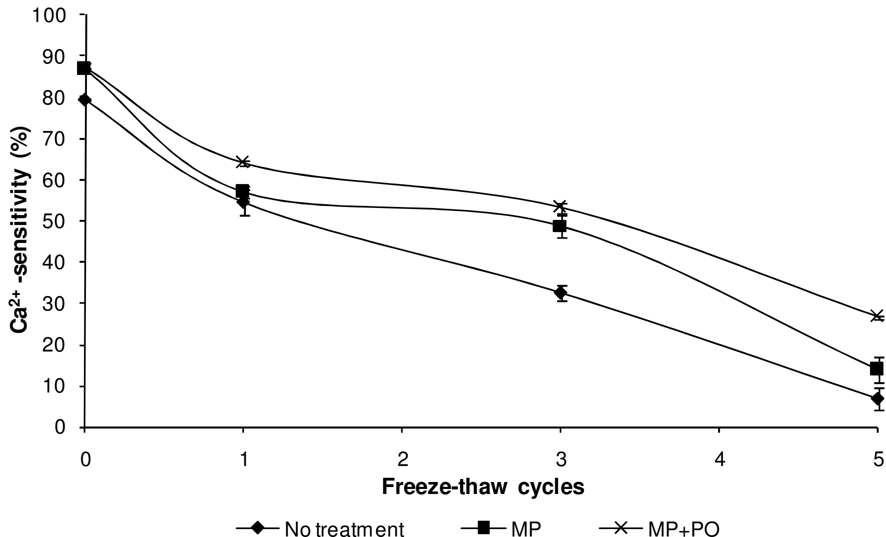


Figure 44. Changes in Ca^{2+}-sensitivity of natural actomyosin extracted from Pacific white shrimp soaked in 1% NaCl containing mixed phosphates in combination without and with ProfixO and subjected to different-freeze thaw cycles subjected to different freeze-thaw cycles. Bars represent the standard deviation from triplicate determinations.

6.4 Changes in surface hydrophobicity of Pacific white shrimp after freeze-thawing

The changes in surface hydrophobicity (SoANS) of NAM extracted from shrimps as influenced by freeze-thaw cycles are shown in Figure 45. Surface hydrophobicity of shrimp NAM increased when the freeze-thaw cycles increased ($p<0.05$). The increase was more pronounced in the shrimp with no treatment, compared with other samples. Increased surface hydrophobicity indicates an exposure of the interior of the molecule due to denaturation or aggregation (Multilangi *et al.*, 1996). At the same freeze-thaw cycle, SoANS of shrimp with no treatment was greater than that of MP and MP+PO, suggesting that treatment with mixed phosphates and ProfixO could prevent unfolding of protein and the exposure of hydrophobic aliphatic and aromatic amino acids to some extent. This might be associated with the ability of

those compounds in binding the water in the muscle. As a result, the water remaining could stabilize protein structure to some degree as indicated by lowered increase in SoANS.

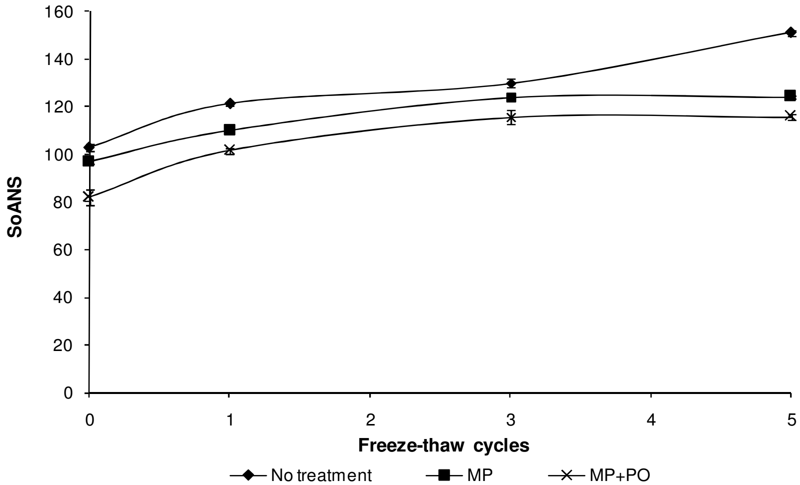


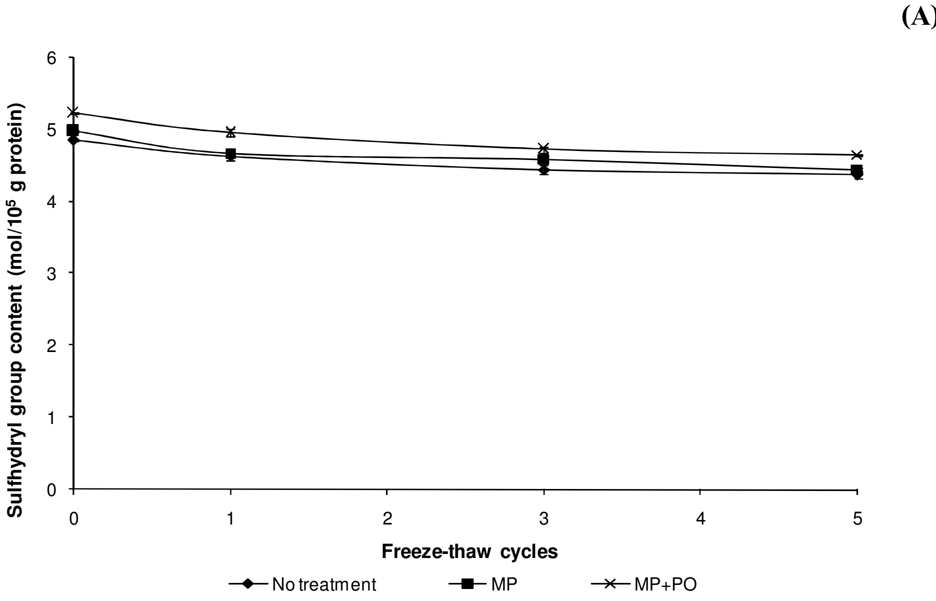
Figure 45. Changes in hydrophobicity of Pacific white shrimp soaked in 1% NaCl containing mixed phosphates in combination without and with ProfixO and subjected to different-freeze thaw cycles subjected to different freeze-thaw cycles. Bars represent the standard deviation from triplicate determinations.

6.5 Changes in sulfhydryl group content and disulfide bond of Pacific white shrimp after freeze-thawing

Sulfhydryl group content of NAM extracted from shrimps decreased with increasing freeze-thaw cycles ($p<0.05$) (Figure 46A). Sulfhydryl group content of shrimp with no treatment, MP and MP+PO NAM decreased to 4.37, 4.45 and 4.66 mole/10^5 g protein, respectively after 5 freeze-thaw cycles. The decrease in sulfhydryl group content was in agreement with the increase in disulfide bond content (Figure 46B). It was postulated that sulfhydryl group more likely underwent oxidation and disulfide bonds were found. With repeated freeze-thawing, the conformational changes, in which the reactive SH groups were exposed to

oxidation, might result in the increased disulfide bond formation. Cysteine is perhaps the most susceptible amino acid residue and it is usually one of the first to be oxidized (Thanonkaew *et al.,* 2006). Another sulfur-containing amino acid, methionine, is readily oxidized to methionine sulfoxide derivative (Vogt, 1995). Benjakul and Bauer (2001) reported that lipid oxidation took place to a greater extent with increasing freeze-thawing. Oxidation products might accelerate the oxidation of sulfhydryl groups. The amino acid with reactive side chain (sulfhydryl, thioether, amino group, imidazole ring and indole ring) are particularly susceptible to oxidation initiated by oxidizing lipid and their products (Gardner, 1979). This result indicated that the freeze-thawing process accelerated the formation of disulfide bonds. It was noted that shrimp with no treatment contained the highest content of disulfide bond, followed by MP shrimp and MP+PO shrimp, respectively. Therefore, shrimp treated with mixed phosphates in combination with ProfixO could retard the oxidation of sulfhydryl group of shrimp muscle proteins. This was in agreement with the lowered decreases in Ca^{2+}- and Mg^{2+}-Ca^{2+}-ATPase activities (Figure 43).

(A)



(B)

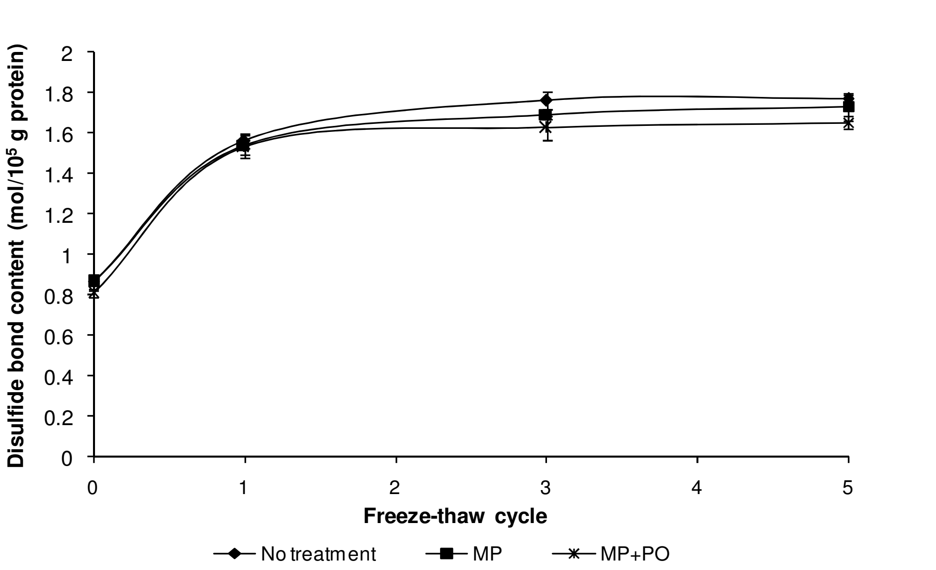
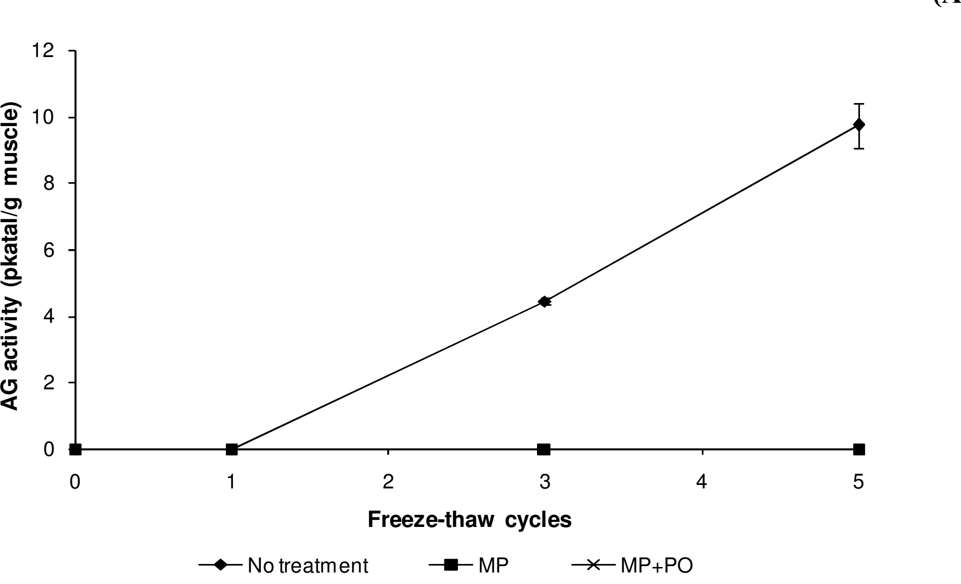


Figure 46. Changes in sulfhydryl group content (A) and disulfide bond (B) of natural actomyosin extracted from Pacific white shrimp soaked in 1% NaCl containing mixed phosphates in combination without and with ProfixO and subjected to different-freeze thaw cycles. Bars represent the standard deviation from triplicate determinations.

6.6 Changes in membrane integrity of Pacific white shrimp after freeze-thawing

AG and NAG have been used as the marker of freezing and thawing process of fish muscle (Benjakul and Bauer, 2000; Rehbein, 1979; Shimomura *et al.,* 1987). Both enzyme activities, as indicator of membrane disintegration, of the shrimp with no treatment increased as the freeze-thaw cycles increased ($p<0.05$) (Figures 47A and 47B). Mitochondrial and lysosomal enzymes have been used to differentiate fresh and frozen muscle. Rehbein (1979) found that the specific activities of α-glucosidase, β-glucuronidase, β-galactosidase and β-N-acetyl-glucosaminidase in pressed juice increased after the freeze-thaw process, while no changes in acid phosphatase were observed. Shimomura *et al*. (1987) reported an increase in activity of AG, NAG and cathepsin D in pressed juice of frozen-thawed fish. The increase in activity of AG and NAG of the shrimp with no treatment indicated the disintegration of membrane structure caused by the freeze-thaw cycles. Freezing and thawing can disrupt muscle cells, resulting in the release of enzymes from lysosomes and mitochondria into the sarcoplasm (Hamm, 1979). The thawing method can also play a role in cell damage, which can be indicated by the release of enzyme. Slow thawing caused a greater release of enzyme from mitochondria into the sarcoplasmic fluid than fast thawing (Benjakul and Bauer, 2000). AG and NAG activities were found only in shrimp with no treatment ($p<0.05$). This was because no exudates was obtained in samples treated with mixed phosphates either without and with ProfixO. It was postulated that the damage of cell might occur to some degree in treated samples, which could not be detected by AG and NAG released in exudates. However, cell disruption induced by repeated freeze-thawing could be retarded by treatment with mixed phosphate, regardless of ProfixO combination.

(A)



(B)

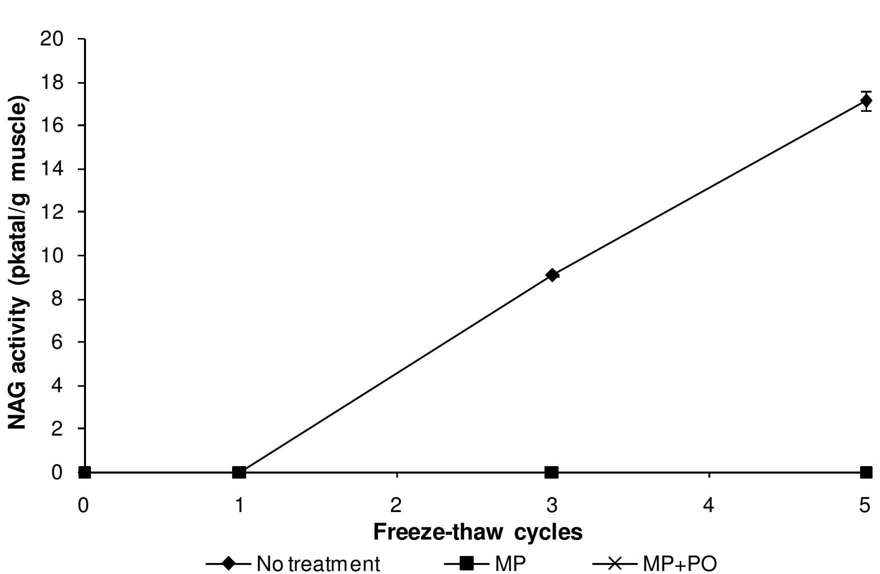


Figure 47. Changes in α-glucosidase (A) and β-N-acetyl-glucosaminidase (B) activity of Pacific white shrimp soaked in 1% NaCl containing mixed phosphates in combination without and with ProfixO and subjected to different-freeze thaw cycles. Bars represent the standard deviation from triplicate determinations.

CHAPTER 4

CONCLUSIONS

1. Type of phosphates determined the efficiency in quality improvement of Pacific white shrimp. The use of SAPP in combination with TSPP or STPP at an appropriate level could reduce translucence of cooked shrimp. 2.5% NaCl containing 2.625% TSPP + 0.875% SAPP was the appropriate soaking solution, which could reduce translucence of cooked shrimps, while still maintained weight gain, cooking loss and cooking yield.
2. The efficacy of mixed phosphates in quality improvement was governed by the freshness of shrimp. The greater translucence was found in shrimp with lower freshness after phosphate treatment, in which M-line was disappeared after heating.
3. The use of 3% ProfixO in combination with mixed phosphates resulted in the greater efficacy in improvement of quality of both fresh and ice-stored Pacific white shrimp, in comparison with the used of only ProfixO. However, the incorporation of ProfixO in soaking solution yielded the shrimp with higher translucence.
4. Concentration of salt used in soaking solution and soaking time affected the quality improvement of Pacific white shrimp. The use of 1% NaCl containing mixed phosphates without and with 3% ProfixO for 240 min resulted in the maximized quality improvement of Pacific white shrimp.
5. Treatment of shrimp with mixed phosphate in combination with or without ProfixO could retard the denaturation of Pacific white shrimp muscle protein and quality loss during frozen storage and repeated freeze-thawing.

REFERENCES

Aaslyng, D. M., Bejerholm, C., Ertbjerg, P., Bertram, C. H. and Andersen, J. H. 2003. Cooking loss and juiciness of pork in relation to raw meat quality and cooking procedure. Food Qual. Pref. 14: 277-288.

AOAC. 2000. Official Method of Analysis. 16th ed. Association of Official Analytical Chemists. Washington DC, USA.

Aktas, N. and Kaya, M. 2001. The influence of marinating with weak organic acids and salts on the intramuscular connective tissue and sensory properties of beef. Eur. Food Res. Technol. 213: 88-94.

An, H., Weerasinghe, V., Seymour, T. A. and Morrissey, M. T. 1994. Cathepsin degradation of Pacific whiting surimi proteins. J. Food Sci. 59: 1013-1017, 1033.

Ang, J. I. T. F. and Hultin, H. O. 1989. Denaturation of cod myosin during freezing after modification with formaldehyde. J. Food Sci. 54: 814-818.

Ashie, I. N. A. and Simpson, B. K. 1997. Purification and characterization of cathepsin L from hepatopancreas of carp *Cyprinus carpio.* Comp. Biochem. Physiol. 115B: 531-537.

Azuma, Y. and Konno, K. 1998. Freeze denaturation of carp myofibril compared with thermal denaturation. Fish. Sci. 64: 287-290.

Badii, F. and Howell, N. K. 2001. A comparison of biochemical changes in cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) fillets during frozen storage. J. Sci. Food Agric. 82: 87-97.

Barely, T. H., Schapira, D. V. and Williams, G. T. 1983. The moisture and proximate composition of commercial fresh and frozen shrimp species as affected by phosphate treatments. J. Food Sci. 51: 878-881.

Beas, V. E., Wagner, J. R., Anon, M. C. and Crupkin, M. 1991. Thermal denaturation in fish muscle proteins during gelling: Effect of spawning condition. J. Food Sci. 56: 281-284.

Bechtel, J. P. 1986. Muscle development and contractile proteins. *In* Muscle as Food (Bechtel, J.P., ed.) pp. 1-35, London, UK: Academic Press.

Badii, F. and Howell, N. K. 2001. A comparison of biochemical changes in cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) fillets during frozen storage. J. Sci. Food Agric. 82: 87-97.

Badii, F. and Howell, N. K 2002. Changes in texture of cod and haddock fillets during frozen storage. Food Hydrocol. 16: 313-319.

Barnoski, E. S., Nip, W. K. and Moy, J. H. 1984. Partial characterization of a crude enzyme extract from the freshwater prawn, *Macrobachrium rosenbergii.* J. Food Sci. 49: 1494-1501.

Beas, V. E., Wagner, J. R., Crupkin, M. and Anon, M. C. 1990. Thermal denaturation of hake (*Merluccius hubsi*) myofibrillar proteins. A differential scanning calorimetric and electrophoretic study. J. Food Sci. 55: 683-687.

Bechtel, J. P. 1986. Muscle development and contractile proteins. *In* Muscle as Food. (Bechtel, J. P., ed.), pp. 1-35. London, UK: Academic Press.

Bendall, J. R. 1954. The swelling effect of polyphosphates on lean meat. J. Sci. Food Agric. 5: 468–475.

Benjakul, S. and Bauer, F. 2000. Physicochemical and enzymatic changes of cod muscle proteins subjected to different freeze-thaw cycles. J. Sci. Food Agric. 80: 1143-1150.

Benjakul, S. and Bauer, F. 2001. Biochemical and physicochemical changes in catfish (*Silurus glanis* Linne) muscle as influenced by different freeze-thaw cycles. Food Chem. 72: 207-217.

Benjakul, S., Seymour, T. S., Morrissey, M. T. and An, H. 1997. Physicochemical changes in Pacific whiting muscle proteins during iced storage. J. Food Sci. 62: 729-733.

Benjakul, S., Visessanguan, W., Aewsiri, T. and Tanaka, M. 2007. Dissociation of natural actomyosin from kuruma prawn muscle induced by pyrophosphate. Food Chem. 102: 295-301.

Benjakul, S., Visessanguan, W., Riebroy, S., Ishizaki, S. and Tanaka, M. 2002. Gel-forming properties of surimi produced from bigeye snapper, *Priacanthus tayenus* and *P. macracanthus*, stored in ice. J. Sci. Food Agric. 82: 1442–1451.

Benjakul, S., Visessanguan, W., Thongkaew , C. and Tanaka, M. 2003. Comparative study on physicochemical changes of muscle proteins from some tropical fish during frozen storage. Food Res. Intern. 36: 787-795.

Busconi, L., Folco, E. J., Martone, C. B. and Sanchez, J. J. 1989. Postmortem changes in cytoskeletal elements of fish muscle. J. Food Biochem. 13: 443-451.

Buttkus, H. 1974. On the nature of the chemical and physical bonds which contribute to some structure properties of protein foods: a hypothesis. J. Food Sci. 39: 484-489.

Chang, C. C. and Regenstein, J. M. 1997. Water uptake, protein solubility, and protein changes of cod mince stored on ice as affected by polyphosphates. J. Food Sci. 62: 305-309.

Connell, J. J. 1995. Control of Fish Quality. p 245. Oxford, England: Fishing News Books.

Crawford, D. L. 1981. Meat yield and shell removal functions of shrimp processing. Available from: http://seagrant.oregonstate.edu/sgpubs/onlinepubs/shrimpyield. html. Accessed December 2006.

Davies, J. R., Bardsley, R. O., Ledward, D. A. and Poulter, R. G. 1988. Myosin thermal stability in fish muscle. J. Sci. Food Agric. 45: 61-68.

Damodaran, S. 1996. Functional properties. *In* Food Proteins Properties and Characterization (Nakai, S. and Modler, H. W., eds.). pp. 167-233. New York, USA: VCH Publishers.

Decker, E. A., Xiong, Y. L., Calvert, J. T., Crum, A. D. and Blanchard, S. P. 1993. Chemical, physical and functional properties of oxidized turkey white muscle myofibrillar proteins. J. Agric. Food Chem. 41: 186-189.

Del Mazo, M. L., Huidobro, A., Torrejon, P., Tejada, M. and Careche, M. 1994. Role of formaldehyde in formation of natural actomyosin aggregates in hake during frozen storage. Z. Lebensm. Unters. Forsch. 198: 459–464.

Dutson, T. R., Pearson, A. M., Merkel, R. A. and Spink, G. C. 1974. Ultrastructural postmortem changes in normal and low quality porcine muscle fibers. J. Food Sci. 39: 32–37.

Dziezak, J. D. 1990. Phosphates improve many foods. Food Technol. 44(4): 80-92.

Ebachi, S., Kodama, A. and Ebashi, F. 1968. Troponin I. Preparation and physiological function. J. Biochem. 64: 465-477.

Eckhoff, M. K., Aidos, I., Hemre, G. I. and Lie, O. 1998. Collagen content in farmed Atlantic salmon (*Salmo salar*, L.) and subsequent changes in solubility during storage on ice. Food Chem. 62: 197-200.

Ellinger, R. H. 1972a. Nomenclature, classification and structure of phosphates used in foods. *In* Phosphates as Food Ingredients. p. 3. CRC Press. Boca Raton, Florida, USA.

Ellinger, R. H. 1972b. The functions and applications of phosphates in food systems. *In* Phosphates as Food Ingredients. p. 31. CRC Press. Boca Raton, Florida, USA.

Ellinger, R. H. 1975. Phosphates in food processing. *In* Hand Book of Food Additives. (Furia, T.E., ed.). pp. 645-698. CRC Press. Cleveland, California, USA.

Ellinger, R. H. 1977. Phosphates in food processing. *In* CRC Handbook of Food Additives. (Furia, T.E., ed.). p. 671. CRC Press. Boca Raton, Florida, USA.

Erdogdu, F., Balaban, M. O., Otwell, W. T. and Laura Garrido, L. 2004. Cook-related yield loss for Pacific white (*Penaeus vannamei*) shrimp previously treated with phosphates: effects of shrimp size and internal temperature distribution. J. Food Eng. 64: 297-300.

Etherington, D. J. 1984. The contribution of proteolytic enzymes to postmortem changes in muscle. J. Anim. Sci. 59: 1644-1650.

Fennema, O. R. 1990. Comparative water-holding properties of various muscle foods. A critical review relating to definitions, methods of measurement, governing factors, comparative data and mechanistic matters. J. Muscle Foods 1: 363–381.

Franks, F. 1995. Protein destabilization at low temperature. Adv. Prot. Chem. 46: 105–139.

Froning, G. W. and Sackett, B. 1985. Effect of salt and phosphates during tumbling of turkey breast muscle on meat characteristics. Poultry Sci. 64: 1328–1333.

Fiske, C. H. and Subbarow, Y. 1925. The colorimetric determination of phosphorous. J. Biol. Chem. 66: 375-400.

Foegeding, E. A., Lanier, T. C. and Hultin, H. O. 1996. Characteristics of edible muscle tissues. *In* Food Chemistry (Fennema, O.R., ed.). pp. 879-942. Marcel Dekker, Inc. New York, USA.

Gardner, H. W. 1979. Lipid hydroperoxide reactivity with proteins and amino acid: a review. J. Agric. Food Chem. 27: 220-229.

Geesink, G. H., Koohmaraie, M. 1999. Effect of calpastatin on degradation of myofibrillar proteins by μ-calpain under postmortem conditions. J. Anim. Sci.77: 2685–92.

Goodband, R. 2002. Functional properties of fish proteins. *In* Seafoods-Quality, Technology and Nutraceutical Applications. (Alasalvar, C. and Taylor, T., eds.). pp. 73-82. Springer. Berlin, Germany.

Greaser, M. 1997. Proceeding of the 13th European Symposium on the Quality of Poultry, September 21-26, Poznan, Poland. pp. 281-289.

Haard, N. F. 1992. Biochemical reactions in fish muscle during frozen storage. *In* Advances in Seafood Biochemistry, Composition and Quality (Flick, G.J. and Martin, R.E., eds.). p. 305. Technomic. New York, USA.

Haard, N. F. 1994. Protein hydrolysis in seafoods. *In* Seafood: Chemistry, Processing Technology and Quality (Shahidi, F. and Botta, J. R., eds.). pp. 10–30. Blackie Academic & Professional. New York, USA.

Ham, A. W. and Cormack, D. H. 1979. Histology. 8th ed. Lippincott Williams & Wilkins. Pennsylvania, USA.

Hamada, I., Tsuji, K., Nakayama, T. and Niwa, E. 1977. Oxidative denaturation of actomyosin. Bull. Japn Soc. Sci. Fish. 43: 1105–1109.

Hamm, R. and Grau, R. 1958. The water binding capacity of mammalian muscle. IV. The action of salts of weak acid. Z. Lebensm. Unters. Forsch. 108: 180-293.

Hamm, R. 1970. Interactions between phosphates and meat proteins. *In* Symposium: Phosphates in Food Processing. (Deman, J.M. and Melnychyn, P., eds.). The AVI Publishing Co. pp. 65-90. Westport. Connecticut, USA.

Hamm, R. 1979. Delocalization of mitochondrial enzymes during freezing and thawing of skeletal muscle. *In* Proteins at Low Temperatures. Advances in Chemistry Series (vol. 180) (Fennema, O.R., ed.). pp. 191. American Chemical Society. Washington DC, USA.

Hasegawa, H. 1987. Laboratory manual on analytical methods and procedures for fish and fish products. Marine Fisheries Research Department SEAFDEC. Singapore.

Hasting, R. J., Rodger, G. W., Park, R., Matthews, A. D. and Anderson, E. M. 1985. Differential scanning colorimetry of fish muscle: the effect of processing and species variation. J. Food Sci. 50: 1503-1510.

Hatae, K., Tobimatsu, A., Takeyama, M. and Matsumoto, J. J. 1986. Contribution of the connective tissues on the texture difference of various fish species. Bull. Japn. Soc. Sci. Fish. 52: 2001-2007.

Hayakawa, S. and Nakai, S. 1985. Contribution of hydrophobicity, netcharge, and sulfhydryl groups to thermal properties of ovalbumin. Can. Inst. Food Sci. Technol. J. 18: 290–295.

Henson, L. S. and Kowalewski, K. M. 1992. Use of phosphate in Seafood. INFOFISH Inter. 5: 52-54.

Hernandez-Herrero, M. M., Duflos, G., Malle, P., and Bouquelet, S. 2003. Collagenase activity and protein hydrolysis as related to spoilage of iced cod (*Gadus morhua*). Food Res. Intern. 36: 141–147.

Heymann, H., Hedrick, H. B., Karrasch, M. A., Eggeman, M. K. and Ellersieck, M. R. 1990. Sensory and chemical characteristic of fresh pork roasts cooked to different centre temperatures. J. Food Sci. 55: 613-617.

Hsieh, Y. L. and Regenstein, J. M. 1989. Texture changes of frozen stored cod and ocean perch minces. J. Food Sci. 54: 824–826.

Honikel, K. O. 1989. The meat aspects of water and food quality. *In*: Water and Food Quality (Hardman TM, ed.). pp 277-304. Elsevier Applied Science. London, UK.

Horowits, R., Kempner, E. S., Bisher, M. E. and Podolsky, R. J. 1986. A physiological role for titin and nebulin in skeletal muscle. Nature. 323: 160-162.

Jiang, S. T., Hwang, B. S. and Chen, C. S. 1988. Effect of storage temperatures on the formation of disulfides and denaturation of milkfish actomyosin (*Chanos chanos*). J. Food Sci. 53: 1333-1335, 1386.

Jittinandana, S.,Kenney, P. B. and Slider, S. D. 2003. Cryoprotection affects physiochemical attributes of rainbow trout fillets. J. Food Sci. 68: 1208-1214.

John, A. and Shashtri, P. N. 1998. Studies on food macromolecules by differential scanning colorimetry: a critical appraisal. J. Food Sci. Technol. 35: 1-14.

Jones, W. and Mandigo, R. W. 1982. Effects of chopping temperature on the microstructure of meat emulsions. J. Food Sci. 47: 1930–1935.

Johnston, I. A. and Frearson, N. G. 1973. The effects of environmental temperature on properties of myofibrillar adenosine triphosphate from various species of fish. J. Biochem. 133: 735-738.

Julavittayanukul, O., Benjakul, S. and Visessanguan, W. 2005. Effect of phosphate compounds on gel forming ability of surimi from bigeye snapper (*Priacanthus tayenus*). Food Hydrocol. 20: 1153-1163.

Karakoltsidis, P. A., Zotos, A. and Constantinides, S. M. 1995. Composition of the commercially important Mediterranean finfish, crustaceans, and molluscs. J. Food Comp. Anal. 8: 258-273.

Karel, M., Fennema, O. R. and Lund, D. B. 1975. Principles of Food Science. Vol. II. p. 485. Marcel Dekker. New York, USA.

Karmas, E. and Turk, K. 1976. Water-binding of cooked fish in combination with various proteins. J. Food Sci. 41:977–979.

Kijowski, J. 2001. Muscle proteins. *In* Chemical and Functional Properties of Food Proteins (Sikorski, Z.E. ed.). pp. 233-270. Technomic Publishing. Philadelphia, USA.

Kijowski, J. M. and Mast, M. G. 1988. Effect of sodium chloride and phosphates on the thermal properties of chicken meat proteins. J. Food Sci. 53: 367-370.

Kilara, A. and Harwarwakar, V. R. 1996. Denaturation. *In* Food Proteins: Properties and Characterization (Nakai, S. and Modler, H. W. eds). pp. 71-165. VCH Publishers Inc. New York, USA.

Knappeis, G. G. and Carlsen, F. 1968. The ultrastructure of M-line in skeletal muscle. J Cell Biol. 38: 202-205.

Kolakowska, E., Szybowicz, Z. and Raczek, K. 1976. Extractability of muscle protein as criterion for Technological evaluation of sea fish Part 1. Changes of protein fraction of Baltic herring and Baltic cod during storage in ice. Food/Nahrung. 21: 485-490.

Kong, B., Diao, X. and Xiong, Y. L. 2006. Postmortem calcium chloride injection alters ultrastructure and improves tenderness of mature Chinese yellow cattle longissimus muscle. J. Food Sci. 71: C124-C129.

Koohmaraie, M. 1992. The role of Ca^{2+}-dependent proteases (calpains) in post-mortem proteolysis and meat tenderness. Biochimie. 74: 239-245.

Kumazawa, Y., Oozaki, Y., Iwami, S., Matsumoto, I. and Arai, K. 1990. Combined protective effect of inorganic pyrophosphate and sugar on freeze-denaturation of carp myofibrillar protein. Nippon Suisan Gakkaishi. 56: 105–113.

Labeit, S., Gautel, M., Lakey, A. and Trinick, J. 1992. Towards a molecular under standing of Titin. Embo. J. 11: 1711-1714.

Ladrat, C., Verrez-Bagnis, V., Noel, J. and Fleurence, J. 2003. *In vitro* proteolysis of myofibirllar and sarcoplasmic proteins of white muscle of sea bass (*Dicentrarchus labrax* L.): effects of cathepsins B, D and L. Food Chem. 81: 517-525.

Lampila, L. A. and Godber, J. P. 1990. Food Phosphates. *In* Food Additives (Branen, A.L., Davidson, P M. and Salminen, S., eds). Marcel Dekker. New York, USA.

Lawrie, R. A. 1998. The eating quality of meat. *In* Lawrie's Meat Sience. 6th ed. (Lawrie R, ed.) pp. 220-258. Woodhead Publishing Limited. Cambridge, UK

Laemmli, U.K. 1970. Cleavage of structure proteins during the assembly of the head of bacteriophage T4. Nature. 277: 680–685.

Li-chan, E. Y. C., Nakai, S. and Hirotsuka, M. 1994. Raman spectroscopy as a probe of protein structure in food systems. *In* Protein structure-Function Relationships in Foods. (Yada, R. Y., Jackman, R. L. and Smith, J.L . eds.). pp. 164-197Blackie Academic and Professional. London, UK.

Lin, T. M. and Park, J. W. 1998. Solubility of salmon myosin as affected by conformational changes at various ionic strengths and pH. J. Food Sci. 63:215–218.

Lindsay, R. C. 1996. Food additives. *In* Food Chemistry. (Fennema, O. ed). pp. 778-785. Marcel Dekker, Inc. New York, USA.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193: 256-275.

Luther, P. K., and Squire, J. M. 2002. Muscle Z-band ultrastructure: Titin Z-repeats and Z-band periodicities do not match. J. Mol. Biol. 319: 1157–1164.

Mackie, I. M. 1994. Fish protein. *In* New and Developing Sources of Food Proteins. (Hudson, B. F. J., ed.). pp. 95-143. Chapman & Hall. New York. USA.

Martinez, I., Friis, T. J. and Careche, M. 2001. Post mortem muscle protein degradation during ice-storage of Arctic (*Pandalus borealis*) and tropical (*Penaeus japonicus and Penaeus monodon*) shrimps: a comparative eletrophoretic and immunological study. J. Sci. Food Agric. 81: 1199-1208.

Masaki, T. and Takaiti, O. 1972. Purification of M-protein. J. Biochem. 71:355-357.

Matsukawa, M., Hirata, F., Kimura, S. and Arai, K. 1995. Effect of sodium pyrophosphate on gelling property and cross-linking of myosin heavy chain in setting-heating gel from six transglutaminase from ordinary muscles of various fishes and marine invertebrates. Comp. Biochem. Physiol. 118B: 313-317.

Matsukawa, M., Sakurada, K. and Arai, K. 1992. The role of sodium pyrophosphate in gel formation and cross-linking of myosin heavy chain of salt-ground meat from walleye pollack. Nippon Suisan Gakkaishi. 58: 735–742.

McCormick, R. J. 1994. Structure and properties of tissue. *In* Muscle Foods Meat Poultry and Seafood Technology (Kinsman, D. M., Kotula, A. W. and Breidenstein, B. C., eds.). pp. 25-62. Chapman & Hall. New York, USA

Miller, M. F., Davis, G. W., Seideman, S. C., Ramsey, C. B. and Rolan, T. L. 1986. Effects of various phosphates on the palatability, appearance and storage traits of flaked and formed restructured beef steaks. J. Food Sci. 51: 1435–1438.

Mizuta, S., Yamada, Y., Miyagi, T. and Yoshinaka, R. 1999. Histological changes in collagen related to textural development of prawn meat during heat processing. J. Food Sci. 64: 91-995.

Mizuta, S., Yoshinaka, R., Sato, M., and Sakaguchi, M. 1997. Histological and biochemical changes of muscle collagen during chilled storage of the kuruma prawn *Penaeus japonicus*. Fish. Sci. 63: 784-793.

Mohan, C. O., Ravishankar, C. N., Bindu, J., Geethalakshmi, V.and Srinivasa Gopal, T. K. 2006. Effect of thermal process time on quality of shrimp kuruma in retortable pouches and aluminum cans. J. Food Sci. 71: S496-S500.

Morrison, C. R. 1993. Fish and Shellfish. *In* Frozen Food Technology (Mallett, C.P., ed.). p. 196. Chapman & Hall. New York, USA.

Multilangi, W. A. M., Panyam, D. and Kilara, A. 1996. Functional properties of hydrolysates from proteolysis of heat-denatured whey protein isolate. J. Food Sci. 61: 270-274.

Nakai, S. and Li-chan, E. Y. C. 1988. Hydrophobic Interactions in Food Systems. CRC Press. Boca Raton, Florida, USA.

Nambudiri, D. D. and Gopakumar, K. 1992. ATPase and lactate dehydrogenase activitiesin frozen stored fish muscle as indices of cold storage deterioration. J. Food Sci. 57: 72-76.

Nave, R., Fürst, D. O, and Weber, K. 1989. Visualization of the polarity of isolated Titin molecules; single globular head as the M-band anchoring domain. J Cell Biol. 109: 2177-2180.

Nilsson, L. B. and Ekstrand, B. 1995. Frozen storage and thawing methods affect biochemical and sensory attributes of rainbow trout. J. Food Sci. 63: 627-635.

Nip, W. K., Lan, C. Y. and Moy, J. H. 1985. Partial characterization of a collagenolytic enzyme fraction from the hepatopancreas of the freshwater prawn, *Macrobrachium rosenbergii*. J. Food Sci. 50: 1187-1188.

Nip, W. K. and Moy, J. H. 1988. Microstructural changes of ice-chilled and cooked freshwater prawn, *Macrobarachim rosenbergii*. J. Food Sci. 53: 319-322.

Ochiai, Y. and Chow, C. J. 2000. Myosin ATPase. *In* Seafood Enzymes (Haard, N. F. and Simpson, B. K., eds.). pp. 69-90. Marcel Dekker, Inc. New York, USA.

Offer, G. and Knight, P. 1988. The structural basis of water-holding in meat. *In* Developments in Meat Science 4 (Lawrie R, ed). pp 63–243. Elsevier Applied Science. London. UK.

Offer, G. and Trinick, J. 1983. On the mechanism of water holding in meat: the swelling & shrink of myofibrils. Meat Sci. 8: 245-281.

Official Journal of the European Communities. 1995. European Parliament and Council Directive No. 95/2. EC of 20 February 1995 on Food additives other than colours and sweetness.

Okada, T., Inoue, N. and Akiba, M. 1986. Electron microscopic observation and biochemical properties of carp myosin B during frozen storage. Bull. Japn. Soc. Sci. Fish. 52: 345-353.

Ogawa, M., Tamiya, T. and Tsuchiya, T. 1994. Structural changes of carp myosin during heating. Fish Sci. 60: 723-727.

Olafsdottir, G., Martinsdottir, E., Oehlenschlager, J., Dalgaard, P., Jensen, B., Undeland, I., Mackie I. M., Henehan, G., Nielsen, J. and Nilsen, H**.** 1997. Methods to evaluate fish freshness in research and industry. Trends Food Sci. Techbnol. 8: 258–265.

Olsson, G. B., Olsen, R. L. and Ofstad, R. 2003. Post-mortem structural characteristics and water-holding capacity in Atlantic halibut muscle. Lebensm. Wiss. U. Technol. 36: 125–133

Pacheco-Aguilar, R., Lugo-Sanchez, M. E. and Robles-Burgueno, M. R. 2000. Postmortem biochemical characteristic of Monterey sardine muscle stored at 0°C. J. Food. Sci. 65: 40–47.

Park, J. W. 2000. Ingredient technology & formulation development. *In* Surimi and Surimi Seafood. (J.W. Park, ed.). pp. 343-391. Marcel Dekker. New York, USA.

Park, J. W. 2005. Ingredient technologt for surimi and surimi seafood. *In* Surimi and Surimi Seafood 2nd Ed. (Park, J. W. ed.) pp 649-707. CRC Press. New York, USA.

Park, J.W. and Lin, J. 2005. Surimi: Manufacturing and Evaluation. *In* Surimi and Surimi Seafood 2nd Ed. (Park, J. W. ed.). pp 33-106. CRC Press. New York, USA.

Peterson, B. C., Parrish, F. C., and Stromer, M. H. 1988. Effects of salt and pyrophosphate on the physical and chemical properties of beef muscle. J. Food Sci. 53: 1258–1265.

Pipatsattayanuwong, S., Park, J. W. and Morrissey, M. T. 1995. Functional properties and shelf life of fresh surimi from Pacific whiting. J. Food Sci. 60: 1241-1244.

Pearson, A. M. and Young, R. B. 1989. Muscle and Meat Biochemistry. *In* Food Science and Technology - A Series of Monographs (Schweigert, B.S. and Taylor, S.L. eds.). Academic Press, Inc. San Diego. California, USA.

Poulter, R. G., Ledward, D. A., Godber, S., Hall, G. and Rowlands, B. 1985. Heat stability of fish muscle protein. J. Food Technol. 20: 203-217.

Ramirez, J. A., Martin-Polo, M. O. and Bandman, E. 2000. Fish myosin aggregation as affected by freezing and initial physical state. J. Food Sci. 65: 556-560.

Rehbein, H. 1979. Development of an enzymatic method to differentiate fresh and sea-frozen and thawed fish fillets I. Comparison of the applicability of some enzymes of fish muscle. Z. Lebensm. Unters. Forsch. 169:263-265.

Robe, G. H. and Xiong Y. L. 1992. Phosphates and muscle fiber type influence thermal transitions in porcine salt-soluble protein aggregation. J. Food Sci. 57: 1304-1310.

Robinson, H. W. and Hodgen, C. G. 1940. The biuret reaction in the determination of serum protein I. A study of the condition necessary for the production of the stable color which bears a quantitative relationship to the protein concentration. J. Biol. Chem. 135:707–25.

Roura, S. I. and Crupkin, M. 1995. Biochemical and functional properties of myofibrils from pre and post-spawned hake (*Merluccius hubbsi Marini*) stored on ice. J. Food Sci. 60: 269-272.

Rowland, B., Finne, G. and Tillman, R. 1982. A morphological study of muscle proteolysis in the tails of *Macrobrachium rosenbergii*. Proc. 7th Annual Trop. Subtrop. Fish. Technol. Conf. Amer. TAMU-SG-82-10. p. 105.

Scott, D. N., Porter, R. W., Kudo, G., Miller, R. and Koury, B. 1988. Effect of freezing and frozen storage of Alaska pollock on the chemical and gel-forming properties of surimi. J. Food Sci. 53: 353-358.

Sato, K., Ohashi, C., Ohtsuki, K. and Kawabata, M. 1991. Type V collagen in trout (*Salmo gairdneri*) muscle and its solubility change during chilled storage of muscle. J. Agric. Food Chem. 39: 1222-1225.

Shahidi, F. 1994. Seafood proteins and preparation of protein concentrates. *In* Seafoods chemistry, processing technology and quality. (Shahidi, F. and Botta, J. R., eds.) pp. 3-9. Chapman & Hall. London, U.K.

Shahidi, F., Xiao-Qing, H. and Synowiecki, J. 1995. Production and characteristics of protein hydrolysates from capelin (*Mallotus villosus*). Food Chem. 53: 285–293.

Shenouda, S. Y. K. 1980. Theories of protein denaturation during frozen storage of fish flesh. Adv. Food Res. 26: 275-311.

Shimomura, H., Takahashi, T., Morishita, T. and Ueno, R. 1987. Investigation of the differentiation of frozen-thawed fish from unfrozen fish by comparison of lysosomal enzyme activity. Nippon Suisan Gakkaishi 53:1841-1845.

Sikorski, Z. E. 2001. Functional properties of proteins in food Systems. *In* Chemical and Functional Properties of Food Proteins. (Sikorski, Z.E., ed.). pp. 113-135. Technomic Publishing Comp. Inc. Lancaster. Pennsylvania, USA.

Sikoroski, Z. E. and Borderias, J. A. 1990. Collagen in the muscles and skin of marine animals. *In* Seafood: Resources, Nutritional Composition and Preservation (Sikorski, Z.E., ed.). pp. 58-70. CRC Press, Inc. Florida, USA.

Sikorski, Z., Kolakowska, A. and Burt, J. R. 1990a. Postharvest biochemical and microbial changes. *In* Seafood: Resources, nutritional composition and preservation (Sikorski, Z.E., ed.). pp. 55–57. CRC Press, Inc. Florida, USA.

Sikorski, Z. E., Kolakoska, A. and Pan, B. S. 1990b. The nutritive composition of the major groups of marine food organisms. *In* Seafood: Resources, Nutritional Composition and Preservation (Sikorski, Z.E., ed.). p. 29-54. CRC Press, Inc. Florida, USA.

Smyth, A. B., Smith, D. M. and O´Neill, E. 1998. Disulfide bonds influence the heat-induced gel properties of chicken breast muscle myosin. J. Food Sci. 63: 584-588.

Sriket, P., Benjakul, S., Visessanguan, W. and Kijroongrojana, K. 2007a. Comparative studies on chemical compositions and thermal properties of black tiger shrimp (*Penaeus monodon*) and white shrimp (*Penaeus vannmei*) meats. Food Chem. 103: 1199-1207.

Sriket, P., Benjakul, S., Visessanguan, W. and Kijroongrojana, K. 2007b. Comparative studies on the effect of the freeze–thawing process on the physicochemical properties and microstructures of black tiger shrimp (*Penaeus monodon*) and white shrimp (*Penaeus vannamei*) muscle. Food Chem. 104: 113-121.

Srinivasan, S. and Hultin, H. O. 1997. Chemical, physical and functional properties of cod proteins modified by a nonenzyme free-radical-generating system. J. Agric. Food Chem. 45:310-320.

Srinivasan, S., Xiong, Y.L., Blanchard, S.P. 1997. Effects of freezing and thawing methods and storage time on thermal properties or freshwater prawns (*Machrobrachium rosenbergii*). J. Sci. Food Agric. 75: 37-44.

Stabursvik, E. and Martens, H. 1980. Thermal denaturation of proteins in post rigor muscle tissue as studies by differential scanning colorimetry. J. Sci. Food Agric. 31: 1934-1042.

Starr, R. and Offer, G. 1983. H-protein and X-protein: two new component of the thick filaments of vertebrate skeletal muscle. J. Mol. Biol. 170: 675-698.

Steel, R. G. D. and Torrie, J. H. 1980. Principle and Procedures of Statistics: a Biometrical Approach. p. 1–633. McGraw- Hill. New York, USA.

Sultanbawa, Y. and Li-Chan, E. C. Y. 2001. Structural changes in natural actomyosin and surimi from ling cod (*Ophiodon elongatus*) during frozen storage in the absence or presence of cryoprotectants. J. Agric. Food Chem. 49: 4716-4725.

Suzuki, T. 1981. Fish and Krill Protein. *In* Protein Technology. Appl. Sci. Publ, Ltd. London. UK.

Thannhauser, T. W., Konishi, Y. and Scheraga, H. A. 1987. Analysis for disulfide bonds in peptides and proteins. Method. Enzymol. 143: 115-118.

Thanonkaew, A., Benjakul, S. and Visessanguan, W. 2006. Chemical composition and thermal property of cuttlefish (*Sepia pharaonis*) muscle. J. Food Comp. Anal. 19: 127-133.

Thawornchinsombut, S. and Park, J. W. 2005. Role of ionic strength in biochemical properties of soluble fish proteins isolated from cryoprotected Pacific whiting mince. J. Food Biochem. 29: 132–151.

The Customs Department. 2006. Import statistics of shrimps and shrimp products 2005. Ministry of Finance, Thailand. Available from: http://www.customs.go.th/Statistic/StatisticIndex.jsp. Accessed December 2006.

Thorarinsdottir, K. A, Arason, S, Geirsdottir, M., Bogason, S.G. and Kristbergsson, K. 2002. Changes in myofibrillar proteins during processing of salted cod (*Gadus morhua*) as determined by electrophoresis and differential scanning calorimetry. Food Chem. 77: 377–385.

Thorarinsdottir, K. A., Gudmundsdottir, G., Arason, S., Thorkelsson, G. and Kristbergsson, K. 2004. Effects of added salt, phosphates, and proteins on the chemical and physicochemical characteristics of frozen cod (*Gadus morhua*) fillets. J. Food Sci. 69: FEP144-FEP152.

Torigai, M. and Wilding, P. 1996. Pyrophosphate accelerated denaturation mechanism in myofibril. Fish Sci. 62:307-311.

Trout, G. R. and Schmidt, G. R. 1983. Utilization of phosphates in meat products. Proc. Recipr. Meat Conf. 36: 24-28.

Trout, G. R. and Schmidt, G. R. 1984. Effect of phosphate type and concentration, salt level and method of preparation on binding in restructured beef rolls. J. Food Sci. 49: 687-694.

Trout, G. R. and Schmidt, G. R. 1986. Effect of phosphates on the functional properties of restructured beef rolls: the role of pH, ionic strength, and phosphate type. J. Food Sci. 51: 1416-1423.

Trout, G. R. and Schmidt, G. R. 1987. The effect of cooking temperature on the functional properties of beef proteins: The role of ionic strength, pH, and pyrophosphate. Meat Sci. 20: 129-135.

Van Wazer, J. R. 1971. Chemistry of the phosphate and condense phosphates. *In* Symposium: Phosphates in Food Processing. (Denma, J. M. and Malnyehyn, P., eds.). p.1.The AVI Pub. Co., Inc. Westport. Connecticut, USA.

Vogt, W. 1995. Oxidation of methionyl residues in protein: tool, target, and reversal. Free Rad. Biol. Med. 18: 93-105.

Vojdani, F. 1996. Solubility. *In* Method of Testing Protein Functionality (Hall, G.M., ed.) pp. 11-60. Chapman & Hall. London, USA.

Wright, D. J., Leach, L. B. and Wilding, P. 1977. Differential scanning calorimetric studies of muscle and its constituent proteins. J. Sci. Food Agric. 28: 557-561.

Wright, D. J. and Wilding, P. 1984. Differential scanning calorimetric study of muscle and its proteins: myosin and subfragments. J. Sci. Food Agric. 35: 357-372.

Xiaowen, L. 1996. The impact of polyphosphates on the water retention of fresh fillets and frozen cod minces. Dis. Abstr. In.t B. 56: 4078–4079.

Woyewoda, A. D and Bligh, E. G. 1986. Effect of phosphate blends on the stability of cod fillets in frozen storage. J. Food Sci 51: 932-935.

Wu, M. C., Akahane, T., Lanier, T. C. and Hamann, D, D. 1985. Thermal transitions of actomyosin and surimi prepared from Atlantic croaker as studied by differential scanning calorimetry. J. Food Sci. 50: 10-13.

Xiong, Y. L. and Decker, E. A. 1995. Alteration of muscle protein functionality by oxidative and antioxidative process. J. Muscle Foods. 6: 139-160.

Xiong, Y. L. 1997a. Phosphate-mediated water uptake, swelling and functionality of the myofibril architecture. *In* Quality Attributes of Muscle Foods. (Xiong, Y. L., Ho, C. T. and Shahidi, F., eds.). pp. 319-334. Kluwer Academic. New York, USA.

Xiong, Y. L. 1997b. Protein denaturation and functionality losses. *In* Quality in Frozen Food. (Erickson, M,C, and Hung, Y.C., eds.). pp. 111-140. Chapman & Hall. New York, USA.

Xiong, Y. L. 1997c. Structure-function relationships of muscle proteins. *In* Food Proteins and Their Applications. (Domodoran, S. and Paraf, A., eds.). pp. 341-392. Marcel Dekker, Inc. New York, USA.

Xiong, Y. L. 2005. Role of myofibrillar proteins in water-binding in brine-enhanced meats. Food Res. Intern. 38: 281–287.

Xiong, Y. L. and Kupski, D. R. 1999a. Monitoring phosphate marinade penetration in tumbled chicken filets using a thin-slicing, dye-tracing method. Poultry Sci. 78:1048–1052.

Xiong, Y. L. and Kupski, D. R. 1999b. Time-dependent marinade absorption and retention, cooking yield, and palatability of chicken fillets marinated in various phosphates solutions. Poultry Sci. *78*: 1048-1052.

Xiong, Y. L., Lou, X., Harmon, R. J., Wang, C. and Moody,W. G. 2000. Salt- and pyrophosphate-induced structural changes in myofibrils from chicken red and white muscles. J. Sci Food Agric. 80: 1176-1182.

Uchiyama, H. and Kakuda, K.1984. A simple and rapid method for measuring for measuring K value, a fish freshness index. Bull. Japn. Soc. Fish. 50: 263-267.

VanWazer, J. R. 1971. Chemistry of the phosphates and condensed phosphates. *In* Symposium: Phosphates in Food Processing. (Deman, J. M. and Malnychyn, P., eds.). p. 1. The AVI Pub. Co., Inc. Westport, Connecticut, USA.

Vojdani, F. 1996. Solubility. *In* Method of testing protein functionality (Hall, G.M., ed.). p. 11-60. Chapman & Hall. London, UK.

Yanar, Y. and Celik, M. 2005. Seasonal variations of fatty acid composition in wild marine shrimps (*Penaeus semisulcatus* De Haan, 1844 and *Metapenaeus monoceros* Fabricus, 1789) from the Eastern Mediterranean Sea. Food Sci. Technol. Intern. 11: 391-395.

Yanar, Y. and Celik, M. 2006. Seasonal amino acid profiles and mineral contents of green tiger shrimp (*Penaeus semisulcatus* De Haan, 1844) and speckled shrimp (*Metapenaeus monoceros* Fabricus, 1789) from the Eastern Mediterranean. Food Chem. 94: 33-36.

Yongswawatdigul, J. and Park, J. W. 2003. Biochemical and conformation changes of actomyosin from threadfin bream stored in ice. J. Food Sci. 67: 895-990.

Yoshikawa, K., Inoue, N., Kowai, Y. and Shinano, H. 1995. Changes of solubility and ATPase activity of carp myofibrils during frozen storage. Fish. Sci. 61: 813-815.

Young, L. L, Lyon, C. E., Searcy, G, K. and Wilson, R. L. 1987. Influence of sodium tripolyphosphates and sodium chloride on moisture retention and textural characteristics of chicken breast meat patties. J. Food Sci. 52: 571-574.

Zotos, A., Hole, M., and Smith, G. 1985. The effect of frozen storage of mackerel (*Scomber scombrus*) on its quality when hot-smoked. J. Sci. Food Agric. 67: 43-48.

APPENDIX

pH OF DIFFERENT SOLUTIONS

Table 1. pH of different mixed phosphate solutions in 2.5% NaCl

Solutions	Concentration (%)				pH of solution
	SAPP	TSPP	STPP	SHMP	
1	-	3.5	-	-	10.12
2	0.875	2.625	-	-	7.34
3	1.75	1.75	-	-	5.99
4	2.625	0.875	-	-	5.26
5	3.5	-	-	-	3.83
6	-	-	3.5	-	8.54
7	0.875	-	2.625	-	7.02
8	1.75	-	1.75	-	5.92
9	2.625	-	0.875	-	5.22
10	-	-	-	3.5	5.45
11	0.875	-	-	2.625	4.99
12	1.75	-	-	1.75	4.61
13	2.625	-	-	0.875	4.30

Table 2. pH of 2.5% NaCl solution containing ProfixO at different concentration in the absence and present mixed phosphates (0.875% SAPP + 2.625% TSPP)

Solutions	ProfixO (%)	Mixed phosphates	pH of solution
1	0	W/O	7.25
2	1	W/O	9.73
3	3	W/O	9.76
4	5	W/O	9.66
5	0	W	7.32
6	1	W	7.66
7	3	W	8.14
8	5	W	8.36

ANALYTICAL METHODS

1. Determination of moisture content (AOAC, 1999)

Method

1. Dry the empty dish and lid in the oven at 105 °C for 3 h and transfer to a desiccator to cool. Weigh the empty dish and lid.
2. Weigh about 3 g of sample to the dish. Spread the sample to the uniformity.
3. Place the dish with sample in the oven. Dry for 3 h at 105°C.
4. After drying, transfer the dish with partially covered lid to the desiccator to cool. Reweigh the dish and its dried sample.

Calculation

$$\text{Moisture content (\%)} = \frac{(W1-W2) \times 100}{W1}$$

where: W1 = weight (g) of sample before drying

W2 = weight (g) of sample after drying

2. Determination of protein content (AOAC, 1999)

Reagents

- Kjeldahl catalyst: Mix 9 part of potassium sulphate (K_2SO_4) with 1 part of copper sulphate ($CuSO_4$)
- Sulfuric acid (H_2SO_4)
- 40% NaOH solution
- 0.2 N HCl solution
- 4% H_3BO_3
- Indicator solution: Mix 100 ml of 0.1% methyl red (in 95% ethanol) with 200 ml of 0.2% bromocresol green (in 95% ethanol)

Method

1. Place sample (0.5-1.0 g) in digestion flask.
2. Add 5 g of Kjeldahl catalyst and 200 ml of conc. H_2SO_4
3. Prepare a tube containing the above chemical except sample as blank. Place flasks in inclined position and heat gently unit frothing ceases. Boil until solution clears.
4. Cool and add 60 ml of distilled water cautiously.
5. Immediately connect flask to digestion bulb on condenser and with tip of condenser immersed in standard acid and 5-7 drops of mix indicator in receiver. Rotate flask to mix content thoroughly; then heat until all NH_3 is distilled.
6. Remove receiver, wash tip of condenser and titrate excess standard acid distilled with standard NaOH solution.

Calculation

$$\text{Protein content (\%)} = \frac{(A-B) \times N \times 1.4007 \times 6.25}{W}$$

Where
A = volume (ml) of 0.2 N HCl used sample titration
B = volume (ml) of 0.2 N HCl used in blank titration
N = Normality of HCl
W = weight (g) of sample
14.007 = atomic weight of nitrogen
6.25 = conversation factor

3. Determination of ash content (AOAC, 1999)

Method

1. Place the crucible and lid in the furnace at 550°C overnight to ensure that impurities on the surface of crucible are burned off.
2. Cool the crucible in the desiccator (30 min).
3. Weigh the crucible and lid to 3 decimal places.
4. Weigh about 5 g sample into the crucible. Heat over low Bunsen flame with lid half covered. When fumes are no longer produced, place crucible and lid in furnace.
5. Heat at 550°C overnight. During heating, do not cover the lid. Place the lid after complete heating to prevent loss of fluffy ash. Cool down in the desiccator.
6. Weigh the ash with crucible and lid when the sample turns to gray. If not, return the crucible and lid to the furnace for the further ashing.

Calculation

$$\text{Ash content (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

4. Determination of fat content (AOAC, 1999)

Reagents

- Petroleum ether

Method

1. Place the bottle and lid in the incubator at 105°C overnight to ensure that weight of bottle is stable.
2. Weigh about 3-5 g of sample to paper filter and wrap.
3. Take the sample into extraction thimble and transfer into soxhlet.

4. Fill petroleum ether about 250 ml into the bottle and take it on the heating mantle.
5. Connect the soxhlet apparatus and turn on the water to cool them and then switch on the heating mantle.
6. Heat the sample about 14 h (heat rate of 150 drop/min).
7. Evaporate the solvent by using the vacuum condenser.
8. Incubate the bottle at 80-90 $^{\circ}$C until solvent is completely evaporated and bottle is completely dry.
9. After drying, transfer the bottle with partially covered lid to the desiccator to cool. Reweigh the bottle and its dried content.

Calculation

$$\text{Fat content (\%)} = \frac{\text{Weight of fat} \times 100}{\text{Weight of sample}}$$

5. Biuret method (Robinson and Hodgen, 1940)

Reagents

- Biuret reagent: Combine 1.50 g $CuSO_4.5H_2O$, 6.00 g sodium potassium tartrate, and 500 ml distilled water in a beaker and stir, add while stirring 300 ml of 10 % NaOH (w/v), transfer to a 1 liter volumetric flask and bring to 1 liter with distilled water.
- Standard reagent: 10 mg/ml bovine serum albumin (BSA)

Method

1. To 0.5 ml of sample, add 2.0 ml of the biuret reagent and mix well.
2. Incubate the mixture at room temperature for 30 min.
3. Read the absorbance at 540 nm.

Table 3. Experimental set up for the Biuret's assay

Tube number	water (μl)	10 mg/ml BSA (μl)	BSA concentration (mg/ml)
1	500	0	0
2	400	100	2
3	300	200	4
4	200	300	6
5	100	400	8
6	0	500	10

6. Preparation of natural actomyosin (Benjakul *et al*., 1997)

Reagents

- 0.6 M KCl, pH 7
- Distilled water

Method

1. Homogenize 10 g of muscle in 100 ml chilled (4°C) 0.6 M KCl, pH 7.0 for 4 min.
2. Place the beaker containing the sample in ice.
3. Blend every 20 sec, followed by a 20 sec rest interval to avoid overheating during extraction.
4. Centrifuge the extract at 5,000xg for 30 min at 4°C.
5. Add three volumes of chilled distilled water to precipitate actomyosin.
6. Collect actomyosin by centrifuging at 5,000xg for 20 min at 4°C.
7. Dissolve the pellet by stirring for 30 min at 4°C in an equal volume of chilled 0.6 M KCl, pH 7.

7. Electrophoresis (SDS-PAGE) (Leammli, 1970)

Reagents

- 30 % Acrylamide-0.8 % bis Acrylamide
- 2 % (w/v) Ammonium persulfate
- 1 % (w/v) SDS
- TEMED (N,N,N'N'-tetramethylenediamine)
- Sample buffer: Mix 30 ml of 10 % of SDS, 10 ml of glycerol, 5 ml of β-mercaptoethanol, 12.5 ml of 50 mM Tris-HCl, pH 6.8, and 10 mg Bromophenol blue. Bring the volume to 100 ml with distilled water and stored at -20°C.
- 0.5 M Tris-HCl, pH 6.8
- 1.5 M Tris-HCl, pH 8.8
- Electrode buffer: Dissolve 3 g of Tris-HCl, 14.4 g of glycine and 1 g of SDS in distilled water. Adjust to pH 8.3. Add distilled water to 1 liter total volume.
- Staining solution: Dissolve 0.04 g of Coomassie blue R-250 in 100 ml of methanol. Add 15 ml of glacial acetic acid and 85 ml of distilled water.
- Destaining solution l: 50 % methanol-7.5 % glacial acetic acid
- Destaining solution ll: 5 % methanol-7.5 % glacial acetic acid

Method

Pouring the separating gel:

1. Assemble the minigel apparatus according to the manufacturer's detailed instructions. Make sure that the glass and other components are vigorously clean and dry before assembly.
2. Mix the separating gel solution by adding as defined in following Table.
3. Transfer the separating gel solution using a pasture pipette to the center of sandwich is 2 cm from the top of the shorter glass plate.

4. Cover the top of the gel with a layer of isobutyl alcohol by gently squirting the isobutyl alcohol by gently squirting the isobutyl alcohol against the edge of one of the spacers. Allow the resolving gel to polymerize fully (usually 45 min).

Pouring the stacking gel:

1. Pour off completely the layer of isobutyl alcohol.
2. Prepare a 4 % stacking gel solution by adding as defined in table.
3. Transfer stacking gel solution to tickle into the center of the sandwich along an edge of one of the spacers.
4. Insert a comb into the layer of stacking gel solution by placing one corner of the gel and slowly lowering the other corner in. Allow the stacking gel solution to polymerize 45 min at room temperature.

Reagents	10 % running gel	4 % stacking gel
30 % Acrylamide-bis	3.333 ml	0.665 ml
1.5 M Tris-HCl buffer, pH 8.8	2.5 ml	-
0.5 M Tris-HCl buffer, pH 6.8	-	1.25 ml
10 % SDS	100 µl	50 µl
Distilled water	4.012 ml	3 ml
10 % Ammonium persulfate	50 µl	25 µl
TEMED	5 µl	3 µl

Sample preparation:

1. Weigh 3 g of sample and homogenize with 5 % (w/v) SDS in a final volume of 30 ml.
2. Incubate the mixture at 85°C for 1 h.
3. Centrifuge at 3,500xg for 5 min at ambient temperature and collect supernatant.

Loading the gel:

1. Dilute the protein to be 1:1 (v/v) with sample buffer in microcentrifuge tube and boil for 1 h.
2. Remove the comb without tearing the edge of the polyacrylamide wells.
3. Fill the wells with electrode buffer.
4. Place the upper chamber over sandwich and lock the upper buffer chamber to the sandwich. Pour electrode buffer into the lower buffer chamber. Place the sandwich attached to the upper buffer chamber into the lower chamber.
5. Fill the upper buffer chamber with electrode buffer so that the sample wells of the stacking gel are filled with buffer.
6. Use a 10-25 μl syringe with a flat-tipped needle, load the protein sample in to the wells by carefully applying the sample as a thin layer at the bottom of the well.
7. Fill the remainder of the upper buffer chamber with additional electrode buffer.

Running the gel:

1. Connect the power supply to the anode and cathode of the gel apparatus and run at 15 mA per gel.
2. After the bromophenol blue tracking dye has reached the bottom of the separating gel, disconnect the power supply.

Disassembling the gel:

1. Remove the upper buffer chamber and the attached sandwich.
2. Orient the gel so that the order of the sample well is known, remove the sandwich from the upper buffer chamber, and lay the sandwich on a sheet of absorbent paper or paper towels. Carefully slide the spacers out from the edge
   the sandwich along its entire length.
3. Insert a spatula between the glass plates at one corner where the spacer was, and gently pry the two plates apart.
4. Remove the gel from the lower plate. Place the plate with the gel attached into the shallow dish of fixing agent of dye and swishing the plate.

Staining the gel:

1. Place the gel in a small plastic box and cover with the staining solution. Agitate slowly for 3 h or more on a rotary rocker.
2. Pour off the staining solution and cover the gel with a solution of destaining solution I. Agitate slowly for 15 min.
3. Pour off the destaining solution I and cover the destaining solution II. Discard destaining solution and replace with fresh solution. Repeat until the gel is clear except for the protein bands.

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Rattanasatheirn, N., Benjakul, S., Visessanguan, W. and Kijroongrojana, K. 2008. Properties, translucence and microstructure of Pacific white shrimp treated with mixed phosphates as affected by freshness and deveining. J. Food Sci. 73(1): S31-S40.

Rattanasatheirn, N., Benjakul, S., Visessanguan, W. and Kijroongrojana, K. 2007. Thermal denaturation of Pacific white shrimp (*Litopenaeus vannamei*) muscle proteins as affected by mixed phosphates and quality changes. The 7th National Graduate Research Conference (GRAD-RESEARCH 2007). Prince of Songkla University, Surat Thani Campus, Surat Thani, Thailand. 4 to 5 April 2007.

Rattanasatheirn, N., Benjakul, S., Visessanguan, W. and Kijroongrojana, K. 2007. Effects of commercial non-phosphate compounds and/or mixed phosphates on the properties of fresh and ice-stored Pacific white shrimp. 10th ASEAN Food Conference 2007. University Putra Malaysia, Kuala Lumpur, Malaysia. 21 to 23 August 2007.