Effect of Washing Solution and Processes on Gelling Properties and Frozen Stability of Yellowtail Barracuda Surimi

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A Thesis Submitted in Partial Fulfillment of the Requirements
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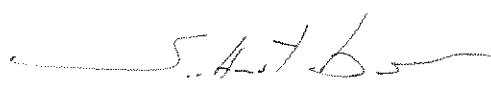
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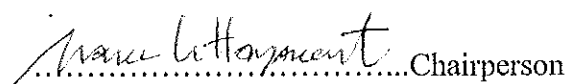**Thesis Title**   Effect of Washing Solution and Processes on Gelling Properties and Frozen Stability of Yellowtail Barracuda Surimi

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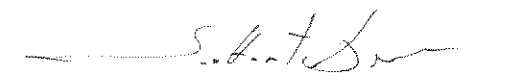
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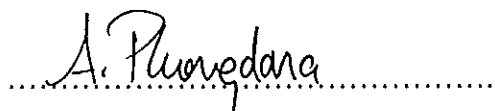
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ชื่อวิทยานิพนธ์	ผลของชนิดเกลือและวิธีการล้างต่อสมบัติของเจลซูริมิและความคงตัว
	ระหว่างการแช่เยือกแข็งของซูริมิปลาเข็มทอง
ผู้เขียน	นายโกศล เลิศวิทยานนท์
สาขาวิชา	วิทยาศาสตร์และเทคโนโลยีอาหาร
ปีการศึกษา	2553

บทคัดย่อ

จากการศึกษาผลของความเข้มข้นของโซเดียมคลอไรด์ที่ระดับต่าง ๆ ในขั้นตอนการล้างครั้งที่สามต่อการกำจัดน้ำและสมบัติของเจลที่ผลิตจากซูริมิปลาเข็มทอง พบว่า เมื่อความเข้มข้นของโซเดียมคลอไรด์ในสารละลายที่ใช้สารครั้งที่สามเพิ่มขึ้นทำให้ปริมาณโซเดียมคลอไรด์และพีเอชของเนื้อปลาบดที่ผ่านการล้างเพิ่มขึ้น ($p<0.05$) อย่างไรก็ตามเมื่อความเข้มข้นของโซเดียมคลอไรด์ในสารละลายที่ใช้ล้างครั้งที่สามเพิ่มขึ้นส่งผลให้ปริมาณความชื้นและซาร์โคพลาสมิกโปรตีนในเนื้อปลาบดลดลง ($p<0.05$) รวมทั้งมีผลให้ T_{max} ของไมโอซินและแอคตินมีค่าลดลง ($p<0.05$) ดังนั้นการล้างเนื้อปลาบดด้วยสารละลายโซเดียมคลอไรด์ร้อยละ 0.45 ในการล้างครั้งที่สามสามารถผลิตซูริมิที่มีเจลซึ่งมีค่าแรงและระยะทางก่อนเจาะทะลุสูงสุดซึ่งสอดคล้องกับปริมาณของเหลวที่สูญเสียจากการบีบอัดลดลง

เมื่อศึกษาผลของสารละลายเกลือซึ่งประกอบด้วยโซเดียมคลอไรด์ร้อยละ 0.45 ผสมกับแคลเซียมคลอไรด์หรือแมกนีเซียมคลอไรด์ที่มีความเข้มข้น 0, 4, 8, 12, 16 และ 20 มิลลิโมลาร์ซึ่งใช้เป็นสารละลายล้างครั้งที่สามเพื่อเพิ่มการกำจัดน้ำและปรับปรุงความสามารถในการเกิดเจลของซูริมิที่ผลิตจากปลาเข็มทอง พบว่า เมื่อเติมแคลเซียมคลอไรด์หรือแมกนีเซียมคลอไรด์ร่วมกับสารละลายโซเดียมคลอไรด์ทำให้ปริมาณของแคลเซียมหรือแมกนีเซียมที่พบในเนื้อปลาบดที่ผ่านการล้างเพิ่มขึ้น ($p<0.05$) ในขณะที่พีเอชของเนื้อปลาบดที่ผ่านการล้างลดลงเล็กน้อย ($p<0.05$) การล้างเนื้อปลาบดด้วยสารละลายที่มีแคลเซียมคลอไรด์สามารถกำจัดน้ำออกจากเนื้อปลาบดได้มากกว่าการล้างด้วยสารละลายที่มีแมกนีเซียมคลอไรด์ที่ระดับความเข้มข้นเดียวกัน ($p<0.05$) การล้างเนื้อปลาบดด้วยสารละลายโซเดียมคลอไรด์ร้อยละ 0.45 ผสมกับแมกนีเซียมคลอไรด์ที่มีความเข้มข้น 20 มิลลิโมลาร์สามารถผลิตเจลซูริมิซึ่งให้เจลที่มีแรงเจาะทะลุสูงสุดทั้งเจลที่ให้ความร้อนโดยตรง (ให้ความร้อนที่ 90 องศาเซลเซียสเป็นเวลา 20 นาที) และให้ความร้อน 2 ขั้นตอน คือ ให้ความร้อนที่ 40 องศาเซลเซียสเป็นเวลา 30 นาที แล้วตามด้วยการให้ความร้อนที่ 90

องศาเซลเซียสเป็นเวลา 20 นาที โดยพบว่าแรงเจาะทะลุของเจลเพิ่มขึ้นร้อยละ 46 และ 33 ตามลำดับเมื่อเปรียบเทียบกับเจลของซูริมิชุดควบคุม อย่างไรก็ตามเมื่อล้างเนื้อปลาบดด้วยสารละลายแคลเซียมคลอไรด์ที่มีความเข้มข้นเพิ่มขึ้น (0-20 มิลลิโมลาร์) มีผลให้ระยะทางก่อนเจาะทะลุของเจลที่ให้ความร้อน 2 ขั้นตอนลดลง ($p<0.05$) การล้างเนื้อปลาบดด้วยสารละลายที่ประกอบด้วยแมกนีเซียมคลอไรด์มีผลให้สีของเจลซูริมิมีค่าลดลงเล็กน้อย ($p<0.05$)

การศึกษาผลของกระบวนการล้างต่างๆต่อองค์ประกอบของเนื้อปลาบดที่ผ่านการล้างและสมบัติของเจลซูริมิ ซึ่งกระบวนการล้างต่าง ๆ ประกอบด้วย 1) Tap water/Tap water/0.45% NaCl (T1) 2) Tap water/Tap water/0.45% NaCl+20 mM MgCl$_2$ (T2) 3) 0.15% NaCl+0.2% NaHCO$_3$/Tap water/0.45% NaCl (T3) 4) 0.15% NaCl+0.2% NaHCO$_3$/Tap water/0.45% NaCl+20 mM MgCl$_2$ (T4) 5) 0.30% NaCl+0.20% NaHCO$_3$/Tap water/0.45% NaCl (T5) 6) 0.30% NaCl+0.20% NaHCO$_3$/Tap water/0.45% NaCl+20 mM MgCl$_2$ (T6) พบว่า พีเอชของสารละลายที่ใช้ในการล้างมีผลโดยตรงต่อพีเอชของเนื้อปลาบดที่ผ่านการล้าง การล้างครั้งแรกด้วยสารละลายโซเดียมไบคาร์โบเนตทำให้ปริมาณความชื้นของเนื้อปลาบดที่ผ่านการล้างเพิ่มขึ้นสูงสุด แต่อย่างไรก็ตามการล้างครั้งที่สามด้วยสารละลายที่ประกอบด้วยแมกนีเซียมคลอไรด์สามารถกำจัดน้ำได้อย่างมีประสิทธิภาพ กระบวนการล้างต่างๆมีผลต่อสมบัติทางความร้อนของเนื้อปลาบดที่ผ่านการล้างแตกต่างกัน ($p<0.05$) ค่าแรงก่อนเจาะทะลุของเจลซูริมิเพิ่มขึ้นประมาณ 33% ด้วยกระบวนการล้าง T6 เมื่อเปรียบเทียบกับกระบวนการล้าง T1 ในขณะที่ค่าระยะทางก่อนเจาะทะลุของเจลซูริมิที่ผลิตด้วยกระบวนการล้างต่างๆไม่มีความแตกต่างกัน ($p<0.05$)

เมื่อศึกษาผลของกระบวนการล้างที่คัดเลือกต่อองค์ประกอบทางเคมีของเนื้อปลาบดที่ผ่านการล้างและสมบัติของเจลซูริมิที่เตรียมจากปลาซึ่งเก็บในน้ำแข็งเป็นเวลาต่างๆ โดยเตรียมซูริมิด้วยกระบวนการล้าง T6 และ T1 ซึ่งเป็นกระบวนการล้างดั้งเดิม พบว่าพีเอชของเนื้อปลาบดที่ผ่านและไม่ผ่านการล้างมีค่าเพิ่มขึ้นเมื่อเก็บรักษาปลาในน้ำแข็งนานขึ้น กระบวนการล้าง T6 มีประสิทธิภาพในการกำจัดน้ำแม้ว่าความสดของปลาลดลง กระบวนการล้าง T6 ทำให้ความคงตัวของไมโอซินและแอคตินลดลง ในขณะที่กระบวนการล้าง T1 มีผลให้ความคงตัวของแอคตินลดลง เมื่อระยะเวลาการเก็บในน้ำแข็งเพิ่มขึ้น ค่าแรงและระยะทางก่อนเจาะทะลุของเนื้อปลาบดที่ผ่านการล้างด้วยวิธีการล้างทั้งสองวิธีลดลง กระบวนการล้าง T1 ทำให้ปริมาณของเหลวที่สูญเสียจากการบีบอัดเจลซูริมิเพิ่มขึ้นเล็กน้อย ในขณะที่กระบวนการล้าง T6 ทำให้ปริมาณของเหลวจากการบีบอัดเจลซูริมิเพิ่มขึ้นเล็กน้อย

จากการศึกษาผลของกระบวนการล้างที่คัดเลือกต่อสมบัติทางเคมีกายภาพและความสามารถในการเกิดเจลของซูริมิที่ผลิตจากปลาเข็มทองระหว่างการเก็บรักษาแบบแช่เยือกแข็งที่

-20°ซ พบว่า ซูริมิที่เตรียมโดยใช้กระบวนการล้างต่างๆ คือ กระบวนการล้างควบคุม (Tap water/Tap water/Tap water) (TC), กระบวนการ T1, T2 และ T6 ไม่มีผลต่อกิจกรรมของ Ca^{2+}-ATPase ในซูริมิระหว่างการเก็บรักษาแบบแช่เยือกแข็งเป็นเวลา 12 สัปดาห์ ปริมาณหมู่ซัลฟ์ไฮดริลในซูริมิที่ผ่านกระบวนการล้างต่าง ๆ ลดลงระหว่างสัปดาห์ที่ 3-6 ของการเก็บรักษาซึ่งการสอดคล้องกับเพิ่มขึ้นของพันธะไดซัลไฟด์ อันตรกิริยาไฮโดรโฟบิกในซูริมิที่ผ่านกระบวนการล้าง T2 มีค่าสูงกว่ากระบวนการล้างอื่น ๆ กระบวนการล้าง T2 ทำให้ค่า TBARS ของซูริมิสูงกว่ากระบวนการล้างอื่น ๆ ตลอด 12 สัปดาห์ของการเก็บรักษา กระบวนการล้าง TC ทำให้ซูริมิมีค่าการละลายสูงสุดและกระบวนการล้าง T6 มีผลให้ซูริมิมีค่าการละลายต่ำสุด ภายหลัง 12 สัปดาห์ของการเก็บรักษากระบวนการล้างต่าง ๆ คือ TC, T1, T2 และ T6 ทำให้ค่าแรงก่อนเจาะทะลุของเจลซูริมิลดลงตามลำดับ ดังนี้ 17.03, 25.42, 20.24 และ 15.29% ส่วนค่าระยะทางก่อนเจาะทะลุลดลง คือ 5.89, 9.16, 5.36 และ 7.86%

      ดังนั้นกระบวนการล้างมีบทบาทสำคัญต่อความสามารถในการเกิดเจลของซูริมิปลาเข็มทอง อย่างไรก็ตามการล้างมีผลต่อความคงตัวของซูริมิเล็กน้อยเท่านั้นระหว่างการเก็บรักษาภายใต้สภาวะแช่เยือกแข็งเป็นเวลานาน

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Major Program   Food Science and Technology

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ABSTRACT

Effects of various levels of sodium chloride (NaCl) in the third washing medium on dewatering of mince and gel properties of surimi produced from yellowtail barracuda were studied. When NaCl concentration of the third washing media increased, the contents of NaCl and pH value in washed mince increased ($p<0.05$). On the other hand, an increasing NaCl concentration of the third washing medium resulted in the marked decrease in moisture and sarcoplasmic protein content in washed mince ($p<0.05$). T_{max} of both myosin and actin was shifted to the lower values as NaCl levels in washing medium increased ($p<0.05$). The highest breaking force and deformation of surimi gels with coincidental lowered expressible moisture content were obtained when 0.45% NaCl was used in the third washing medium ($p<0.05$).

To enhance the dewatering and gel-forming ability of surimi produced from yellowtail barracuda, 0.45% NaCl containing $CaCl_2$ or $MgCl_2$ at various levels (0, 4, 8, 12, 16, and 20 mM) were used as the third washing media. When $CaCl_2$ or $MgCl_2$ was incorporated into the washing media, the contents of Ca or Mg ions in washed mince increased ($p<0.05$), whereas pH of washed mince slightly decreased ($p<0.05$). The dewatering of mince washed with media containing $CaCl_2$ was much more efficient than that found when $MgCl_2$ was incorporated at the same concentration used ($p<0.05$). Washing mince with 0.45% NaCl containing 20 mM $MgCl_2$ yielded the gel of resulting surimi with the highest breaking force for both direct (90°C for 20 min) and two-step heating (90°C for 30 min, followed by 90°C for 20 min) ($p<0.05$), in which the breaking force increased by 46 and 33%, respectively, compared with that of the control. However, the deformation of gel obtained from

two-step heating decreased when the concentrations of $CaCl_2$ in the washing media increased ($p<0.05$). The whiteness of gel slightly decreased when the mince was washed with washing media containing $MgCl_2$ ($p<0.05$).

Impacts of different washing processes on the compositions of washed mince and properties of surimi were studied. Surimi was prepared by different washing processes comprising 3 steps including 1) Tap water/Tap water/0.45% NaCl (T1) 2) Tap water/Tap water/0.45% NaCl+20 mM $MgCl_2$ (T2) 3) 0.15% NaCl+0.2% $NaHCO_3$/Tap water/0.45% NaCl (T3) 4) 0.15% NaCl+0.2% $NaHCO_3$/Tap water/0.45% NaCl+20 mM $MgCl_2$ (T4) 5) 0.30% NaCl+0.20% $NaHCO_3$/Tap water/0.45% NaCl (T5) 6) 0.30% NaCl +0.20% $NaHCO_3$/Tap water/0.45% NaCl+20 mM $MgCl_2$ (T6). It was found that pH of washing media mainly affected the pH of resulting washed mince. Media containing $NaHCO_3$ in the first washing step resulted in the highest moisture content of washed mince. However, the third washing media containing 20 mM $MgCl_2$ showed the efficiency in dewatering. Various washing processes affected thermal property of washed mince differently ($p<0.05$). The highest breaking force of gel was obtained when T6 process was used. Breaking force increased by 33%, compared with that of gel from T1 process, while no marked difference in deformation of gels was found ($p<0.05$).

Effect of selected washing process on chemical compositions of washed mince and properties of gels prepared from ice-stored fish was investigated. Selected process (T6) was used to prepare surimi in comparison with typical washing process (T1). pH of unwashed and washed mince increased as storage time increased. T6 process had the efficacy in dewatering of washed mince although the freshness of fish decreased. T6 process resulted in the lower stability of both myosin and actin, whereas T1 process caused only the lowered stability of actin. The decrease in both breaking force and deformation of gels produced from mince washed with both washing processes was obtained when fish stored in ice for a longer time was used ($p<0.05$). The slight increase in expressible moisture content of gel was obtained in surimi from T1 process, while T6 process caused the slight decrease in expressible moisture content of gel.

Influences of selected washing processes on physicochemical properties and gel-forming ability of surimi were produced from yellowtail barracuda

during frozen storage at -20°C were studied. Surimi prepared using different washing processes including control process (tap water/tap water/tap water) (TC), T1, T2 and T6 processes. No change in Ca^{2+}-ATPase activity of mince with various washing processes was obtained during 12 weeks of storage ($p<0.05$). The decrease in sulfhydryl group content during weeks 3-6 was coincidental with the increase in disulfide bond content. Higher surface hydrophobicity of surimi from T2 process was observed ($p<0.05$). The higher TBARS value was found in surimi from T2 process throughout 12 weeks of storage ($p<0.05$). The highest solubility was observed in surimi from T1 process and the lowest solubility was found in surimi from T6 process. After 12 weeks of storage, breaking force of surimi gel decreased by 17.03, 25.42, 20.24 and 15.29% and deformation of surimi gel decreased by 5.89, 9.16, 5.36 and 7.86%, for surimi prepared using TC, T1, T2 and T6 processes, respectively.

Therefore, washing process played an essential role in gel-forming ability of surimi from yellowtail barracuda. However, it exhibited a little effect on stability of surimi during extended frozen storage.

ACKNOWLEDGEMENT

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CHAPTER 1

INTRODUCTION

Surimi is served as a potential raw material for a variety of seafood products, which become more increasingly popular due to their unique textural properties as well as high nutrition value. However, raw materials for surimi production have been continuously decreasing (Yanpakdee *et al.*, 2009). Recently, yellowtail barracuda has been used as a new raw material, due to its high yield and white color of gel. However, during dewatering process by screw press, moisture content of resulting washed mince remains at a level above the standard. To enhance the dewatering efficacy, 0.03 to 0.6% NaCl was used as washing medium (Okada and Tamato, 1986). Nevertheless, the excessive moisture content is still obtained in yellowtail barracuda surimi, even though NaCl is incorporated in washing media.

In addition, when freshness of raw material during extended ice storage decreased, moisture content of surimi tended to increase, due to the water-retention properties of muscle proteins (Toyoda *et al.*, 1992). Consequently, the use of other salts in conjunction with NaCl could pave a way for effective dewatering of washed mince. Solberg *et al.* (1990) washed cod mince using water containing $CaCl_2$ and $MgCl_2$. The dewatering was found to be much more efficient when washing medium containing $CaCl_2$ was used, rather than that with $MgCl_2$. The highest breaking force of gel with 5 mM $CaCl_2$ was observed but deformation of gel decreased when the concentration of $CaCl_2$ increased. However, a high level of inorganic salts in the wash water, primarily Ca^{2+} and Mg^{2+}, caused the denaturation of actomyosin in Alaska pollock during frozen storage (Tamato, 1971) as well as reduced thermal stability during washing (Saeki, 1986).

Additionally, a mixture of NaCl and $NaHCO_3$ containing both salts may be added in the first washing step to increase the pH of muscle proteins (Shimizu *et al.*, 1992). It has been suggested that gelation is improved by this washing process because the solubility of the sarcoplasmic protein is increased, and there is a decreased rate of denaturation as the muscle pH is increased. This process also

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releases more fat than washing without alkali salt. Furthermore, the addition of salt aids in the removal of heme pigments (Hultin and Kelleher, 2000a,b; Chaijan *et al.* 2004).

However, effect of salts on the forces, which stabilize the tertiary and quaternary conformation of protein molecule, could be responsible for protein denaturation (Park, 1994). During freezing process, the amount of unfreezable water depends on the concentration and type of solutes. Increased salt concentration in unfrozen water phase directly affect the denaturation of proteins. This leads to modification of protein and losses in functional properties (Xiong, 1997a). Thus, better understanding on the effect of various salts in the different washing media and washing processes on dewatering and chemical composition of mince, and gel-forming ability of surimi produced from yellowtail barracuda (*Sphyraena flavicauda*) can be useful for improving the quality of surimi. Impacts of washing processes on properties of surimi produced from yellowtail barracuda with different freshness and the stability of surimi prepared by selected washing processes during frozen storage can be elucidated.

Review of Literature

1. Fish muscle proteins

Protein is a major composition of fish muscle, ranging from 15 to 20% (wet weight), but protein content is reduced in a spawning period (Almas, 1981). Protein compositions of fish vary depending upon muscle type, feeding period and spawning, etc. Generally, fish muscle protein can be divided into three major groups on the basis of solubility characteristic including sarcoplasmic protein (water-soluble), myofibrillar protein (salt soluble) and stroma protein (insoluble) (Xiong, 1997b).

1.1 Sarcoplasmic proteins

Sarcoplasmic proteins are located inside the sarcolemma and are soluble in low salt concentrations (<0.1 M KCl). Sarcoplasmic proteins comprise about 30-35% of the total muscle proteins (Xiong, 1997b), including myoglobin, enzymes and other albumin (Sikorski, 1994). Sarcoplasmic enzymes are responsible for quality deterioration of fish after death. These include glycolytic and hydrolytic enzymes. Various proteinases found in the sarcoplasmic fraction may further catalyze the degradation of nitrogenous compounds in the muscle tissues. The activity of these enzymes depends on the fish species, type of muscle tissue as well as seasonal and environmental factors (Shahidi, 1994). Furthermore, sarcoplasmic proteins have an adverse effect on the strength and deformability of myofibril protein gels via an interference with myosin cross-linking during gel matrix formation (Sikorski, 1994). Myoglobin has been known to be a major contributor to the color of muscle, depending upon its derivatives and concentration (Faustman et al., 1992; Postnikova et al., 1999). The stability of myoglobin also affects the color of meat (Chen, 2003). Haemoglobin is lost rather easily during handling and storage, while myoglobin is retained by the muscle intracellular structure (Livingston and Brown, 1981). Therefore, color changes in meat are mainly due to the reaction of myoglobin with other muscle components, especially myofibrillar proteins (Hanan and Shaklai, 1995).

1.2 Myofibrillar proteins

Myofibrillar proteins are the major proteins in fish muscle. Normally, these proteins account for 65-67% 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 homogenate (Sikorski et al., 1990; 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 protein. Kalakowska et al. (1976) recommended that NaCl solution at 2.5-3.0% could be used for extraction of myofibrillar proteins. Myofibrillar proteins include several proteins.

- Myosin

Myosin is the protein which forms the thick filament. A molecular weight is about 500,000 daltons. It is the most abundant myofibrillar component, constituting approximately 40-60% of total protein content. Myosin consists of six polypeptide subunits, two large heavy chains and four light chains arranged into an asymmetrical molecule with two pear-shaped globular heads attached to long α-helical rod like tail (Xiong, 1997b). The rod portion is responsible for the assembly of myosin into thick filaments, and the two globular heads contain both the enzymatic active site and the actin-binding region. Thus, myosin has the biochemical properties of both a globular and a fibrous protein (Pearson and Young, 1989). The myosin molecule is approximately 150 nm in length, with a diameter of approximately 8 nm in the globular region and around 1.5-2.0 nm in the α-hilical region. Myosin contains two identical heavy chains (approximately 220,000 daltons each) and two sets of light chains that range in size from 14,000-20,000 daltons, depending on species and type of muscle (Pearson and Young, 1989). If solutions of myosin are treated with either trypsin or chymotrypsin, the two new species are generated. One of them is called light meromyosin (LMM) and the other is heavy meromyosin (HMM) (Watabe et al., 1994). The HMM fragment contains all ATPase activity and actin-binding ability.

The LMM portion is responsible for packing of myosin into the body of thick filaments. The treatment of HMM with papain results in formation of two additional fragments termed S1 and S2 (Watabe *et al.*, 1994). Both of S1 and S2 fragments contain the portion of the α-helical region between the head and the LMM. Generation of HMM, LMM, rod, S1 and S2 proteolytic fragments of myosin are shown in Figure 1.
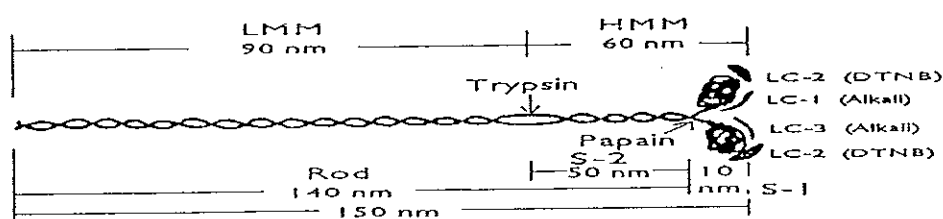
Figure 1. Schematic presentation of myosin molecule. Light meromyosin (LMM), Heavy meromyosin (HMM), rod, S-1, S-2 subfragments of HMM, the light chains, and the hinge regions susceptible to trypsin and papain are indicated.

Source: Xiong (1997b)

- Actin

Actin is the second most abundant myofibrillar protein, constituting about 22% of the myofibrillar mass. Actin can exist either as monomer (G-actin) or a fibrous form (F-actin). Normally, actin exists as double-helical filaments (F-actin) composed of polymerized globular monomers. Each monomer has a molecular weight of approximately 43,000 daltons (Xiong 1997b). In muscle tissue, actin is naturally associated with tropomyosin and 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 meat (Sikorski, 1994).

- Actomyosin

When actin and myosin are mixed *in vitro*, a complex, call actomyosin, is formed. The complex is bound not by covalent, but by electrostatic bonding with the contribution of phosphate groups (Xiong, 1997b). The reconstituted actomyosin

produced from both component proteins demonstrates many biochemical and physicochemical features of myosin, however does not exhibit physicochemical and function features of F-actin (Kijowski, 2001). This complex can be dissociated by the addition of ATP or high ionic strengths (Xiong, 1997b). Actomyosin is the main state of actin and myosin in postmortem muscle because ATP is depleted by postmortem metabolism (Ochai and Chow, 2000).

- **Tropomyosin**

Tropomyosin is a dimeric molecule consisting of two dissimilar subunits designated α- and β- tropomyosin with molecular weights of about 34,000 and 36,000 daltons, respectively. 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). Tropomyosin is about 5% of myofibrillar protein. Each tropomyosin molecule consists of 7 molecules of G-actins (Foegeding *et al.*, 1996).

- **Troponin**

Troponin is an asymmetrical protein and consists of three subunits. Troponin T (molecular weight of 37,000 dalton), which is also bound to troponin subunits C and I, links the troponin molecule to the tropomyosin molecule in the I-band. Troponin C (molecular weight of 18,000 daltons) binds Ca^{2+} and confers Ca^{2+} sensitivity to the troponin-tropomyosin-actin complex. Troponin I (molecular weight of 23,000 daltons), the inhibitory subunit, binds tightly to troponin C and actin and only slightly to tropomyosin or troponin T (McCormick, 1994) (Figure 2).

1.3 Stroma proteins

The stroma is composed of connective tissue proteins, such as collagen and elastin representing approximately 3% of total protein content of fish muscle (Suzuki, 1981). The stroma is the residue after extraction of the sarcoplasmic and myofibrillar proteins. Generally, the stroma is insoluble in dilute solutions of hydrochloric acid or sodium hydroxide (Sikorski *et al.*, 1990).
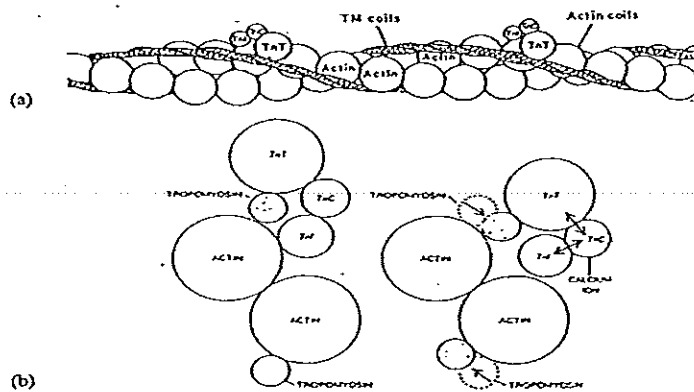
Figure 2. Thin filament of muscle formed by the filament of tropomyosin molecules wound in each of the two grooves of the actin helix (a) and proposed model for configuration of actin, tropomysin and troponin (Tn) subunits (b).

Source: McCormick (1994)

Tn T = troponin-tropomyosin subunit,

Tn I = troponin-inhibitory subunit,

Tn C = troponin-calcium-binding subunit

2. Gelation of fish muscle proteins

Fish muscle proteins typically undergo heat-induced gelation, which plays a role in texture development of many seafood gelly products. Among fish muscle proteins, myofibrillar proteins mainly contribute to functional properties including gelation (Benjakul *et al.*, 2001; Ko *et al.*, 2007). Myosin or actomyosin are the major protein in myofibrils and mainly involve in gelation of fish proteins. Generally, myosin alone form excellent gels and actin has a synergistic or antagonistic on myosin gelation, depending upon the myosin/actin ratio in the gelling system (Grabowska and Sikorski, 1976; Matsumoto, 1980). A specific actin/myosin weight ratio (~1/15) favors the formation of a stronger gel than that from myosin alone (Ishioroshi *et al.*, 1980; Yasui *et al.*, 1980).

Gelation of fish protein generally involves denaturation and aggregation. Denaturation is a process, in which proteins undergo conformational changes,

primarily unfolding without alteration of the amino acid sequence. Then, protein-protein interactions, known as association, aggregation and polymerization, take place and a three-dimensional network can be formed. Normally, gels are formed when partially unfolded proteins interact at specific points to form a three dimensional cross-linked network (Zayas, 1997) and capable of holding water. Table 1 gives a summary of changes, which may occur during the heat denaturation of natural actomyosin.

Table 1. Conformational change occurring during the thermal denaturation of natural actomyosin

Temperature (°C)	Protein (s) or segment involved	Description of events
30-35	Native tropomyosin	Thermal dissociation from the F-actin backbone
38	F-actin	Super helix dissociates into single chain
40-45	Myosin	Dissociated into light and heavy chains
	Head	Possibly some conformational change
	Hinge	Helix to random coil transformation
45-50	Actin, myosin	Actin-myosin complex dissociates
50-55	Light meromyosin	Helix to coil transformation and rapid aggregation
>70	Actin	Major conformational changes in the G-actin monomer

Source: Ziegler and Aton (1984)

2.1 Denaturation or dissociation

Muscle proteins undergo structural and conformational changes during heating, leading to promoting the interaction among protein molecules (Xiong, 1997b). Conformational changes of proteins occurring on heating are usually called denaturation (Tornberg, 2005). When protein denatures, both inner- and intramolecular bonds are disrupted or the protein is thought to change its conformation from a highly ordered state to a less ordered counterpart (Kilara and Harwalkar, 1996). Thermal

transition temperatures (T_{max}) represent point where conformational changes in protein structure take place. Generally, three major transition peaks are found in fish muscle, depending on species and are related to the environmental at which fish live. Peak transition temperature (T_{max}) for the protein in cod muscle was reported by Thorainsdottir *et al.* (2002). The first transition (zone A) at 43.5°C is attributed to myosin (Figure 3). The second transition (zone B), which occurs at 59.3°C, was assigned to collagen or sarcoplasmic proteins. This peak is a smaller peak usually seen at a temperature intermediate between the myosin and actin denaturation peaks. The third transition (zone C) has been assigned to actin and was found at 73.6°C. 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). However, fish muscle proteins are less stable than mammalian protein (Ogawa *et al.*, 1994). Kijowski and Mast (1988) reported that chicken breast muscle had T_{max} of 58.9, 68.7 and 78.7°C. In general, myosin is unstable and prone to denaturation than actin (Hasting *et al.*, 1985).
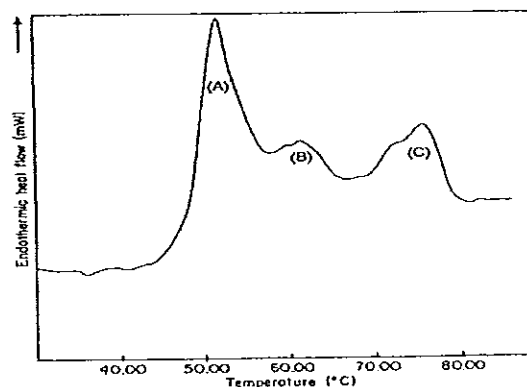
Figure 3. A typical thermal curve of whole muscle involving three major zones: myosin (A) subunits; sarcoplasmic proteins or collagen (B); actin (C)

Source: Adapted from Howell *et al.* (1991)

During the dissociation, protein is unfolded or denatured by modifying the charged groups through pH changes or addition of salt or water-competitive compounds under appropriate conditions (Zayas, 1997). Normally, addition of salt in combination of heating process is two major factors involved in denaturation and gelation of muscle proteins. Salts have been used as important ingredient for surimi gel production. Salts can disrupt the ionic linkages and assist in the dispersion of the proteins, which are necessary for the development of an elastic structure in heat-set-gel (Niwa, 1992). The salts ion (Na^+ and Cl^-) selectively bind to the oppositely charged groups exposed on the protein surface (Figure 4). As a consequence, the intermolecular ionic lingkage among the myofibrillar proteins are ruptured, and the proteins are dissolved in water because of their increased affinity for water (Lanier, 2000). The addition of salt shifted the denaturation transitions to lower temperatures and decreased the enthalpies of heat denaturation. Hastings *et al.* (1985) reported that salt treatment of herring muscle decreased transition temperature by 5-10 K and peak area was also decreased by salt addition. This might cause a partial unfolding of proteins and increased sensitivity to denaturation (Park and Lanier, 1989). Park and Lanier (1990) reported that 3% salt addition in washed tilapia muscle shifted endothermic peaks to lower temperature.
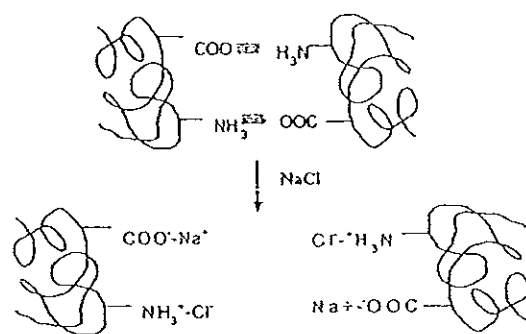
Figure 4. Model of salt linkage formation among myofibrillar protein and the role of NaCl in solubilization.

Source: Adaped from Niwa (1985)

2.2 Aggregation

Aggregation of protein results from the association of protein molecules. Denatured proteins begin to interact noncovalently to form a fine elastic network when surimi sol is subjected to heating (Lanier, 2000). Differences in cross-linking of myosin heavy chain contribute to the differences in gel forming ability among the muscles of various fish (Benjakul *et al.*, 2001). Different composition and structure of MHC among various fish species may be responsible for species-to-species differences in gelation (Visessanguan *et al.*, 2000). Samejima *et al.* (1981) reported that heat-induced gelation of myosin can be represented by two reactions. One is the aggregation of the globular head portion of the molecule which is complementary to and closely associated with the oxidation of SH groups and the other is the inevitable network formation by the thermal unfolding of the helical tail portion (Samejima *et al.*, 1981). Aggregation of myosin during heating is solely due to the association of the head portions (Samejima *et al.*, 1981). In the sol state, the rod chains have 100% helix-coil transition, which is sufficient to provide cross-links for a continuous network. The unfolding tends to prevent the gel from becoming progressively more tightly cross-linked. Chan *et al.* (1993) reported that thermal aggregation of fish myosin was coincidental with an increase in the surface hydrophobicity of the unfolded domains of myosin molecules and was affected by the temperature at which these domains unraveled. Temperature and ionic strength have a profound effect on the hydrophobic interaction. Both HMM and LMM are involved in the thermal aggregation of cod and herring myosin. Thermal aggregation may be initiated by the unfolding and interaction of HMM S-2, and further aggregation may be mediated through the interaction of LMM to form clusters of aggregate at higher temperatures (Figure 5). Gill and Conway (1989) concluded that the tail rather head portion of myosin was involved in the thermal aggregation of cod myosin. Furthermore, Sano *et al.* (1994) reported that the natural actomyosin (NAM) molecule extracted from carp muscle began to unfold around 30°C and substantial unfolding occurred from 30°C to 50°C, which was mainly attributed to the interaction of LMM.
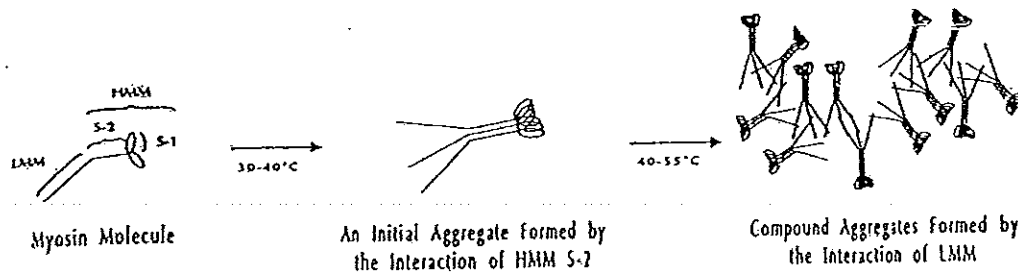
Figure 5. Schematic representation of the thermal aggregation of fish myosin

Source: Chan *et al.* (1993)

2.3 Bondings involved in gelation

The formation of gel network during gelation process is associated with various bonds. Types and numbers of bonds directly affect the characteristics and properties of protein gels.

- Hydrogen bonds

Hydrogen bonds are weaker dipole bonds not responsible for the gelation of myofibrillar proteins but are important in the stabilization of bound water within the hydrogel. Normally, hydrogen bonds between proteins are more numerous when the gel is colder, but it is destabized by heating (Lanier, 2000). A large amount of water molecules are hydrogen bonded to the polar amino acid residues, which are abundantly exposed on the molecular surface of heat-denatured proteins (Lanier, 2000). Hydrogen bonds between amino acids also help stabilize the internal (secondary) structure of individual protein molecules in water. The α helix of native and partially denatured proteins and the β-pleated structure that forms on heating and cooling are both stabilized by hydrogen bonds (Bouraoui *et al.*, 1997).

- Ionic linkages

Ionic linkages are the attraction of positively charged sites to negatively charged site on the protein surface. At the normal pH of surimi, the carboxyl groups (COO⁻) of glutamic acid and aspartic acid, two amino acids on the protein chain, are negatively charged, whereas the amino groups (NH_3^+) of lysine and arginine are positively charged. Therefore, an ionic attraction will be formed between these groups, and the myofibrillar proteins associate with each other to form an aggregate, which is insoluble in water.

Sodium chloride must be added to break the ionic linkages and assist in the dispersion of the proteins because an even dispersion of the proteins is necessary for the development of an elastic structure in the heat-set gel (Niwa, 1992). Calcium chloride has been used to enhance the gel strength of surimi. Calcium ion can form salt linkages between negatively charges localized on the adjacent proteins, resulting in the strengthening of surimi gel by calcium bridge (Figure 6) (Wan *et al.*, 1994). Furthermore, adding calcium salts to improve the gelling properties of surimi is more actually based on the effect of calcium as a cofactor for an endogeneous cross-linking enzyme (transglutaminase) in the muscle. Benjakul *et al.* (2004) reported that the addition of $CaCl_2$ (up to 20 mmol/kg) resulted in the increases in breaking force of surimi produced from bigeye snapper, threadfin bream, barracuda and bigeye croaker.

Charged Protein Stands   Ion Bridge Crosslinks
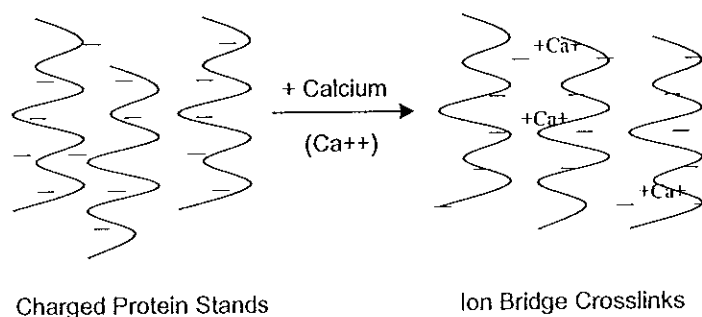
Figure 6. Formation of calcium cross-links between proteins.
Source: Adaped from Lanier (2000)

- **Hydrophobic interaction**

Hydrophobic interactions play an important role in strengthening of surimi gel. Generally, hydrophobic interactions are formed by increasing temperature at least to near 60°C (Lanier, 2000). About 25% of the amino acids that constitute the myosin molecule are hydrophobic amino acids such as alanine, valine, leucine, isoleucine, proline, tryptophan and phenylalanine. Normally, the interior portion of the folded protein chain has the greater density of hydrophobic amino acids. Conversely, the amino acids on the surface of the undenatured protein are largely hydrophilic. When a protein denatures (unfold) during heating, the hydrophobic core is exposed to the surface, leading to hydrophobic interaction between the hydrophobic residue. Sano *et*

al. (1994) found that carp myosin started to unfold hydrophobic domains when heated to 30°C and extensively increased when heated to 50°C. The increase in hydrophobicity also indicated the involvement the hydrophobic interaction in gel formation (Chan *et al.*, 1992). Yongsawatdikul and Park (2003) reported that the hydrophobic interaction of threadfin bream actomyosin took place at 70°C. Hydrophobic interaction is a major force involved in aggregation of arrowtooth flounder myosin at high temperature (Visessanguan *et al.*, 2000b). Benjakul *et al.* (2001) suggested that the higher surface hydrophobicity was in agreement with the higher aggregation of bigeye snapper natural actomyosin.

- Disulfide bonds

During heating at high temperatures (cooking at >40°C), disulfide bonds are the predominant covalent bond contributing to gel formation of proteins. Generally, the rate of disulfide bond formation of most native protein is low. It can be increased by unfolding the native structure via applying high pressure, elevated temperature, shears force and addition of component that affect the solvent quality (Visschers *et al.*, 2005). In general, most of sulfhydryl groups (~68%) in myosin are located in the head portion of myosin (Lowry *et al.*, 1969). S-1 (globular head) and rod (fibrillar tail) fractions of myosin primarily involved in the dimerization and polymerization of myosin, respectively (Kishi *et al.*, 1997). Benjakul *et al.* (2001) found that disulfide bonds in natural actomyosin from *P. tayenus* and *P. macracanthus* were formed at temperatures of 45°C and 40°C, respectively. Yongsawatdikul and Park (2003) found that reactive SH increased as heating progressed and decreased at 50°C, suggesting the formation of inter- or intramolecular disulfide bonds of threadfin bream actomyosin at >50°C. Ko *et al.* (2007) reported that the decreases in the total sulfhydryl group content of tilapia actomyosin observed at 55°C and 65°C due to the formation of disulfide bonds.

- Non-disulfide covalent bond

The cross-linking reactions other than disulfide bonding involve in the setting phenomenon (Niwa, 1992) at temperature ranging from 0 to 40°C (Lanier, 1992). The unique setting ability of surimi is thought to mainly result from the enzymatically catalyzed formation of nondisulfide covalent bonds between protein molecules, induced by endogenous transglutaminase (TGase). TGase is a

transferase that catalyzes the acyl transfer reaction between γ-carboxyamide groups of glutamine serving as the acyl donor and ε-amino groups of lysine acting as the acceptor, resulting in protein polymerization via ε-(γ-glutamyl) lysine cross-linkings (Folk, 1980) (Figure 7). Benjakul and Visessanguan (2003) found that setting of surimi paste with and without subsequent heating resulted in the increase in both breaking force and deformation of suwari and kamaboko gel from Bigeye snapper surimi, respectively. However, TGase mediated cross-linking reaction of MHC depends upon species (Araki and Seki, 1993) and setting temperatures (Benjakul and Visessanguan, 2003). The maximum gel strength and MHC cross-liking ability of Alaska pollock surimi was found with setting temperature of 25°C, while the maximum cross-linking by endogenous TGase occurred at 40°C in croaker surimi (Kamath et al., 1992). Benjakul et al. (2004) reported that high temperature setting at 40°C for an appropriate times enhanced gel strength of surimi from some tropical fish. Additionally, endogenous TGase is a Ca^{2+}-dependent enzyme. The addition of Ca^{2+} to fish protein paste has been reported to activate TGase activity and the strengthened gels are obtained (Benjakul and Visessanguan, 2003; Lee and Park, 1998).
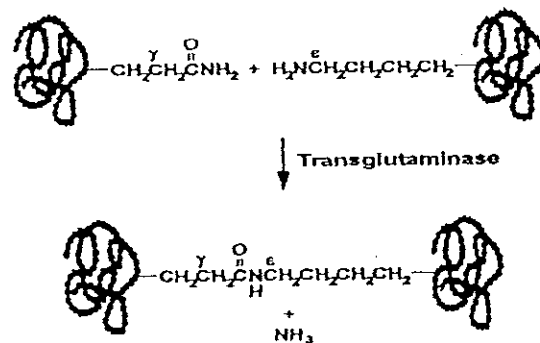
Figure 7. Cross-linking of proteins by transglutaminase

Source: An et al. (1996)

2.4 Factors affecting gelation

In general, the characteristics of gel are governed by many factors including temperature (Sano et al., 1994), heating rate (Yongsawatdigul and Park, 1999),

pH (Shikha *et al.*, 2006a) and type of actomyosin used (Lefevre *et al.*, 2007). Muscle protein gel network is influenced by a number of factors as shown in Figure 8.
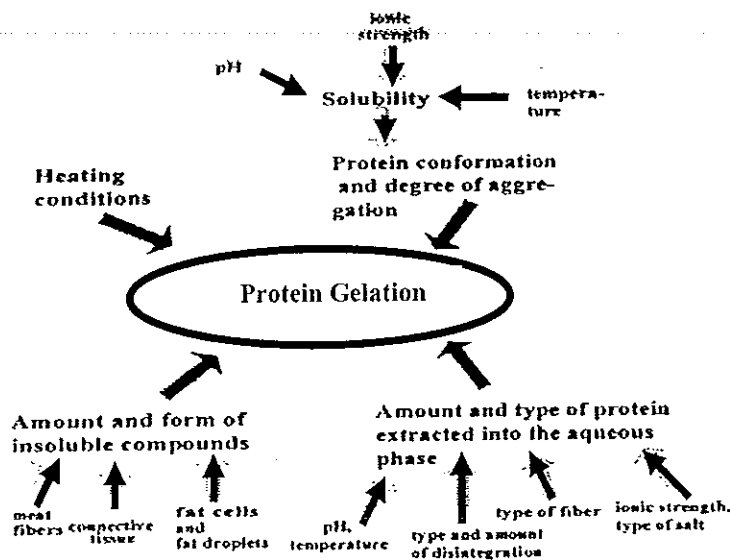
Figure 8. Factors affecting protein gelation

Source: Tornberg (2005)

2.4.1 Effect of muscle type

Differences in thermal gelation properties of muscle protein relate to muscle types. In brown trout muscle, myosin extracted from white muscle form gels at lower temperature than red counterpart (Lefevre *et al.*, 1998). Red muscle protein extracted from salmon had higher transition temperatures (Lefevre *et al.*, 2007). Chaijan *et al.* (2004) reported that surimi produced from Mackerel and Sardine exhibited poor gel quality, because of high content of dark muscle associated with high content of lipid and myoglobin. In addition, dark muscle also has a higher proteolytic activity than white muscle (Shimizu *et al.*, 1992). Ochiai *et al.* (2001) suggested that high-quality surimi with higher gel strength and better whiteness can be obtained when dark muscle is removed as much as possible

2.4.2. Effect of heating rate and time

Heating rate or time of heating affect the unfolding and appear to influence the gel formed. When the rate of aggregation is slow to cause denaturation, heat-denatured proteins are allowed to align in an ordered fashion to form a fine gel

network, resulting in more elastic gels (Hermansson, 1979). Gill *et al.* (1992) demonstrated that slow heating rate yielded more elastic gel of herring muscle. Yongsawatdigul and Park (1996) indicated that the effect of heating rate on textural properties of fish myofibrillar proteins was species-dependent. Myofibrillar proteins of Alaska pollock also formed the stronger gels at slower heating rate (e.g. 0.5°C/min) while those of pacific whitting exhibited higher shear stress and shear strain at higher heating rate (e.g. 30°C/min). This was attributed to the differences in predominant endogenous enzyme in each species. Yongsawatdigul and Park (1999) concluded that slow heating rate activated endogenous protease associated with whiting myosin, leading to the hydrolysis of myosin and lowering gel elasticity. However, it promoted the aggregation of cod myosin and enabled aggregates to form a more elastic gel.

2.4.3 Effect of pH and ionic strength

The pH of the salted paste is one of the most important factors in producing a strong elastic kamaboko gel. Postmortem lowering of muscle pH induced the denaturation of the myofibrillar protein. The solubility was decreased with lowering pH of fish meat. Shimizu *et al.* (1954) reported that the gel strength was high at pH 6.5-7.0 for flying fish and became low by acidifying. The net charge of protein at its isoelectric point is equal to zero. The greater the net charge on the protein molecules, the greater the electrostatic repulsion between molecules is obtained, preventing the interactions required to form a gel matrix (Totosaus *et al.*, 2002). Shikha *et al.* (2006b) reported that gel strength of surimi produced from Walleye pollack decreased with decreasing pH value. Neutralization of acidified surimi (pH 6.0 reneutralized to pH 7.2) could improve gel strength, however it was considerably lower than original gel strength (pH 7.2). Park *et al.* (2008) found that the solubility of myosin purified from pacific sardine was low at pH range of 2.5-5.0, which was close to the pI of myosin (pH 4.6-6.2), resulting in the poor gel forming ability.

The ionic strength affects state of the myosin molecules. Myosin molecules associate and form filaments at low ionic strength (<0.3 M). However, dispersion of filaments to the monomers was observed at ionic strength above 0.3 M. Sano *et al.* (1990) suggested that the interaction between myosin molecules occurred more easily on increasing the ionic strength. In the presence of 0.1-0.2 M KCl,

interfilament aggregation of myosin heads on filament surfaces is responsible for gel formation, and gel strength is positively correlated with salt concentration (Samejima, 1981). Acceleration of gelation was observed at low ionic strength (0.2 M KCl) with the formation of a fine-stranded gel microstructure, with greater rigidity than at high ionic strength (0.6 M KCl) (Hermansson, 1986).

2.4.4 Effect of enzymes

Temperature plays an important role in surimi gelation. In addition to its effects on the conformation of myofibrillar proteins, temperature can activate endogenous enzyme. Fish muscles from various species revealed similar responses to temperature: a structure-setting reaction for gelation below $40^{\circ}C$ (suwari) and a structure-disintegration reaction at $50-70^{\circ}C$ (modori). Low temperature setting is associated with tranglutaminase (TGase) activity, while modori is induced by endogenous thermal stable proteinases that can degrade myosin rapidly (Jiang, 2000).

TGase can catalyze the formation of ε-(γ-glutamyl) lysyl crosslinks and can also form isopeptide bonds among fish myosin molecules, which is considered to be related to the gel strength of minced fish (Seki et al., 1990). TGase isolated from Alaska pollack and could induce the gelation of minced fish (Seki et al., 1990). Tsukamasa and Shimizu (1990) further reported that the strong gel-forming ability of sardine was due to the formation of the nondisulfide bond, which later was shown to be due to the action of TGase. Jiang (2000) also reported that the gel strength of Mackerel surimi was greatly improved by the addition of pig plasma TGase. Addition of calcium to activate the endogenous TGase or incubation at higher than $35^{\circ}C$ to activate endogenous TGase during setting process indicate that this enzyme contributes greatly to the gelation of pollack paste (Takeda, 1996). Ca-independent microbial TGase (MTGase) from *Streptoverticillium mobarense* or from *Streptoverticillium ladakanum* has shown high potential to increase the gel strength of fish surimi (Jiang, 1998a).

Endogenous proteinases resulted in a texture often described as soft or mushy (Figure 9). Proteolytic disintegration of surimi gels is characterized by high activity at temperatures above $50^{\circ}C$ and by the rapid and severe degradation of myofibrillar proteins, particularly myosin (Jiang, 2000). This disintegration has detrimental effects on surimi quality, which substantially lowers the strength and elasticity (Morrissey et al., 1993). Among numerous proteinases presented in muscle,

cysteine proteinases have the most serious effect on texture due to their thermostability and the ability to cleave the internal peptide bonds, while exopeptidase hydrolyzes terminal peptide bonds (Jiang, 2000). The most active proteinases in fish muscle that can soften the surimi gels vary with species, but are generally categorized into two major groups: cathepsins (Seymour, 1994) and heat-stable alkaline proteinases (Jiang, 2000). Benjakul et al. (2003b) reported that heat-activated alkaline protease played a role in degradation of bigeye snapper surimi.
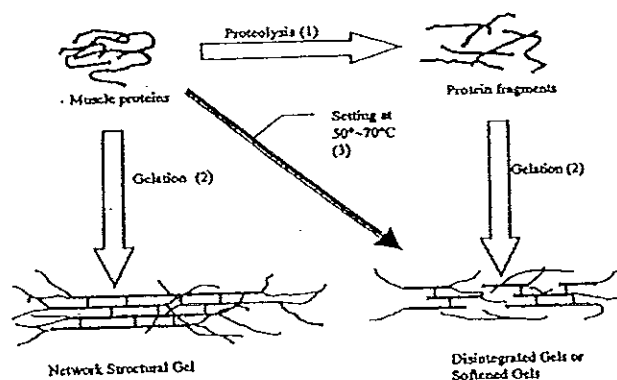
Figure 9. Proposed model of the gelation and disintegration of surimi gels. (1) Proteolysis by calpains, cathepsins, and other proteinases during storage ; (2) gelation ; (3) proteolysis by heat stable alkaline proteinases (HAP), cathepsins B, L, and L-like during setting at 50-70°C ; (4) modori or disintegration by HAP, cathepsins, calpains, and other proteinases if the formed network structure is not fixed by heating at 85-100°C.

Source: Jiang (2000)

3. Washing processes for surimi production

The purpose of washing of fish muscle is to remove water-soluble matter, lipids, and blood from the mince to improve the color and flavor as well as to increase the gel strength of the surimi (Toyoda et al., 1992). The water-soluble matter includes sarcoplasmic proteins, digestive-enzymes (primarily proteases), inorganic salts, and low-molecular organic substances such as trimethylamine oxide. The removal of sarcoplasmic proteins increases the concentration of myofibrillar proteins, which is

primarily responsible for gel formation (Park and Lin, 2005). A proper washing process is vital to achieve high-quality surimi with high recovery. An insufficient washing process could result in a substantial loss of gel quality during frozen storage (Toyoda et al., 1992). On the other hand, over-washing could case a substantial loss of fine particles and excessive moisture content. Washing efficiency is often affected by various factors such as water/meat ratio, the number of washing, freshness of fish, structure of the washing unit, etc. (Park and Lin, 2005).

3.1 Typical washing process

The typical washing process involves mixing minced meat with cold water (5°C) and removing water by screening and dehydrators or centrifuging to about 5-10% solids. The screw press draws water out with compression to a level of 82-85% moisture (Park and Morrisey, 2000). This process is repeated by water two or three times, leading to the lower salt content of washed mince and the higher hydrophilic tendency (Okada, 1981). The ionic strength of the washing water affects the swelling tendency of the washed meat. The greater ionic strength of the washing water, the easier the water is removed from the meat. The minimum hydrophilic condition occurs at ionic strength between 0.005 and 0.1 (0.03 and 0.6% NaCl) (Tamato, 1986).

However, Park and Lanier (1989) showed that the addition of salt shifted the denaturation transition to lower temperatures and decreased the enthalpies of heat denaturation. These results suggest that the addition of salt might cause a partial unfolding of proteins and increase sensivity to denaturation. Lin and Park (1996) evaluated the effect of salt concentrations and washing cycles on the extraction of proteins. Sarcoplasmic proteins were soluble in water (0% NaCl) and removed in the initial washing steps. Control of water/meat ratio, washing time and washing cycle was critical in reducing the loss of myofibrillar proteins. Washing with 0.25, 0.5 and 1.0% NaCl solutions reduced the loss of myofibrillar proteins. However, these solutions were not effective in removing sarcoplasmic proteins when washing cycle increased. Washing with 2.0% NaCl resulted in low removal of sarcoplasmic proteins and myofibrillar proteins were severely lost (Lin and Park, 1996).

3.2 Washing process with oxidizing agent

Ozone water in surimi manufacturing reduces bacteria counts as well as increases whiteness of surimi (Park and Lin, 2005). During ozonation, the porphyrin structure of the heme pigment is destroyed and consequently decolored (Buckley *et al.*, 1975). Jiang *et al.* (1998) found that ozonation for thirty min increased the whiteness (51.7 to 60.1) and L value (53.9 to 62.6), and decreased myoglobin (47.8 to 12.2 mg/100g) and total pigments (78.5 to 15.5 mg/100g) of surimi from mackerel surimi. However, the deformation of surimi decreased from 0.90 to 0.45 cm, even though there was no change in breaking force. The decrease in deformation might be due to the increase in disulfide bond, which decreased the solubility of mackerel muscle proteins. In addition, Chen (2002) studied washing mackerel mince in combination with flotation using nitrogen, air and ozone. The gel-forming ability of mince with N_2 or air flotation washing effectively improved gel strength in heated surimi but poor gel was observed with ozone flotation washing. Strong oxidation chacteristics of ozone accelerate changes in chemical bonding and cause denaturation of proteins. Phatcharat *et al.* (2006) investigated the effects of washing with hydrogen peroxide (H_2O_2) and sodium hypochlorite (NaOCl) solutions on the gel-forming ability and physiochemical properties of surimi produced from Bigeye snapper, stored in ice for up to 14 days. Washing with 20 ppm NaOCl resulted in the highest increase in both the breaking force and the deformation of surimi gel produced from fish stored in ice. Natural actomyosin (NAM) extracted from NaOCl-washed mince had higher surface hydrophobicity and disulfide bond content than that of water-washed mince. The results suggested that washing mince with the appropriate type and concentration of oxidizing agent can improve the gelling ability of surimi, particularly from low quality fish.

3.3 Alkaline saline washing process

Alkaline saline leaching was a process to improve the gel-forming ability of dark-fleshed species (Shimizu, 1965). This process involves suspending fish mince in about four volumes of cold and dilute alkaline salt solution (0.15% NaCl containing 0.2% $NaHCO_3$ of which the ionic strength is 0.05 and the final pH is in the range of 6.8-7.3) for 15-20 min with occasional stirring. The material is dewatered through

screens, and may be leached an additional one or two times in water or 0.2-0.3% NaCl if needed. The temperature is kept below 10°C throughout all leaching steps (Shimizu, 1965). This improvement is a result of a decreased rate of denaturation as the muscle pH increased and solubility of the sarcoplasmic proteins of dark-fleshed species increased. The enhanced removal of sarcoplasmic proteins also results in a lighter colored surimi due to removal of heme pigments. Additionally, the flavor is improved as the removal of carbonyl compounds is enhanced (Tokunaga, 1982). Suzuki (1987) studied a new processing method from the conventional surimi process in three ways. First, the muscle disintegrates into very small particles, on the myofibril level (1-2μ in thickness), by a vacuum homogenizer just prior to leaching. The particle reduction is carried out with the meat particles suspended in the leaching medium, under a vacuum of about 10 mmHg to reduce oxidation of lipid and proteins. Second, the mixture of sodium pyrophosphate and sodium bicarbonate (0.05-0.1 and 0.1%, respectively) was used as the leaching medium. Sodium bicarbonate is used for regulating the pH of the sardine meat to avoid denaturation of the myofibrillar protein. Sodium pyrophosphate is added to induce dissociation of actomyosin to myosin and actin. Third, vacuum leaching was the leaching vessel and controlled removal of gases from the mixture. As the gases escape (violently) from the muscle particles at low atmospheric pressures, compounds such as heme, lipids, and odorous substances are also released. The vacuum level is maintained at about 30 torr during this operation, which lasts for 20-30 min. The result is an enhanced removal of color and flavor/odor and reduced lipid content in surimi. Chen et al. (1997) studied the effect of different washing processes on color and gel-forming properties of horse mackerel surimi. Improvement in gel strength occurred for mince washed with alkaline solution for 90 min and undesirable gel strength occurred for mince washed with ozone. Balange and Benjakul (2009) reported that breaking force and deformation of gels varied with washing processes. Alkaline washing process led to the concentrated myosin heavy chain. The gel of alkaline-saline washing process surimi added with 0.25% OTA had the increases in breaking force and deformation by 166.2 and 45.9%, respectively, compared with that of conventional washing process surimi without OTA addition. Thus, oxidized tannic acid showed the synergistic effect with alkaline washing process in improving the gel properties of mackerel surimi.

3.4 pH shift process

The pH shift process has been implemented to obtain maximal recovery and functional protein isolates from dark-muscle fish. The overall process concept is the protein of muscle tissue are first solubilized. The solubilization can be accomplished in 5 to 10 volumes of water with alkali to obtain approximately pH 10.5 or higher, or with acid added to about pH 3.5 or lower. It is usually necessary to choose the pH at which the consistency of the solution decreases to a value that allows the removal of undesirable material. If it is desired to remove cellular membrances, it is generally necessary to go to a consistency of about 50 mPa.sec or lower. The mixture is then centrifuged. This allows the light oil fraction to rise to the top of the suspension. At the same time, the lipids of the membrane are removed in the sediment. Thus, both lipid fractions are removed due to density differences compared to the main protein solution. Other insoluble impurities, such as bone or skin, are also sedimented at this stage. The muscle proteins are precipitated and collected by a process such as centrifugation (Hultin *et al.*, 2005).

The easiest way to precipitate proteins is by adjusting the pH to a value near the isoelectric point of the majority of the proteins, which is about 5.2 to 5.5 (Hultin *et al.*, 2005). Almost all muscle proteins become insoluble under these conditions. This includes the sarcoplasmic proteins, which are mostly washed away during conventional surimi manufacture. The non-protein-soluble materials from the muscle tissue remain in the supernatant fraction after centrifugation and can subsequently be removed. Additional washes of the sedimented protein at the same pH can be used to decrease the concentration of soluble impurities if necessary (Hultin *et al.*, 2005).

Kim *et al.* (2003) reported that Pacific whiting protein solubility was significantly affected as the pH shifted away from the isoelectric point (5.5). The highest breaking force of gels was measured for fish proteins treated at pH 11, while high deformation values were obtained at pH 2 and 11. Pacific whiting proteins were highly degraded by acid-aided process (AC) and alkali-aided process (AL). High activity of cathepsin B-like enzyme as detected from AC, while strong cathepsin L-like activity was found in fish proteins treated at pH 10.5. Yongsawatdigul and Park (2000) indicated that disulfide linkages occurred at a greater extent in gel of rockfish surimi prepared by AL, resulting in higher breaking force and deformation.

Kristinsson *et al.* (2005) showed that both AC and AL led to higher recoveries of channel catfish muscle and larger reduction in lipids, compared with conventional washing (CW). The AL led to higher whiteness than AC and CW, respectively. The AC led to higher yellowness than the other two processes. Kristinsson and Liang (2006) found that the AC led to higher recoveries of protein from Atlantic croaker (*Micropogonias undulates*) than the AL and CW, respectively. Lipid reductions were highest for the AL followed by AC, while CW gave the least reduction. Perez-Mateos and Lanier (2006) founded that greater endogenous tranglutaminase activity was found in surimi from Atlantic menhaden (*Brevoortia tyrannus*) prepared by CW and AL than that prepared by AC. Salt addition improved CW gels, but seemed to adversely affect MTGase activity in AC and AL surimi. AC and AL surimi gels had lower whiteness than were CW surimi gels. Rawdkuen *et al.* (2009) reported that solubility and recovery of protein from Tilapia (*Oreochromis* ssp.) was found to be highest by CW, followed by AL and AC, respectively. Decrease in myoglobin and lipid contents were found in AL and AC, as compared to the CW. The highest breaking force and deformation of kamaboko and modori gels was found in the gels prepared by CW.

3.5 Washing process with adjusted hardness and ionic strength

The ionic salt level (hardness) is associated with water quality for surimi production. Generally, soft water with minimum levels of minerals, such as Ca^{2+}, Mg^{2+}, Fe^{2+}, and Mn^{2+}, is recommended for washing. Hard water causes deterioration of texture and color quality during frozen storage (Park and Morrissey, 2000). Ca^{2+} and Mg^{2+} are responsible for the color change (Lee, 1990). In addition, a high level of inorganic salts in the wash water, primaly Ca^{2+} and Mg^{2+}, affects the gel-forming ability of surimi by causing the denaturation of actomysin in Alaska pollock during frozen storage (Tomato, 1971), and by reducing thermal stability during washing (Saeki *et al.*, 1986). High Ca^{2+} level (higher than 20 mM) causes the increase in firmness but decrease in cohesiveness of surimi from Alaska pollack. Park (2000) reported that it is common to use a mixture of NaCl and/or $CaCl_2$ of 0.1-0.3% in the final wash water. However, special care must be given because too much salt in the wash water could cause solubilization of myofibrillar proteins. Furthermore,

Solberg *et al.* (1990) showed that higher removal of moisture from Cod (*Gadus morhua*) was obtained when Ca^{2+} or Mg^{2+} levels in washing water increased. The highest values of stress and strain at failure were obtained when approximately 10 mM $CaCl_2$ and 15 mM $MgCl_2$ were used in washing water. Okada (1981) hypothesized that the superior quality of kamaboko was likely due to the water quality which had a calcium content of 20-40 mg/100g. This has been supported by the fact that a calcium-dependent-acyl-tranfer reaction of cross-linking of myosin heavy chain was catalyzed by TGase during the setting of surimi paste (Kimura *et al.*, 1991; Benjakul and Visessanguan, 2003).

The activity of endogeneous TGase, a calcium-depentdent, can be optimized during the setting phenomenon by adding calcium to fish paste before incubation at 25-40°C. It was found that the shear stress property of surimi gels from warm-water fish species, such as Mexican flounder and Northen kingfish, was improved by adding 0.2% calcium chloride (Morales *et al.*, 2001). The shear stress of surimi gels from cold water fish species, such as Pacific whiting and Alaska pollock, was increased by adding 0.2% calcium chloride (Lee and Park, 1998).

4. Effect of salts on protein functional properties

Protein functionality can be defined as any physicochemical properties of protein that allows protein molecules to interact among themselves and their environment to produce or improve the quality and stability of final products (Xiong, 1997a). Salts have been known to affect the molecular properties of proteins, thereby influencing the functionality of protein.

4.1 Solubility

Solubility is prerequisite for all functionalities of proteins. Solubility of protein is governed by many factors. Ionic strength effect on protein solubility probably involves solvation, electrostatic, salting in and salting out phenomena (Kinsella, 1979). Chloride ions increase solubility by electrostatic repulsion after binding to the positively charged protein groups. Low concentrations of neutral salts at molarities of the order of 0.5-1.0 M may increase the solubility of proteins but

above 0.15 M can reduce it. In salts solutions, protein solubility initially increases (salting in) and after a maximum of solubility it starts to decrease (salting out). Increase in salt concentration greater than 1.0 M causes a decrease in protein solubility. Water molecules are strongly bound to the salt and there is competition between the salt ions and the protein molecules for the water molecules (Zayas, 1997).

Ions of neutral salts decrease the electrostatic attraction between the countercharged surface domains of neighboring protein molecules. Salts of divalent ions, such as $MgCl_2$ are far more effective at salting-in than salts of monovalent ions, such as NaCl, NH_4Cl and KCl. On the other hand, as the ionic strength is increased, the solubility of a protein begins to decrease. At sufficiently high ionic strength, a protein can be completely precipitated from solution. The great numbers of ions that must be solvated when dissolved in solution remove water molecules from hydration shells and thus promote interaction among partially dehydrated protein molecules. However, some ions are especially effective at salting-in, some others at salting-out. The ions can be ordered in the so-called Hofmeister series, according to their effectiveness at salting-in and salting-out (Milewski, 2001).

Increasing salting-out effect

$$\longleftarrow \underline{\hspace{6cm}}$$

$PO_4^{3-}, SO_4^{2-}, CH_3COO^-, Cl^-, Br^-, NO_3, ClO_4, I^-, SCN^-$

$NH^{4+}, Rb^+, K^+, Na^+, Cs^+, Li^+, Mg^{2+}, Ca^{2+}, Ba^{2+}$

$$\underline{\hspace{6cm}} \longrightarrow$$

Increasing salting-in effect
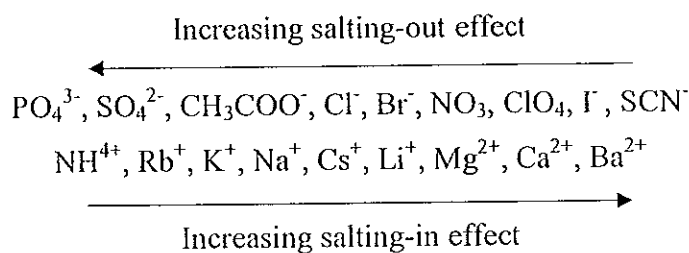
4.2 Water holding capacity

Water holding capacity of meat is influenced by the structure of myofibrils. The structure of cross-bridges between myosin and actin in the rigor state is believed to affect myofibril swelling and water-holding ability (Offer and Trinick, 1983). Certain structural components of the myofibril, such as the Z-disks that connect the actin filaments and the M-lines that link myosin filaments together, probably provide constraints to swelling. Some other structural myofibrillar constituents, including C-protein, may play a role in regulating water imbibing by myofibrils. Thus, the removal

of many of scaffold proteins, as occurs in postmortem muscle due to proteolysis, can lead to improving water-holding capacity in meat (Xiong, 1997a).

There are two major types of forces that contribute to water retention in meat: polarity, including surface charges, and capillary effects. Binding of water to the surface of protein through hydrogen bonds between water molecules and charged dipolar amino acid residues seems to be insignificant for water retention in meat. However, the capillary forces are believed to account for most of the water contained in meat. An increase in the electrostatic charges would lead to an increase in the repulsive force between myofilaments and increase in myofibril swelling and water-holding capacity. Hence, any change in the surroundings of myofibrils that result in increased protein charges or dipoles (e.g., high concentrations of salt and pH away from the protein isoelectric points) would lead to increased water retention in meat (Xiong, 1997a).

4.3 Stability

Quantitative ion binding by proteins is a function of several variables including the nature of the protein, the pH, the size and charge of the ion, and the ion concentration. The binding of chloride ion to proteins varies with the type of protein and as a function of pH. The relation of ions in stabilizing proteins against conformational change follows the Hofmeister series. The conformational stability of the protein is intimately related to its solubility (Kilara and Harwalkar, 1996). Decreased conformational stability is associated with greater solubility of the protein. Ions may react directly with protein, or they may alter the structure of the solvent such as the alteration of structure and orientation of water molecules around apolar site chains of protein (Kilara and Harwalkar, 1996).

4.4 Gelling properties

The presence of salts strongly affects the strength, deformability, and appearance of protein gel (Lupano et al., 1992; Kuhn and Foegeding, 1991). Effect of cations on protein molecules is the formation of short-range hydration repulsion. This result from the energy needed to dehydrate cation on the protein surface as two protein molecules approach each other (Israelachvili, 1985). Polar parts of the protein

will be prone to this repulsion, but the effect is likely to be greater when kosmotrpic (water-ordering) cations are bound to the protein (Bringe and Kinsella, 1991). Divalent cations, such as Ca^{2+} and Mg^{2+}, can be used to form protein gels. These ions from cross-links between negatively charged groups of protein molecules (Damodaran, 1996).

5. Changes in physico-chemical properties of muscle proteins and gelling properties of surimi during frozen storage.

5.1 Physico-chemical changes in muscle proteins during frozen storage

Freezing is a physical prcess involving the transformation of water molecules from an amorphous state to highly structured ice crystals. The phase change can lead to protein denaturation caused by alterations in the physical and chemical environment of proteins (Table 3) (Xiong, 1997a).

Table 2. Causes of freeze-induced protein denaturation and possible prevention methods

Cause	Mode of action	Method to minimize denaturation
Ice formation	Mechanical damage	Fast freezing rate
	Cell-wall diruption	Low thawing temperature
Recrystallization	Mechanical damage	Low temperature storage
		Prevent temperature fluctuation
Dehydration	Increased exposure of hydrophobic group : unfolding	Cryoprotectants
Salt concentration	Modified electrostatic interaction ; unfolding	Cryoprotectants
Oxidation	Modified amino acid side chains peptide bond cleavage	Antioxidants
Lipids	Protein-lipid complexes	Inactivate trimethylamine
Metabolites	Formaldehyde-methylene bridge	demethylase

Source: Xiong (1997a)

5.1.1 Mechanical damage

The formation of ice crystals from either intracellular or extracellular water can result in mechanical damage caused by irregular ice crystals protruding through and disrupting the cell walls. The size and location of ice crystals formed during freezing is greatly influenced by the freezing rate and temperature fluctuations (Xiong, 1997a). As crystallization proceeds, extracellular salt become more concentrated, creating an osmotic pressure gradient across the cell membrane. In an attempt to balance the chemical potential, intracellular (hypoosmotic) moisture flows outward, leading to dehydration and an increase in the ionic strength of the cell (Xiong, 1997a). Water drawn from the interior of the cell will freeze onto the existing extracellular ice crystals, causing them to grow, thus distorting and damaging the membrane and protein.

During frozen storage, especially when temperature fluctuates, ice crystal can undergo metamorphic changes referred to as recrystallization. In general, small ice crystals have a tendency to melt, recrystalize or aggregate onto existing larger crystals (nuclei) because of differences in the surface energy. This phenomenon is often observed for food stored at close to the critical freezing zone (-0.8 to -5°C). Because recrystallization involves enlargement in size, changes in shape, orientation, and movement of ice crystals, it usually causes tissue damage and accelerates protein denaturation (Xiong, 1997a).

5.1.2 Dehydration

Dehydration caused by ice formation can lead to protein denaturation, which can be explained in thermodynamic terms. In biological tissues, where moisture is the most abundant cellular constituent, proteins exposed to the aqueous environment have a hydrophobic interior and a polar surface. Most hydrophobic amino acid residues are occluded inside and most charged or polar side chains tend to stay outside. This structural assemblage is entropy driven, thereby facilitating hydration of protein by interacting with water molecules via hydrogen bond and lowers the total free energy of the system (Privalov and Makkatadze, 1993). In a dehydration state, protein-solvent interactions are disrupted and protein molecules are exposed to an essentially 'organic' environment (less polar than water). This will result in increased exposure of hydrophobic side chains and segments, leading to

changes in protein conformation. To maintain the lowest possible free energy status upon migration of water to form ice crystal, increased protein-protein interactions via hydrophobic and ionic forces occur, resulting in further denaturation of protein and formation of protein aggregates (Xiong, 1997a). Hydrophobic interactions are generally weakened as the temperature decreases. Hence, lowering the temperature to subfreezing temperatures will destabilize proteins whose native structures are critically maintained by hydrophobic forces (Privalov et al., 1986; Franks, 1995).

5.1.3 Concentration of salts

Water in biological tissues, cellular suspensions, or protein aqueous solution can be devided loosely into free and bound types. During freezing to -10°C or below, as much as 10% water in muscle tissue could stay unfrozen and is more or less "bound" to protein or other nonaqueous cellular components (Lawrie, 1991). However, the amount of unfreezable water depends on the concentration and type of solutes. As freezing proceeds, proteins are exposed to increase concentration of salts, and this process can theoretically continue until the final eutectic temperature (at which all solutes are also crystallized out) is reached. Because electrostatic interactions are one of the major bonds maintaining protein tertiary and quaternary structures, an abrupt increase in ionic strength or salt concentration in the nonfrozen phase can cause competition with existing electrostatic bonds, which in turn leads to extensive modification of the protein native structure (Xiong, 1997a). Disruption of energetically and entropically favorable native protein conformation usually results in denaturation, dissociation of subunits, and, ultimately, aggregation of denatured molecules. The extent of protein damage is a function of the type of salts, the freezing rate, the temperature and time of storage, and the characteristics of the protein involved in the interaction (Xiong, 1997a). For instance, freeze denaturation of carp myosin and myofibrillar proteins was shown to increase with KCl and NaCl concentration, reaching maxima at about -11 to -13°C for KCl, and -8 to -18°C for NaCl (Inove et al., 1992; Takahashi et al., 1993). Since these temperatures correspond to the eutectic points of the salts, the concentration effects by both salts are implicated.

5.1.4 Oxidation

Oxidative processes contribute to protein denaturation during frozen storage. Proteins exposed to oxidizing environments are very susceptible to chemical modifications such as amino acid destruction, peptide scission, and formation of protein-lipid complexes. Among the various oxidizing agents, enzymes, heme, and transition metals can be released from confined cellular organelles or derived from inactive compound during freezing and frozen storage. These oxidizing agents can react with proteins either directly or indirectly through lipid and nonlipid radicals, leading to physical and chemical alterations in proteins (Xiong, 1997a). Many lipid degradation product, including malondialdehyde, are also capable of cross-linking polypeptides and thus are responsible for the generation of insoluble protein aggregates (Buttkus, 1970).

Perez-Villarreal and Howgate (1991) found that the increase in TBARS and peroxide value in European hake muscle during frozen storage. Benjakul *et al.* (2005a) reported that TBARS in muscle of frozen fish inculding threadfin bream (*Nemipterus bleekeri*), bigeye snapper (*Priacanthus tayenus*), lizardfish (*Sauruda micropectoralis*) and croaker (*Pennahai macrophthalmus*) increased as the storage time increased. The rate of increase varied, depending upon species. The differences in lipid oxidation possibly resulted from the different fatty acid compositions. Polyunstaurated fatty acids are more prone to oxidation, compared to saturated fatty acids (Benjakul *et al.*, 2005a). Lipid oxidation occurred during frozen storage might cause the denaturation of proteins. Proteins exposed to oxidizing environments are very susceptible to chemical modification, such as amino acid destruction, peptide scission and formation of protein–lipid complexes (Saeed and Howell, 2002).

5.1.5 Cellular metabolites

Increased meat toughness and sponginess are common textural problems associated with freezing and frozen storage of fresh meat. The texture deterioration is a result of protein denaturation and loss of protein solubility. Some muscle-cellular metabolites, such as free fatty acids (FFA) and formaldehyde are directly responsible for such changes (Xiong, 1997a). FFA is able to bind to proteins and cause an increase in the hydrophobicity of proteins. Some of the nonpolar amino acids or hydrophobic patches occluded in the native protein may in fact be exposed because of

the hydrophobic attraction by FFA. Subsequent conformational changes would lead to insolubilization of the proteins through protein polymerization and aggregation (Xiong, 1997a). Myofibrillar proteins in frozen muscle are more susceptible than sarcoplasmic protein to FFA-induced denaturation or loss in solubility. The rate of FFA–myofibrillar protein interaction and subsequent protein solubility loss are related directly to the degree of unsaturation of FFA and storage time and inversely related to the chain length of FFA (Shenouda, 1980). Both oleic (C18;1) and myristic (C14;0) acids could bind to the hydrophobic regions of fish actomyosin during frozen storage, resulting in major losses of ATPase activity, solubility and viscosity of the protein (Careche and Tejada, 1994). Shaabanpour et al. (2008) reported that FFA content of silver carp surimi increased during 6 month of frozen storage at -18°C. The content of thiobarbituric acid (TBA) also increased until 6th month. However, the values of TBA during 6 month of frozen storage were very far from spoilage stage. In addition, moisture content and total lipid of mince did not change significantly during 6 month of frozen storage. Hoke et al. (2000) reported that, FFA values increased over storage time of 4 month at -14°C both washed and unwashed mince from Channel catfish. After the first month, FFA of unwashed mince was higher than washed mince. This is probably the result of the higher fat and blood/iron content in unwashed mince.

Formaldehyde (FA) can be formed in some fish species during frozen storage mediated by TMAO demethylase, which convert TMAO to FA. FA acts as cross–linker, leading to the poor functionalities (Castell et al., 1873). FA reacts with different functional groups of protein side chains, followed by the formation of intra- and inter-molecular methylene bridges (Sotelo et al., 1995). Leelapongwattana et al. (2005) reported that FA and dimethylamine (DMA) contents increased with a concomitant decrease in TMAO content as the storage time increased. Benjakul et al. (2005a) found a continuous decrease in the breaking force and deformation of gel, especially at extended storage time. Among all the species, surimi from lizardfish had a higher rate of decrease in gel-forming ability as the storage time increased. These changes were coincidental with the formation of FA in lizardfish muscle. FA could react with different functional groups of protein side chains and form intra- and intermolecular methylene bridges. However, Benjakul et al. (2005b) reported that the use of cysteine at levels of 0.05%, 0.1%, 0.05%, and 0.1% in surimi prepared from

frozen croaker, lizardfish, threadfin bream, and bigeye snapper, respectively, could improve the breaking force and deformation of the gels. The addition of cysteine resulted in the reduction of disulfide bonds formed during extended frozen storage.

5.2 Use of cryoprotectants

The addition of cryoprotectants is required in order to retain the functional properties of proteins (Macdonald and Lanier, 1994), during freezing and frozen storage (Huda et al., 2000, 2001). Many compounds, such as some low molecular weight sugars, polyols, amino acids, carboxylic acids and polyphosphates, have been found to be cryoprotective (Park et al., 1987; Sych et al., 1991).

Sorbitol and sucrose: Incorporation of sucrose (4%), sorbitol (4%) and polyphosphates (0.2%) has been known to protect fish myofibrillar protein during long periods of frozen storage (Sultanbawa and Li-Chan, 1998).

Lactitol: Lactitol is the product of hydrogenation of lactose and disaccharide polyol (Marshall et al., 2003). The use of lactitol in cod surimi (Godus morhua) could be reduced from 8% to 5.7-6.4% without a significant alteration of the stabilizing effect.

Polydextrose: Polydextrose is an odorless, white to light cream amorphous powder, with virtually no sweetness and an energy value of only 1 kcal g-1 (Roller and Jones, 1996). Herrera and Mackie (2004) reported that polydextrose was highly effective in preventing changes in natural actomyosin extracted from rainbow trout during frozen storage.

Trehalose: Trehalose is a dietetic sugar and non-reducing disaccharide synthesized by a variety of organisms (Arnoldi, 2004). Trehalose has been found to have properties protective against thermal inactivation of enzymes. This was correlated with its large hydration volume (MacDonald et al., 2000). Osako et al. (2005) reported that an addition of 5.0 to 7.5% trehalose increased the amount of unfrozen water and prevented freezing-induced denaturation of proteins. Trehalose exhibited the greatest protective effects on protein denaturation in comparison with sucrose and sorbitol. The greatest breaking force and deformation were obtained in surimi added with 8% trehalose during frozen storage for 24 weeks (Zhou et al., 2006).

Phosphates: Phosphates increase moisture retention and increase the ability of a protein to reabsorb liquid when the surimi is thawed or tempered. Phosphates increase the pH slightly, gel strength and cohesiveness because of an increase in water-holding capacity at a higher pH. Polyphosphate added at 0.5% provides the greatest gel strength but 0.3% is optimal level used in combination with sodium tripolyphosphate and trisodium pyrophosphate (Hui, 2006). Julavittayanukul *et al.* (2006) reported that a fine network in gel prepared from bigeye snapper was formed with addition of PP in combination with $CaCl_2$.

5.3 Changes in gelling properties of surimi during frozen storage

Freezing and frozen strorage decreased the gelling ability of fish proteins. The loss of the gel-forming capacity in frozen fish has been related to denaturation and aggregation of the myofibrillar proteins. The temperature fluctuation during frozen storage caused reduction in gel-forming capacity of fish proteins. Effect of different salts on the stability of actomyosin in surimi during frozen storage at -30°C are shown in Figure 10. The denaturation rate was highest in surimi to which $CaCl_2$ was added and lowest in the surimi to which no salts were added. Thus the inorganic salt ions such as Ca^{2+} and Mg^{2+} accelerate freeze denaturation of myofibrillar proteins during frozen storage (Nishiya *et al.*, 1960; Tomoto, 1971). Ions come from the natural components of muscle as well as from hard water used for leaching. Furthermore, Mathew and Prakash (2006) studied the effect of calcium sulfate and calcium chloride on the enzymatic and structural properties of actomyosin isolated from sardine. Mince prepared from sardine in the presence of calcium chloride and calcium sulfate at different levels during frozen storage at -20°C was monitored. The solubility of proteins decreased during storage. The reduction in solubility was less for samples treated with calcium chloride. However, sardine mince showed better functionality during storage in the presence of calcium compounds. The ATPase enzyme activity of actomyosin increased with increasing concentration of calcium and decreased after reaching the maximum value. Thus, the reduction in ATPase did not correlate with the loss of functionality of proteins. The secondary structural content of actomyosin was not altered in the presence of both calcium chloride and calcium sulfate as evidenced by circular dichroic measurements.
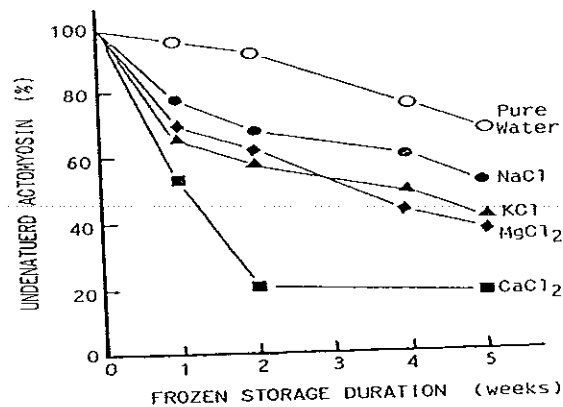
Figure 10. Effect of various inorganic salts on actomyosin extractability during frozen storage of cod surimi. Cod was washed once with 0.1% each salt solution at a 2:1 ratio of water to mince, 5% sucrose added, and storage at -20°C.

Source: Tomoto (1971)

Panayotis *et al.* (2008) showed that kamaboko gels of acceptable quality could be produced from sardines frozen stored up to 20 days. A gradual increase in gel hardness and cohesiveness was found after 10 days of storage due to protein aggregation. Hardness and cohesiveness, as well as whiteness, were positively affected by the addition of $CaCl_2$ and microbial transglutaminase (MTGase), while springiness was only affected by MTGase addition. Turan and Sonmez (2007) reported that surimi was prepared from the thornback ray and divided into two groups. The first group was prepared with 4% sorbitol, 4% sucrose and 0.3% sodium tripolyphosphate as a cryoprotectant, while surimi in second group was prepared with 8% sorbitol and 0.3% sodium tripolyphosphate. The total volatile basic nitrogen, trimethylamine nitrogen, thiobarbituric acid, and pH values of surimi increased during 6 months of frozen storage but remained within the acceptable limits.

Objectives

1. To study the chemical composition and thermal properties of yellowtail barracudar muscle.

2. To investigate the effect of type of salts in washing media and different washing processes on dewatering and gel forming ability of yellowtail barracuda surimi.

3. To study the effect of iced storage of yellowtail barracuda on dewatering and gel-forming ability of surimi produced by selected washing processe.

4. To monitor the changes in physicochemical properties and gel forming ability of surimi during frozen storage as influenced by selected washing process.

CHAPTER 2

MATERIALS AND METHODS

1. Materials

1.1 Fish samples and preparation

Yellowtail barracuda (*Sphyraena flavicauda*) with an average weight of 80-90 g were caught from Songkla-Pattani Coast along the Gulf of Thailand. The fish, off-loaded approximately 12 h after capture, were placed in ice with a fish/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai within 2 h. Upon arrival, fish were washed, deheaded, eviscerated and used for surimi preparation.

1.2 Chemicals

Calcium chloride ($CaCl_2$) and magnesium chloride ($MgCl_2$) were obtained from Ajax Finechem Pty Ltd. (NSW, Australia). Sodium dedocyl sulfate (SDS), β-mercaptoethanol (βME), trichloroacetic acid (TCA), 8-anilino-1-naphthalenesulphonic acid (ANS), guanidine thiocyanate, adenosine 5'-triphosphate (ATP), 5-5'-dithio-bis (2-nitrobenzoic acid) (DTNB), sodium hydrogen sulfite, tris-maleate, sodium hydroxide, hydrochloric acid and thiobarbituric acid (TBA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA), ,*N,N,N',N'*-tetramethyl ethylenediamine (TEMMED), acrylamide, bis-acrylamide, urea, nitric acid and glutaraldehyde were procured from Fluka (Bucsh, Switzerland).

2. Instuments

Instuments	Model	Company/Country
Electrophoresis apparatus	Mini-Protein II	Bio-Rad, USA
Spectrofluorometer	RF-1501	SHIMADZU, Japan
Texture analyzer	TA-XT2	Stable Micro Systems,England
Homogenizer	T25B	Ultra turrax, Malaysia
Refrigerated centrifuge	RC-5B plus	Sorvall, USA
Magnetic stirrer	RO-10 power	KIKAL labortechnik, Germany
Basket centrifuge	CE 21K	Grandimpianti, Italy
Mixer	MK-K77	National, Japan
pH meter	Denver 15	Scientific, USA
Scanning Electron Microscope	JSM5800LV	JEOL, Japan
Microcentrifuge	MIKR 020	Zentrifugan, Hettich, Germany
Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)	Optima 4300 DV	Perkin Elmer, USA
Colorimeter	ColorFlex	HunterLab Reston, VA, USA
Mixer	MK-K77	National, Tokyo, Japan
Water bath	W350	Memmert, Schwabach, Germany

3. Methods

3.1 Determination of chemical composition and thermal properties of yellowtail barracuda mince

3.1.1 Chemical composition and protein pattern

3.1.1.1 Proximate analysis

Fish mince was determined for moisture, protein, ash and fat contents according to AOAC method (AOAC, 1999). The values were expressed as % (wet weight basis).

3.1.1.2 Determination of TVB and TMA contents

TVB and TMA contents were determined using the Conway microdiffusion assay according to the method of Conway and Byrne (1936). Sample

(5g) was mixed with 20 ml of 4% trichloroacetic acid (TCA). The mixtures were homogenized at 11,000 rpm using an Ultra Turrax homogenizer (IKA Laboratechnink, Selangor, Malaysia) for 1 min. The homogenate was filtered using Whatman No. 4 filter paper and the filtrate was used for analysis. To determine the TMA content, formaldehyde was added to the filtrate to fix ammonia present in sample. TVB and TMA were released after addition of saturated K_2CO_3 and diffused into the boric acid solution. The titration of solution was performed and the amount of TVB and TMA were calculated.

3.1.1.3 Determination of K-value

K-value was determined by using ion exchange chromatography (Uchiyama and Kakuda, 1984). Sample (5g) was homogenized at a speed of 2000-3000 rpm in 10 ml of 10% perchloroacetic acid (PCA) for 2 min. The sample was centrifuged at 3,000xg for 5 min. The extraction was repeated for three times. The extract was subjected to ion exchange chromatography with Anion exchange resin-AG (R) 1-X4, 400 mesh Cl-form. Elution was carried out and the eluate was determined for ATP and derivatives. K-value was then calculated.

3.1.2 Thermal properties of muscle proteins

Thermal transition of yellowtail barracuda mince proteins was determined using the differential scanning calorimetry (DSC) (Perkin-Elmer, Model DSCM, 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 denaturation enthalpies (ΔH) were estimated by measuring the area under the DSC transition curve.

3.2 Effect of sodium chloride at various levels in the third washing media on dewatering of mince and properties of surimi

Surimi from yellowtail barracuda mince was prepared as shown in Figure 11. For the third washing step, the mince was washed with sodium chloride solution at different concentrations (0, 0.15, 0.3, 0.45 and 0.6%, w/v).

Yellowtail barracuda mince

↓

Wash with cold water (5-8°C) with a fish/water ratio of 1:3 (w/v) for 2 min
and two times

↓

Wash with sodium chloride solution at different concentrations (5-8°C)

With a fish/water ratio of 1:3 (w/v) for 2 min

↓

Filter through cheesecloth and centrifuge at 860xg for 5 min

↓

Add with 4% sorbitol and 4% sucrose

↓

Mix for 4 min, pack and store at -20°C

↓
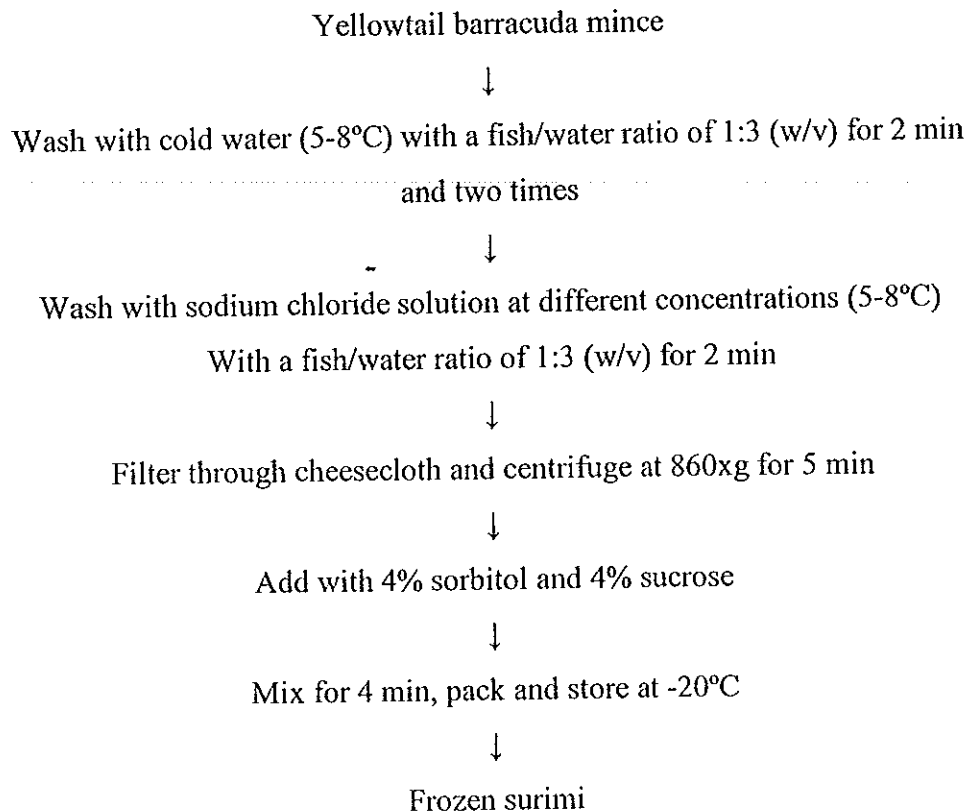
Frozen surimi

Figure 11. Scheme for frozen surimi preparation

3.2.1 Chemical composition and physical properties

The washed mince obtained was subjected to determination as follows:

1. Salt content as per AOAC method (AOAC, 1999).

2. pH according to the method of Benjakul *et al.* (1997).

3. Thermal properties using differential scanning colorimetry (DSC) (Kelleher, 1991).

4. Moisture content according to AOAC method (AOAC, 1999).

5. Fat content according to AOAC method (AOAC, 1999).

6. Sarcoplasmic protein content following the method of Hashimoto *et al.* (1979).

7. Whiteness as per the method of Park (1994).

8. Protein patterns by SDS-PAGE (Laemmli, 1970).

3.2.2 Gel forming ability

3.2.2.1 Surimi gel preparation

To prepare the surimi gel, the frozen surimi was thawed using running tap water until the core temperature reached 0-2°C. To the surimi, 2.5% NaCl (w/w) was added and the moisture was adjusted to 80%. The mixture was chopped for 4 min at 4°C to obtain a homogeneous sol. The sol was then stuffed into polyvinylidine casing with a diameter of 3.6 cm, and both ends of the casing were sealed tightly. Surimi gels were prepared by two-step heating condition (40°C for 30 min, followed by 90°C for 20 min). The gels were cooled in iced water and stored overnight at 4°C prior to analysis as follows:

3.2.2.2 Analysis

1. Texture analysis

The texture of surimi gels was determined according to the method of Benjakul *et al.* (2002). Gels were equilibrated and evaluated at room temperature (28-30°C). Seven cylinder-shaped samples with a length of 2.5 cm were prepared and subjected to determination. Breaking force (strength) and deformation (cohesiveness/elasticity) were measured using a texture analyzer TA-XT2 (Stable Micro System, Surrey, UK) equipped with a spherical plunger (diameter 5 mm, depression speed 60 mm/min and 60% compression).

2. Whiteness

Color of gel was determined using a HunterLab (ColorFlex, Hunter Associates Laboratry, VA, USA). Illuminant C was used as the light source of measurement. CIE L*(lightness), a*(redness/greenness) and b*(yellowness/blueness) were measured and whiteness was calculated as described by Park (1994) as follows:

$$\text{Whiteness} = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$$

3. Expressible moisture content

Expressible moisture content was measured according to the method of Ng (1987). Cylindrical gel samples were cut into a thickness of 5 mm, weighed (X) and placed between three pieces of Whatman paper (No. 1) at the bottom

and two pieces of paper on the top. A standard weight (5 kg) was placed on the top of the sample for 2 min, and then the sample was removed from the papers and weighed again (Y). Expressible moisture content was calculated and expressed as percentage of sample weight as follows:

$$\text{Expressible moisture content} = [(X-Y)/X] \times 100$$

4. Microstructure

Gel samples ($0.25 \times 0.25 \times 0.25$ cm^3) were prepared. The specimens were then fixed with 2.5% glutaraldehyde in 0.2 M phosphate buffer, pH 7.2 for 2 h at room temperature. The specimens were rinsed with distilled water and dehydrated in graded ethanol solutions with serial concentrations of 25, 50, 75, 95 and 100%. Dehydration was conducted for 1 h in each solution, except for 100% ethanol, the dehydration was performed twice. The specimens were dried using CO_2 as transition fluid (Balzers mod. CPD 030, Balzers Process Systems, Liechtenstein). The dried specimens were mounted on aluminum stubs and sputter-coated with gold. The prepared samples were examined on a JSM 5200 scanning electron microscope (JEOL, Ltd., Akishima, Japan) at a magnification of x10,000 (15 kV).

3.3 Effect of calcium chloride and magnesium chloride at various levels in the third washing media on chemical compositions of washed mince and properties of surimi

Surimi from yellowtail barracuda mince was prepared as described in Figure 11. For the third washing media, the appropriate sodium chloride solution (section 3.2) was used in combination with calcium chloride or magnesium chloride at different concentrations as follows:

- $CaCl_2$ (0, 4, 8, 12, 16 and 20 mM)
- $MgCl_2$ (0, 4, 8, 12, 16 and 20 mM)

The washed mince obtained from washing process using different salts in the third washing step were determined for chemical compositions (section 3.2.1) and the contents of calcium or magnesium ions were also determined.

Calcium and magnesium contents of washed mince were determined according to the method described by Thompson (1969). The samples were ashed, dissolved in 20% nitric acid and boiled on hot plate for 15 min before analysis using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (model Optima 4300 DV, Perkin Elmer, USA).

The surimi of all treatments were used for gel preparation as mentioned in section 3.2.2.1, but surimi gels were prepared by two heating conditions : 1) direct heating (90°C for 20 min) and 2) two-step heating (40°C for 30 min, followed by 90°C for 20 min). The gels were cooled in iced water and stored overnight at 4°C prior to analysis as described in section 3.2.2.2.

3.4 Effect of different washing processes on the chemical compositions of washed mince and properties of surimi

To produce surimi, yellowtail barracuda mince was washed with the first washing media containing sodium chloride and sodium bicarbonate at different concentrations.

Different washing processes of yellowtail barracuda mince

Treatments	Washing step		
	I	II	III
T1 (Control)	Tap water	Tap water	0.45% NaCl
T2	Tap water	Tap water	0.45% NaCl + 20 mM $MgCl_2$
T3	0.15% NaCl + 0.2% $NaHCO_3$	Tap water	0.45% NaCl
T4	0.15% NaCl + 0.2% $NaHCO_3$	Tap water	0.45% NaCl + 20 mM $MgCl_2$
T5	0.30% NaCl + 0.2% $NaHCO_3$	Tap water	0.45% NaCl
T6	0.30% NaCl + 0.2% $NaHCO_3$	Tap water	0.45% NaCl + 20 mM $MgCl_2$

For the third washing step, sodium chloride solution containing calcium chloride or magnesium chloride, yielding the most effective dewatering and highest gel-forming ability (section 3.3), was used. Washed minces was determined for chemical compositions as described in section 3.2.1 and the gel properties were analyzed as described in section 3.2.2.2.

3.5 Effect of iced storage on chemical compositions of washed mince and gelling properties of surimi prepared from yellowtail barracuda

Fresh whole yellowtail barracuda were kept in ice with a fish/ice ratio of 1:2 (w/w). Fish and ice were placed in a polystyrene box, which were kept at room temperature (28-30°C). The molten ice was removed and replaced with an equal amount of ice every 2 days. The stored fish were taken at day 0, 7 and 14. Fish was subjected to deboning and the mince obtained was washed using the process rendering the highest dewatering and highest gel property (section 3.4). The washed minces were determined for chemical compositions as described in section 3.2.2. Additionally, washed mince was subjected to determinations as follows:

- TVB and TMA contents were determined using the Conway microdiffusion assay according to the method of Conway and Byrne (1936) as described in section 3.1.1.2.

- K-value was determined using ion exchange chromatography (Uchiyama and Kakuda, 1984) as described in section 3.1.1.3.

- TBARS determination

Thiobarbituric acid reactive substances (TBARS) were determined as described by Benjakul and Bauer (2001). Sample (0.5g) was dispersed in 2.5 ml of a solution containing 0.375% thiobarbituric acid, 15% trichloroacetic acid and 0.25N HCl. The mixture was heated in boiling water for 10 min, followed by cooling in running tap water. The mixture was centrifuged at 3600g for 20 min at room temperature. The absorbance of supernatant was measured at 532 nm using a spectrophotometer (Shimadzu, Kyoto, Japan). TBARS were calculated and expressed as mg malondialdehyde/kg samples.

The surimi gels from yellowtail barracuda stored in ice for different times were analyzed as described in section 3.2.2.2.

3.6 Effect of selected washing processes on physicochemical properties and gel-forming ability of surimi produced from yellowtail barracuda during frozen storage

Surimi was prepared following the conventional method (using water at a ratio of 2:1 for 3 times). Another surimi was prepared using the selected washing

process (section 3.2, 3.3 and 3.4). Both surimis were mixed with cryoprotectants (section 3.2), and packed in a polyethylene bag. The samples were frozen by air-blast freezer and kept at -18°C for different times (0, 3, 6, 9 and 12 weeks). The samples were analyzed as follows:

3.6.1 Ca^{2+}-ATPase activity

Ca^{2+}-ATPase activity was determined according to the method of Benjakul et al. (1997). Natural actomyosin (NAM) prepared as described by Benjakul et al. (1997) was diluted to 2.5-6 mg/ml with 0.6 M KCl, pH 7.0. Diluted NAM solution (1 ml) was added to 0.6 ml of 0.5 M tris-maleate, pH 7.0. The mixture was added with CaCl$_2$ to obtain a final concentration of 10 mM CaCl$_2$ with the total volume of 9.5 ml. To each assay solution, 0.5 ml of 20 mM ATP was added to initiate the reaction. The reaction was conducted for 8 min at 25°C and terminated by adding 5 ml of chilled 15% (w/v) trichloroacetic acid. The reaction mixture was centrifuged at 3500g 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 micromoles inorganic phosphate released/mg protein/min. A blank solution was prepared by adding chilled trichloroacetic acid prior to ATP addition.

3.6.2 Total sulfhydryl group and disulfide bond contents

Total sulfhydryl group content was measured according to method of Ellman (1959) as modified by Benjakul et al. (2001). To 0.25 ml of NAM solutions, 3 ml of 0.2 M Tris-HCl buffer containing 8 M urea, 2% SDS and 10 mM EDTA (pH 6.8) and 0.25 ml of 0.1% DTNB in 0.2 M Tris-HCl buffer (pH 6.8) were added. The mixture was incubated at 40°C for 40 min and the absorbance at 412 nm was read using a spectrophotometer. Reagent blank was prepared by replacing the sample with 50 potassium phosphate buffer containing 0.6 M KCl (pH 7.0). For sample blank, the reaction was run in the same manner except 0.2 M Tris HCl (pH 6.8) was used instead of DTNB solution. Sulfhydryl group content was calculated using a molar extinction coefficient of 13,600 M^{-1}cm^{-1}. Disulfide bond content was determined by using 2-nitro-5-thiosulfobenzoate (NTSB) assay as described Thannhauser et al. (1987). To 0.5 ml of NAM solutions, 3.0 ml of freshly prepared NTSB assay solution were added. The mixture was incubated in the dark at room temperature (25°C) for 25 min.

Absorbance was then measured at 412 nm. Disulfide bond content was calculated from the absorbance using the extinction coefficient of 13,900 $M^{-1}cm^{-1}$.

3.6.3 Surface hydrophobicity

Surface hydrophobicity was measured according to the method of Benjakul et al. (2001a) using 8-anilo-1-napphthalensulfonic acid (ANS) as a probe. NAM solution was diluted to 0.125, 0.25, 0.5 and 1 mg/ml using the same buffer. To 2.0 ml of diluted NAM solution, 10 µl of ANS dissolved in 50 mM potassium phosphate buffer (pH 7.0) were added and the mixtures were mixed thoroughly. Fluorescence intensity was measured using a FP-750 spectrofluorometer (Shimadzu, Kyoto, Japan) at the excitation and emission wavelength of 374 and 485 nm, respectively. Surface hydrophobicity was calculated from initial slope of plot of fluorescence intensity against protein concentration using linear regression analysis. The initial slope was referred to as S_oANS.

3.6.4 TBA value

TBA value was measured according to method of Egan et al. (1981). Samples (10g) were homogenized with 50 ml of distilled water for 2 min and tranformed into distillation flask containing 47.5 ml of distilled water. The mixture was added with 2.5 ml of 4 N HCl to bring the pH to 1-5. A few drops of antifoaming agent and a few glass beads were added. The mixtures were heated by means of an electric mantle and 50 ml of distillate were collected in 10 min. Five ml of distillate were added with 5 ml of TBA reagent. The mixture was heated in boiling water for 35 min. The reaction mixtures were then cooled in running water and the absorbance at 532 nm was measured. TBA value was calculated using the following equation.

TBA value (mg malonaldehyde/kg sample) = 7.8 x A

where A = absorbance of sample

3.6.5 Protein solubility

Protein solubility was determined as described by Benjakul and Bauer (2000). To 2 g sample, 18 ml of 0.6 M KCl were added and the mixture was homogenized at a speed of 11000 rpm for 30 s. The homogenate was stirred at room temperature (25-27°C) for 4 h, followed by centrifuging at 12,000g for 20 min at 4°C. To 10 ml of the 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. Protein content was determined using the Biuret method (Robinson and Hodgen, 1940).

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, IL, USA).

CHAPTER 3

RESULTS AND DISCUSSION

1. Chemical composition and thermal properties of yellowtail barracuda muscle

1.1 Chemical composition

Chemical composition of yellowtail barracuda mince is shown in Table 3. Yellowtail barracuda mince had high moisture content, accounting for approximately 79.17%. Depending on the species and the nutritional status of the marine fish and invertebrate, the muscle consists of 50-80% water (Sikorski et al., 1990). Protein was found as the major constituent (17.54%), indicating that yellowtail barracuda can be the good source of amino acids. Small amounts of ash (1.46%) and lipid (1.53%) were found. The composition of muscle can vary with the season, size, sex, spawning and feeding condition (Pigott and Tucker, 1990). With low fat content, yellowtail barracuda can be classified as the lean fish (Sikorshi et al., 1990).

When the K-value, TVB and TMA contents were determined (Table 3), very low values were observed. This indicated that yellowtail barracuda was very fresh with negligible spoilage. Generally, K-value has been used as a freshness index of fish and is related to degradation of nucleotides by nucleotide-degrading enzymes. The K-value is expressed as the ratio of Ino and Hx to the sum of ATP, ADP, AMP, IMP, Ino and Hx. Generally, K-value of 60% is considered as the rejection limit for consumers. Thus, the low K-value obtained in yellowtail barracuda mince indicated that the low amount of degradation product of nucleotide was formed. Furthermore, the low TVB and TMA contents suggested that the spoilage occurred in yellowtail barracuda at the low extent. In freshly caught marine fish, TVB and TMA contents are negligible (Oehlenschlager, 1992). Benjakul et al. (2002) reported that initial values of TVB in bigeye snapper samples were 5.0-5.4 mg N/100g. The presence of TVB and TMA indicated some decomposition of nitrogeneous compounds occurring during post-mortem handing and storage. The volatile amines such as TMA, NH_3 and DMA were responsible for fishy odor in unfresh fish (Sikorski et al., 1990).

Table 3. Chemical compositions of yellowtail barracuda mince

Compositions	Contents
Moisture (% wet wt. basis)	79.17 ± 0.51*
Ash (% wet wt. basis)	1.46 ± 0.07
Fat (% wet wt. basis)	1.53 ± 0.05
Protein (% wet wt. basis)	17.54 ± 0.31
K-value (%)	9.82 ± 0.18
TMA (mg N/100g sample)	ND
TVB (mg N/100g sample)	3.03 ± 0.40

* Values are given as means ± SD from triplicate determinations.

ND: non-detectable

1.2 Thermal properties of muscle proteins

Thermal transition of proteins in yellowtail barracuda mince was determined using differential scanning colorimetry (Table 4). DSC thermogram of yellowtail barracuda mince revealed two major peaks with T_{max} of 50.58 and 72.33°C with ΔH of 1.70 and 0.37 J/g, respectively. The observed T_{max} were similar to those found in the muscle of various fish species, in which the first and second peaks were postulated to be the transitions of myosin and actin, respectively (Akahane et al., 1985). Whole cod muscle showed two maximal transitions on DSC thermogram with T_{max} values of 46 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). Two species had two major endothermic peaks with T_{max} of 47.7 and 70.6°C for the muscle of P.tayenus and T_{max} of 47.6 and 69.9°C for the muscle of P.macracanthus were reported (Benjakul et al., 2002). Myosin and actin of lizardfish had the T_{max} of 49.55 and 70.94°C, respectively (Tammatinna, 2007). The differences in T_{max} among the fish seem to be correlated with the habitat temperature of the fish (Akahane et al., 1985; Hasting et al., 1985; Davies et al., 1988).

Table 4. T_{max} and enthalpy (ΔH) of yellowtail barracuda mince

Sample	Peak I		Peak II	
	T_{max} (°C)	ΔH (J/g)	T_{max} (°C)	ΔH (J/g)
mince	50.58 ± 0.11	1.70 ± 0.08	72.33 ± 0.24	$0.37 \pm 0.02*$

*Values are given as means $\pm$ SD from triplicate determinations.

2. Effect of sodium chloride at various levels in the third washing medium on dewatering of mince and properties of gel produced from yellowtail barracuda

2.1 Chemical compositions and properties of washed mince

2.1.1 Sodium chloride content

The different concentrations of NaCl in the third washing medium had the effect on NaCl contents of the resulting washed mince as shown in Fig. 12. When concentration of NaCl in washing media increased, the contents of NaCl in resulting washed mince markedly increased ($p<0.05$) (Fig 12). When NaCl was present in washing medium, it more likely contributed to the increase in Cl^-. Shen (1976) reported that the binding affinity of Cl^- to NH_3^+ is greater than that of Na^+ to COO^-. The chloride ion preferentially binds to the protein, thereby increasing electronegativity of the protein. NaCl might affect the taste of mince as well as influenced the physiochemical property of mince proteins.
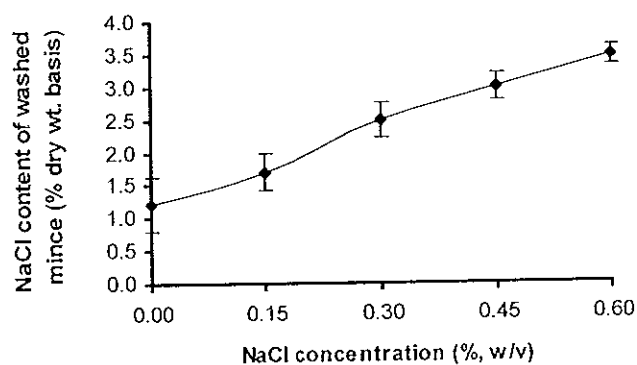
Figure 12. NaCl content of yellowtail barracuda mince washed with the third washing medium containing different levels of NaCl. Bars represent the standard deviation from triplicate determinations.

2.1.2 pH

When the mince was washed with the third washing medium containing various levels of NaCl, different pH was obtained. The pH of resulting washed mince gradually increased from 6.32 to 6.98 when NaCl concentration increased from 0.15 to 0.45%. Slight increase in pH was noticeable when NaCl concentration increased from 0.45 to 0.6% (Figure 13). The increased pH of washed mince might be due to preferential interaction among protein-salt-water. Generally, Na^+ prefers to interact with water, leading to increase surface tension of washing water (Arakawa and Timasheff, 1984). Cl^- might bind with H^+ by ionic interaction. The higher removal of acidic compounds such as lactic acid in the mince with increasing NaCl concentration also led to higher pH of washed mince.
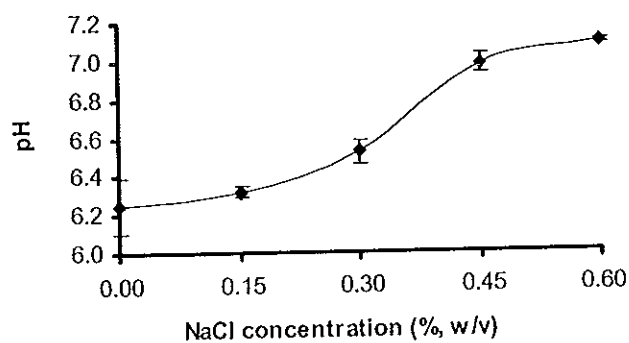
Figure 13. pH of barracuda mince washed with the third washing medium containing different levels of NaCl. Bars represent the standard deviation from triplicate determinations.

2.1.3 Thermal property

DSC data of mince washed with the third washing medium containing different concentrations of NaCl are shown in Table 5. Increasing NaCl content in the third washed mince caused the shift of T_{max} to the lower values for both myosin and actin ($p<0.05$). Nevertheless, no difference in enthalpy of both peaks was observed with different NaCl concentrations used ($p>0.05$). The decrease in T_{max} was presumably due to the loss of the proteins stability, resulting from a partial unfolding of proteins (Park and Lanier, 1990). The thermal stability of proteins can be affected

by its chemical environment, such as the ionic strength (Wright and Wilding, 1984). The addition of salt could lower the stability of protein because Cl⁻ might interact with positively charged domains. The repulsive force between negatively charged proteins might result in the lower thermal stability of both myoin heavy chain and actin.

Table 5. T_{max} and enthalpy (ΔH) of yellowtail barracuda mince washed with the third washing medium containing different levels of NaCl.

NaCl concentration (%) in the third washing medium	Peak I		Peak II	
	T_{max} (°C)	ΔH (J/g)	T_{max} (°C)	ΔH (J/g)
0.00	53.83 ± 0.24 [a]	1.38 ± 0.04 [a]	70.58 ± 0.59 [a]	0.25 ± 0.01 [a]
0.15	53.25 ± 0.35 [a]	1.25 ± 0.41 [a]	70.67 ± 1.18 [a]	0.24 ± 0.11 [a]
0.30	52.08 ± 0.12 [b]	1.58 ± 0.02 [a]	69.17 ± 0.00 [ab]	0.33 ± 0.01 [a]
0.45	51.50 ± 0.00 [bc]	1.68 ± 0.07 [a]	69.17 ± 0.23 [ab]	0.30 ± 0.04 [a]
0.60	51.25 ± 0.35 [c]	1.44 ± 0.31 [a]	68.50 ± 0.47 [b]	0.26 ± 0.14 [a]

Values are given as means ± SD from triplicate determinations. Different superscripts in the same column indicate significant differences ($p < 0.05$).

2.1.4 Moisture content

Dewatering ability of various levels of NaCl in the third washing medium was measured by determining moisture content of washed mince after dewatering process (Table 6). The result showed that when the concentration of NaCl in washing medium increased, moisture content of washed mince decreased ($p < 0.05$). This indicated that NaCl was effectively responsible for removal of water in washed mince. Na^+ and Cl⁻ could bind with the opposite charge of amino acids in the proteins, leading to the less repulsive force between adjacent protein molecules. As a consequence, protein molecules aligned more closely with the concomitant migration of water from the space between protein molecules. Additionally, salt might compete with protein in binding water, termed "salting out" effect. As a result, proteins could interact each other at a higher extent, thereby repelling water from the mince.

Table 6. Moisture, fat, sarcoplasmic protein and whiteness of yellowtail barracuda mince washed with the third washing medium containing different levels of NaCl.

NaCl concentration (%) in the third washing medium	Moisture (%)	Fat (% dry wt. basis)	Sarcoplasmic protein (% dry wt. basis)	Whiteness -
0.00	85.27 ± 0.04 [c]	3.05 ± 0.09 [c]	7.05 ± 0.22 [c]	66.56 ± 1.04 [a]
0.15	83.80 ± 0.16 [b]	2.67 ± 0.09 [b]	6.09 ± 0.43 [d]	67.32 ± 0.71 [a]
0.30	83.53 ± 0.26 [ab]	2.66 ± 0.07 [b]	5.97 ± 0.31 [c]	66.54 ± 0.80 [a]
0.45	83.45 ± 0.30 [a]	2.31 ± 0.09 [a]	5.89 ± 0.34 [b]	68.19 ± 1.22 [a]
0.60	83.39 ± 0.14 [a]	2.29 ± 0.04 [a]	5.78 ± 0.06 [a]	66.58 ± 1.33 [a]

Values are given as means ± SD from triplicate determinations. Different superscripts in the same column indicate significant differences ($p<0.05$).

2.1.5 Fat content

The slight decreases in fat content in the mince washed with higher levels of NaCl were observed, compared with that washed without NaCl ($p<0.05$) (Table 6). The lowest fat content in washed mince was obtained when the mince was washed with 0.45% and 0.6% NaCl ($p<0.05$). Generally, the effectiveness in fat removal was on the basis of density and polarity difference (solubility difference) between mince and the washing solution (Yang and Fronging, 1992). Long-chain fatty acids are practically insoluble in water. Instead, they form a floating film on the water surface (Belitz et al., 2009). Some lipoproteins were also removed to some extent by washing process. Lipoproteins conform aggregate via noncovalent bond such as hydrophobic interactions, ionic interaction and hydrogen bond. Since lipids have a lower density (0.88-0.90 g/ml) than proteins (1.30-1.35 g/ml), they are separated by a stepwise centrifugation in solutions of NaCl into three fractions with different densities as very low density lipoprotein (<1.006 g/ml), low density lipoprotein (1.063 g/ml) and high density lipoprotein (1.21 g/ml) (Belitz et al., 2009). The higher NaCl concentration in the third washing medium might help in solubilizing the proteins associated with fat or lipids. As a result, the lipids could be

more liberated and separated from mince via floating as skim. Therefore, NaCl at appropriate concentration could be responsible for the higher removal of lipid in the washed mince.

2.1.6 Sarcoplasmic protein content

Concentrations of NaCl in the third washing medium affected sarcoplasmic protein content of washed mince differently (Table 6). When the concentration of NaCl in the third washing medium increased, sarcoplasmic protein content (dry wt. basis) of washed mince markedly decreased ($p<0.05$). Sarcoplasmic proteins are located inside the sarcolemma and are soluble in low salt concentrations (<0.1 M KCl). Those include myoglobin, enzymes and other albumin (Sikorski, 1994). The result suggested that NaCl might facilitate the solubilization of muscle proteins, particularly sarcoplasmic proteins. However, the use of high concentration of NaCl may cause the loss in myofibrillar proteins (Hultin, 1995). Chaijan *et al.* (2004) reported that the highest amount of myoglobin from sardine and mackerel mince was removed when the mince was washed with 0.2 and 0.5% NaCl, respectively. The difference in NaCl concentration needed between two species might be due to the different interaction or bonding between myoglobin and muscle. NaCl could weaken those bonds, leading to the release of myglobin from the muscle. As Na^+ and Cl^- are bound to acidic and basic amino acid residues, intermolecular ionic bonds among protein molecules are broken (Lee, 1992).

2.1.7 Whiteness

No difference in the whiteness of washed mince was found when various levels of NaCl in the third washing medium was used ($p>0.05$) (Table 6). For surimi processing, myoglobin plays an essential role in the whiteness (Chen, 2002). Whiteness is one of most important factors in quality of surimi. The removal of sarcoplasmic protein containing pigments can improve the whiteness of washed mince. The washing process is necessary for color improvement and gel strengthening of surimi produced from whole muscle (Chaijan *et al.*, 2004). Washing without NaCl for three times could remove myoglobin or other heme proteins such as haemoglobin effectively. Inclusion of NaCl did not increase the efficacy in whiteness improvement. Kim *et al.* (1996) reported that the color of surimi can be improved by increasing the washing cycle and washing time. However, Jin *et al.* (2007) suggested that two time-

washing was recommended rather than four time-washing because the color of surimi was not much affected by washing cycle, although muscle pigment such as myoglobin was lower in surimi made by four time-washing.

2.1.8 Protein patterns

Protein patterns of mince washed with the third washing medium containing NaCl solution at different concentrations are shown in Figure 14. The increase in MHC band intensity of mince washed with NaCl solution was found, compared with that without NaCl. Washing with NaCl solution might increase the removal of sarcoplasmic proteins, blood, fat, and other nitrogenous compounds from minced fish, leading to the concentrated myofibrillar proteins, especially MHC. However, no difference in MHC and actin band intensity of mince washed with NaCl at various concentrations (0.15-0.6% NaCl). Higher content of MHC has been known to contribute to the higher gel forming ability.
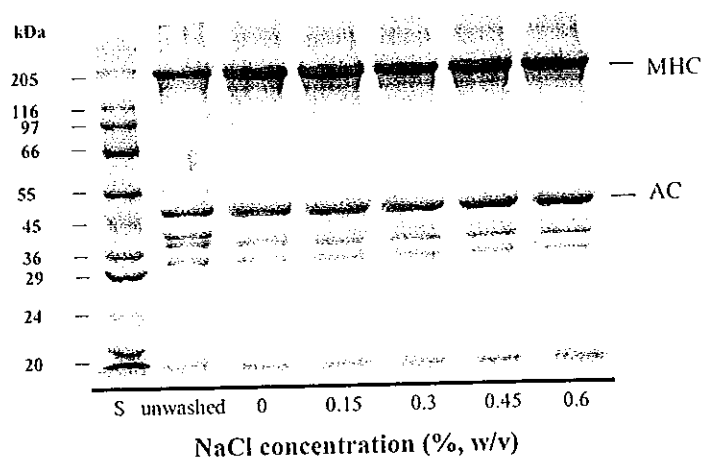
Figure 14. Protein patterns of yellowtail barracuda mince washed with the third washing medium containing different levels of NaCl.

2.2 Properties of surimi gel

2.2.1 Breaking force and deformation

Breaking force and deformation of surimi gels prepared from yellowtail barracuda mince washed with the third washing media containing different NaCl concentrations (0, 0.15, 0.30, 0.45, 0.60%) are shown in Figure 15. The highest breaking force and deformation of surimi gels were obtained when 0.45% NaCl was

used as the third washing medium ($p<0.05$), in which the breaking force and deformation increased by 39 and 10%, respectively, compared with that of the control (without NaCl). Increases in breaking force and deformation were noticeable, when NaCl concentration was increased up to 0.45% ($p<0.05$). This might be due to the greater removal of sarcoplasmic proteins (Table 6). Sarcoplasmic proteins have been reported to detrimentally affect gelation of fish muscle proteins (Hall, 1997; Ismail, 2004). Nevertheless, the use of 0.6% NaCl, as the third washing medium resulted in the lowered breaking force and deformation of resulting gels. It could be due to the imbalance between protein-protein and protein-solvent interactions. Above the optimum concentration of salt, the formation of intermolecular bridges is more rapid, more random, and less ordered, leading to weaker bridging between larger aggregates (Dalgleish and Hunt, 1995). These networks are more readily broken under stress, thereby reducing gel strength and elasticity. Thus, NaCl concentration in washing medium had the pronounced impact on gelation of resulting surimi.
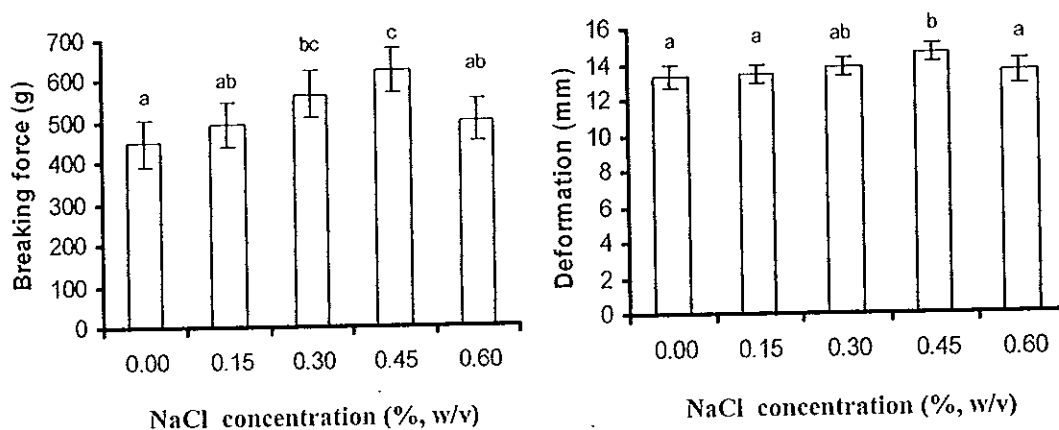
Figure 15. Breaking force and deformation of gels produced from yellowtail barracuda mince washed with the third washing medium containing different levels of NaCl. Bars represent the standard deviation from triplicate determinations.

2.2.2 Whiteness

Whiteness of surimi gel produced from mince washed with the third washing medium having different concentrations of NaCl is shown in Table 7. When concentration of NaCl increased up to 0.3%, the slight decrease in whiteness was

obtained ($p<0.05$). The use of higher NaCl concentration had no further impact on whiteness ($p>0.05$). Na^+ and/or Cl^- ions of salt might affect the Maillard reaction. Maillard reaction is a chemical reaction involving the amino group of protein and carbonyl group of reducing sugar. Subsequently, dark-brown polymeric compounds like melanoidin are formed (Wijewickreme *et al.*, 1997). Rizzi (2008) studied the effects of cationic species on Maillard browning after heating buffered solutions containing amino acid and pentose sugars at 100°C and pH 7.2. Metallic ions of group I metals (Li, Na, K, Rb and Cs) produced a small increase in browning (A_{420}). Therefore, it is considered that the ions from NaCl, particularly Na^+ might increase the rate of Mailard reaction to some degree. This was evidenced by the lowered whiteness of mince washed with NaCl solutions.

Table 7. Whiteness and expressible moisture content of gels produced from yellowtail barracuda mince washed with the third washing medium containing different levels of NaCl.

NaCl concentration (%) in the third washing medium	Whiteness	Expressible moisture content (%)
0.00	78.33 ± 0.53 [c]	4.32 ± 0.20 [c]
0.15	77.53 ± 0.72 [b]	4.19 ± 0.43 [bc]
0.30	77.07 ± 0.57 [a]	4.06 ± 0.17 [abc]
0.45	77.35 ± 0.44 [ab]	3.89 ± 0.18 [ab]
0.60	77.05 ± 0.56 [a]	3.78 ± 0.22 [a]

Values are given as means ± SD from triplicate determinations. Different superscripts in the same column indicate significant differences ($p<0.05$).

2.2.3 Expressible moisture content

Different concentrations of NaCl in the third washing medium affected expressible moisture content of resulting surimi gels (Table 7). The lower expressible moisture content was obtained when the higher concentration of NaCl was used in washing medium ($p<0.05$). Gels with higher breaking force and deformation were able to imbibe more water in the matrix. Although surimi prepared by washing mince

with 0.45% NaCl as the third washing medium had the higher breaking force and deformation of surimi gel, compared to surimi prepared using 0.6% NaCl as washing medium, they showed the similar expressible moisture content ($p>0.05$). At 0.6% NaCl, NaCl might affect water holding capacity of myofibrillar proteins via charge modification in the fashion, which water was bound to protein, though gel matrix might not be well developed. Xiõng (1997a) reported that an increase in the electrostatic charges would lead to an increase in the repulsive force between myofilaments and increases in myofibril swelling and water-holding capacity. Hence, any change in the surroundings of myofibrils that results in increased protein charges or dipoles (such as high concentrations of salt and pH away from the protein isoelectric points) would lead to increased water retention in meat.

2.11 Microstructure

Microstructures of surimi gels from yellowtail barracuda mince washed without and with 0.3, 0.45, and 0.6% NaCl as the third washing media are illustrated in Figure 16. Surimi gels produced from mince washed with media containing 0.45 or 0.6% NaCl had a finer gel network and less number of voids, compared with gel of surimi prepared by washing with 0.3% NaCl and the control gel (surimi prepared from mince washed with water). This might be attributed to the formation of ordered network stabilized by various bonds including electrostatic interaction, hydrophobic interaction, hydrogen bonds as well as covalent bonds. Fine and ordered network of gel prepared from mince washed with 0.45% NaCl correlated well with the highest breaking force and deformation of gel. This suggested that the concentration of NaCl in the third washing medium played a role in gel formation of resulting surimi. Clark and Lee-Tuffnell (1986) reported that the formation of heat-induced protein gels is governed by a balance between attractive and repulsive forces. The attractive forces are considered to be induced by the various functional groups exposed by the thermal unfolding of protein and the repulsive forces are considered to be created by the surface charge. The use of 0.45% NaCl as the third washing was the optimal washing condition to yield the ordered gel network, which showed the high gel strength.

Control (Tap water)   0.3% NaCl

0.45% NaCl   0.6% NaCl
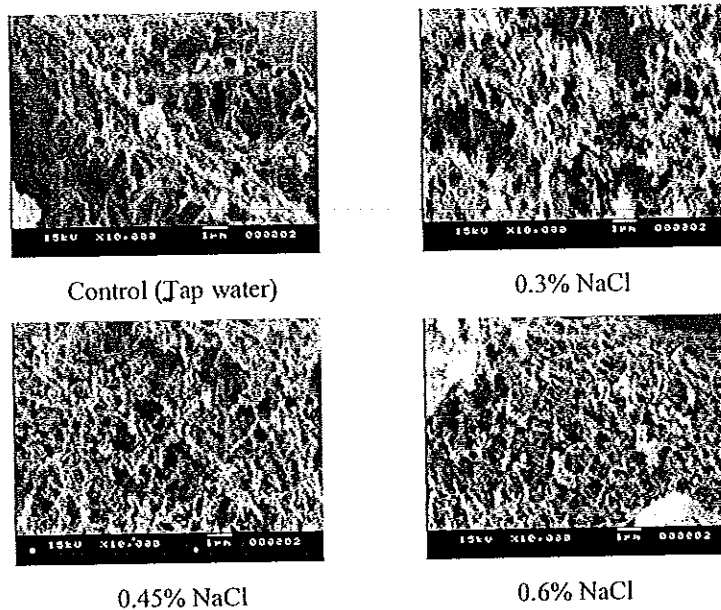
Figure 16. Microstructures of gels from yellowtail barracuda surimi produced from mince washed with the third washing medium containing different levels of NaCl (Magnification: 10,000x).

3. Effect of calcium chloride and magnesium chloride at various levels in the third washing media on chemical compositions of washed mince and properties of yellowtail barracuda surimi

3.1 Chemical composition and properties of washed mince

3.1.1 Calcium and magnesium contents

The types and concentrations of ions in the third washing media containing 0.45% NaCl had the effect on the calcium and magnesium contents of the resulting washed mince ($p<0.05$). When $CaCl_2$ or $MgCl_2$ in the washing media increased, calcium and magnesium contents in the resulting washed mince increased ($p<0.05$) (Table 8). This result was in agreement with Solberg *et al.* (1990) who reported that the higher contents of calcium and magnesium were obtained when the mince was washed with the higher concentration of both salts. At the same level of both salts used, the higher calcium content was found in washed mince than magnesium content. This might be due to the different affinity for protein molecules between Ca^{2+} and Mg^{2+}, where Ca^{2+} showed the higher affinity toword muscle protein

than Mg^{2+}. The preferential interaction of the protein with various ions increased in the order of $Ca^{2+} > Mg^{2+} > Na^+$, respectively (Arakawa and Timasheff, 1984). Na^+ salt prefered to interact with water, leading to a increase surface tension of washing water. Ca^{2+} and Mg^{2+} (divalent cations) prefer to bind with protein via ionic interaction. When the preferential interactions between divalent cation salts and protein overcame the salt exclusion, those ions were retained in the washed mince.

Table 8. Effect of the third washing media (0.45% NaCl) containing $CaCl_2$ or $MgCl_2$ at various levels on calcium and magnesium contents of yellowtail barracuda washed mince.

Ions	Samples	Ions content (g/kg of sample, dry basis)
Ca^{2+}	Unwashed mince	1.77 ± 0.02 [b]
	Mince washed with 0.45% NaCl	1.74 ± 0.03 [a]
	Mince washed with 0.45% NaCl + 4 mM $CaCl_2$	2.97 ± 0.01 [c]
	Mince washed with 0.45% NaCl + 12 mM $CaCl_2$	4.43 ± 0.02 [d]
	Mince washed with 0.45% NaCl + 20 mM $CaCl_2$	5.84 ± 0.03 [e]
Mg^{2+}	Unwashed mince	1.52 ± 0.01 [c]
	Mince washed with 0.45% NaCl	0.68 ± 0.02 [a]
	Mince washed with 0.45% NaCl + 4 mM $MgCl_2$	1.29 ± 0.02 [b]
	Mince washed with 0.45% NaCl + 12 mM $MgCl_2$	2.11 ± 0.01 [d]
	Mince washed with 0.45% NaCl + 20 mM $MgCl_2$	3.03 ± 0.02 [e]

Values are means $\pm$ SD from triplicate determinations. Different superscripts within the same column under the same ion determined indicate significant differences ($p < 0.05$).

3.1.2 pH

The pH of washed mince varied, depending on the type and concentrations of ions in the third washing media (Fig 17). Generally, the pH of washed mince slightly decreased when the mince was washed with 0.45% NaCl containing the increasing concentrations of $CaCl_2$ or $MgCl_2$ (0–20 mM) ($p < 0.05$). Washing with 0.45% NaCl could increase the pH of mince from 6.48 to 6.78. Washing might remove acidic compounds, especially lactic acid in the mince. The

slight differences in pH of mince washed with $CaCl_2$ or $MgCl_2$ at the same levels were observed. Mince washed with media containing $MgCl_2$ had the slightly higher pH than those washed with media comprising $CaCl_2$.

□ Unwashed mince ■ 0 mM ▣ 4 mM ▥ 8 mM ▨ 12 mM ◪ 16 mM ▩ 20 mM
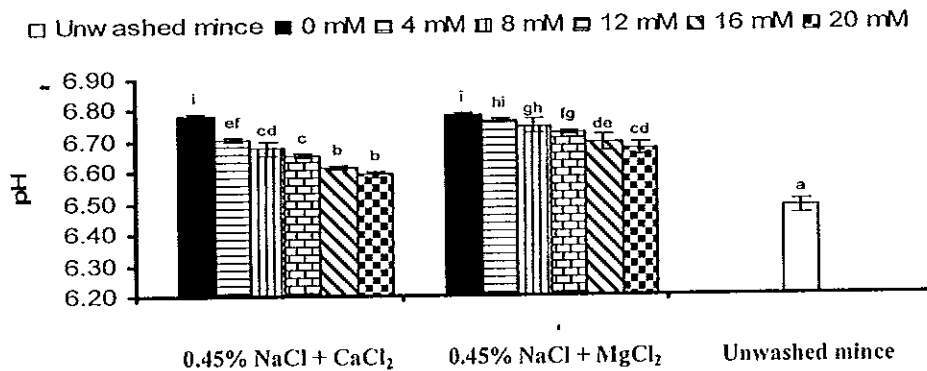
Figure 17. Effect of the third washing media (0.45% NaCl) containing $CaCl_2$ or $MgCl_2$ at various levels on pH of yellowtail barracuda washed mince. Bars indicate the standard deviation from triplicate determinations. Different letters on the bars indicate the significant differences ($p<0.05$).

3.1.3 Thermal property

T_{max} and ΔH determined by DSC of mince washed with the third washing media containing 0.45% NaCl incorporated with $CaCl_2$ or $MgCl_2$ at various concentrations are shown in Table 9. DSC thermogram has been used to indicate the transition of myosin and actin in the muscle (Akahane et al., 1985). Salt was found to decrease the stability of proteins in washed mince. Myosin in mince washed with 0.45% NaCl containing $CaCl_2$ possessed less stability than that in mince washed with $MgCl_2$ when the same level was used. Generally, when the higher concentration of both ions was used, the stability of myosin was lowered ($p<0.05$). No difference in the stability of actin was observed when $CaCl_2$ or $MgCl_2$ was used in the third washing media. Therefore, regardless of concentrations, both types and concentrations of ions affected the stability of proteins to different degrees. Salts influence the electrostatic interactions by providing charged and polar groups and affect the hydrophobic interaction via a modification of the water structure (Hippel and Schleich, 1969; Franks and England, 1975). The degree to which structure is affected depends on the nature of cations and anions and follows the Hofmeister series (Hippel and Wong, 1965). Cations and anions of higher order in the series (e.g., Ca^{2+} and SCN^-) could

reduce the energy required to transfer the nonpolar groups into water. This transfer would weaken intramolecular hydrophobic interaction and enhance the unfolding tendency of proteins (Hippel and Wong, 1965).

3.1.4 Moisture content

The dewatering of mince washed with 0.45% NaCl containing $CaCl_2$ or $MgCl_2$ at various levels was monitored by measuring the moisture content of washed mince (Table 10). Washing mince with 0.45% NaCl containing $CaCl_2$ or $MgCl_2$ enhanced dewatering ability, compared with washing with only 0.45% NaCl. Higher decrease in moisture content was obtained in mince washed with the media containing $CaCl_2$, compared with $MgCl_2$. The result indicated that dewatering of mince washed with media containing $CaCl_2$ was much more efficient than media having $MgCl_2$ ($p<0.05$). The differences in dewatering might be associated with different ability of ions in interaction with the charged groups of proteins. This was in agreement with Dalgleish and Hunt (1995) who reported that the binding of cations on protein molecules is based on the formation of a short-range hydration repulsion. When the charge of protein molecule was neutralized, two protein molecules approached each other, thereby repelling the water from the protein molecules. Ca^{2+} might bind protein via ionic interaction more potentially than Mg^{2+}, leading to the greater charge neutralization. This contributed to the lowering of water binding ability of protein when washing medium containing Ca^{2+} was used.

3.1.5 Fat content

Fat content of washed mince was affected to different degrees when $CaCl_2$ or $MgCl_2$ was added in the third washing media (Table 10). With increasing $CaCl_2$ concentration, significant reduction in fat content of washed mince was observed ($p<0.05$). Ca^{2+} has been reported to interact with phospholipids polar head, thereby repelling those phospholipids from the muscle (Khantaphant *et al.*, 2011). Nevertheless, the incorporation of Mg^{2+} into the washing media had no pronounced impact on lowering the fat content. Therefore, Ca^{2+} was shown to be effective in removal of fat. This would be a means to increase stability of mince or surimi, caused by lipid oxidation, especially during the extended storage. However, high amount of divalent ion may cause the destabilization or the acceleration of protein denaturation during the extended frozen storage.

Table 9. Effect of different washing media (0.45% NaCl) containing $CaCl_2$ or $MgCl_2$ at various levels on thermal property of yellowtail barracuda washed mince

Samples	Peak I		Peak II	
	T_{max} (°C)	ΔH (J/g)	T_{max} (°C)	ΔH (J/g)
Mince washed with 0.45% NaCl (Control)	51.75 ± 0.35 [c]	1.50 ± 0.07 [bcd]	70.00 ± 0.00 [abc]	0.33 ± 0.13 [a]
Mince washed with 0.45% NaCl + 4 mM $CaCl_2$	51.33 ± 0.24 [bc]	1.74 ± 0.07 [def]	69.67 ± 0.47 [ab]	0.44 ± 0.06 [ab]
Mince washed with 0.45% NaCl + 8 mM $CaCl_2$	51.00 ± 0.24 [b]	1.03 ± 0.05 [a]	70.08 ± 0.12 [abc]	0.33 ± 0.04 [a]
Mince washed with 0.45% NaCl + 12 mM $CaCl_2$	50.33 ± 0.24 [a]	1.90 ± 0.03 [ef]	70.00 ± 0.24 [abc]	0.50 ± 0.00 [b]
Mince washed with 0.45% NaCl + 16 mM $CaCl_2$	50.00 ± 0.47 [a]	1.94 ± 0.06 [f]	69.75 ± 0.35 [abc]	0.50 ± 0.03 [b]
Mince washed with 0.45% NaCl + 20 mM $CaCl_2$	49.75 ± 0.12 [a]	1.57 ± 0.18 [bcd]	69.50 ± 0.47 [a]	0.45 ± 0.04 [ab]
Mince washed with 0.45% NaCl + 4 mM $MgCl_2$	51.25 ± 0.12 [bc]	1.47 ± 0.06 [bc]	70.42 ± 0.59 [bc]	0.40 ± 0.00 [ab]
Mince washed with 0.45% NaCl + 8 mM $MgCl_2$	51.58 ± 0.12 [bc]	1.52 ± 0.21 [bcd]	70.42 ± 0.35 [bc]	0.40 ± 0.00 [ab]
Mince washed with 0.45% NaCl + 12 mM $MgCl_2$	51.00 ± 0.24 [a]	1.68 ± 0.10 [bcde]	70.50 ± 0.00 [c]	0.42 ± 0.09 [ab]
Mince washed with 0.45% NaCl + 16 mM $MgCl_2$	50.25 ± 0.35 [a]	1.44 ± 0.01 [b]	69.58 ± 0.35 [a]	0.42 ± 0.02 [ab]
Mince washed with 0.45% NaCl + 20 mM $MgCl_2$	50.33 ± 0.00 [a]	1.69 ± 0.05 [cde]	69.92 ± 0.12 [abc]	0.39 ± 0.03 [ab]

Values are means ± SD from triplicate determinations.

Different superscripts within the same column indicate significant differences ($p < 0.05$).

Table 10. Effect of different washing media (0.45% NaCl) containing $CaCl_2$ or $MgCl_2$ at various levels on moisture content, fat, sarcoplasmic protein and whiteness of yellowtail barracuda washed mince

Samples	Moisture content (%)	Fat (% dry wt basis)	Sarcoplasmic protein (% dry wt basis)	Whiteness
Unwashed mince	80.57 ± 0.10 [a]	7.33 ± 0.25 [a]	14.67 ± 0.13 [a]	46.23 ± 1.62 [a]
Mince washed with 0.45% NaCl (Control)	84.80 ± 0.15 [f]	4.49 ± 0.11 [c]	4.60 ± 0.09 [de]	66.27 ± 0.22 [d]
Mince washed with 0.45% NaCl + 4 mM CaCl₂	84.15 ± 0.08 [de]	4.53 ± 0.17 [c]	4.71 ± 0.24 [de]	66.34 ± 1.00 [d]
Mince washed with 0.45% NaCl + 8 mM CaCl₂	83.68 ± 0.05 [c]	4.48 ± 0.11 [c]	4.93 ± 0.36 [de]	67.64 ± 0.53 [c]
Mince washed with 0.45% NaCl + 12 mM CaCl₂	83.12 ± 0.09 [b]	4.39 ± 0.09 [c]	5.72 ± 0.32 [cde]	67.56 ± 0.99 [c]
Mince washed with 0.45% NaCl + 16 mM CaCl₂	83.04 ± 0.22 [b]	2.55 ± 0.10 [d]	4.01 ± 0.01 [e]	68.15 ± 0.09 [ef]
Mince washed with 0.45% NaCl + 20 mM CaCl₂	82.78 ± 0.34 [b]	0.04 ± 0.05 [e]	4.17 ± 0.01 [e]	68.64 ± 0.41 [f]
Mince washed with 0.45% NaCl + 4 mM MgCl₂	84.18 ± 0.30 [e]	4.87 ± 0.01 [b]	7.16 ± 0.99 [bcd]	66.15 ± 1.07 [cd]
Mince washed with 0.45% NaCl + 8 mM MgCl₂	83.94 ± 0.15 [cde]	4.36 ± 0.04 [c]	6.57 ± 1.50 [bcde]	66.17 ± 0.38 [cd]
Mince washed with 0.45% NaCl + 12 mM MgCl₂	83.78 ± 0.02 [cd]	4.46 ± 0.13 [c]	5.49 ± 0.14 [de]	65.24 ± 1.67 [bc]
Mince washed with 0.45% NaCl + 16 mM MgCl₂	83.68 ± 0.07 [c]	4.78 ± 0.11 [b]	9.14 ± 3.72 [b]	65.06 ± 0.17 [b]
Mince washed with 0.45% NaCl + 20 mM MgCl₂	83.57 ± 0.08 [c]	4.57 ± 0.12 [c]	8.46 ± 0.25 [bc]	65.01 ± 0.63 [b]

Values are means ± SD from triplicate determinations.

Different superscripts within the same column indicate significant differences ($p < 0.05$).

3.1.6 Sarcoplasmic protein content

Sarcoplasmic protein contents of yellowtail barracuda mince washed with the third washing media (0.45% NaCl) containing $CaCl_2$ or $MgCl_2$ at various levels are shown in Table 10. Mince washed with 0.45% containing various levels of $CaCl_2$ had the similar sarcoplasmic protein content ($p > 0.05$). Nevertheless, higher sarcoplasmic protein content of washed mince was obtained in comparison with media washed with $MgCl_2$ ($p < 0.05$). Mg^{2+} might cause the aggregation of sarcoplasmic protein in the presence of NaCl. This led to the loss in solubility of those aggregated sarcoplasmic proteins, which were retained in the resulting washed mince. The effect of calcium and magnesium is quite complex and differs for white and red muscle proteins (Xiong and Brekke, 1991b). In the case of white muscles, myofibrillar protein solubility increased in the presence of 2.5–5 mM $CaCl_2$ or $MgCl_2$ but decreased with further increase in ionic strength. However, in red muscles, solubility reached a maximum at 5 mM concentration and remained the same with a further increase in ionic strength (Xiong and Brekke, 1991a). Presence of calcium and magnesium binding proteins, like myosin light chain, troponin, etc., may be contributing to the different fiber type dependent solubility (Ramachandran et al., 2006). Thus, salt incorporated in washing media had an important role in leaching effect of sarcoplasmic protein in mince.

3.1.7 Whiteness

The concentration of $CaCl_2$ or $MgCl_2$ in conjunction with the third washing media containing 0.45% NaCl affected the whiteness of washed mince differently (Table 10). When the higher level of $CaCl_2$ was added to the washing media, whiteness value of washed mince increased slightly ($p < 0.05$), while the increasing levels of $MgCl_2$ resulted in the decrease in whiteness of washed mince ($p < 0.05$). $CaCl_2$ might form complex with some anions of myofibrillar proteins, resulting in the formation of insoluble particles, leading to the light scattering in resulting gels (Benjakul et al. 2004b). Julavittayanukul et al. (2006) reported that whiteness of kamaboko gel of bigeye snapper surimi was increased with increasing $CaCl_2$ concentrations. Furthermore, Benjakul et al. (2010) also found that whiteness of both kamaboko and modori gels added with 50 mmol $CaCl_2$/kg was greater than that of gels without $CaCl_2$. For $MgCl_2$ effect, the decrease in whiteness of mince washed with washing media containing $MgCl_2$ was closely related with the increase in

sarcoplasmic protein content of resulting washed mince. Sarcoplasmic proteins, especially heme protein such as haemoglobin and myoglobin, retained in washed mince, more likely contributed to the lowered whiteness.

3.1.8 Protein pattern

Protein patterns of washed mince, mince washed with 0.45% NaCl containing $CaCl_2$ or $MgCl_2$ at various levels in the third washing media are depicted in Figure 18. After washing, proteins with MW of 40 and 55 kDa were removed, while band intensity of both MHC and actin slightly increased. No differences in MHC and actin band intensity were noticeable when the mince was washed with 0.45% NaCl containing different concentrations of $CaCl_2$ and $MgCl_2$. This might indicate that washing with 0.45% NaCl containing $CaCl_2$ or $MgCl_2$ had no effect on MHC and actin of resulting washed mince.

Unwashed   0   4   8   12   16   20   Unwashed   0   4   8   12   16   20

0.45% NaCl + $CaCl_2$ (mM)   0.45% NaCl + $MgCl_2$ (mM)
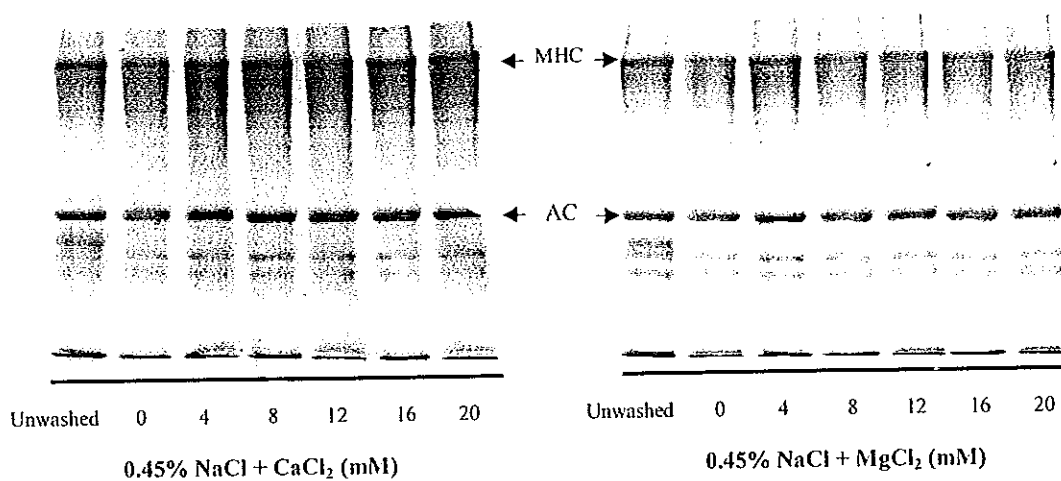
Figure 18. Effect of the third washing media (0.45% NaCl) containing $CaCl_2$ or $MgCl_2$ at different levels on protein patterns of yellowtail barracuda washed mince.

3.2 Properties of surimi gel

3.2.1 Breaking force and deformation

Breaking force and deformation of gel from yellowtail barracuda surimi prepared using different washing media (0.45% NaCl containing $CaCl_2$ or $MgCl_2$ at various levels) with two different heating conditions are depicted in Figure 19. Surimi prepared from mince washed with 0.45% NaCl containing 20 mM $MgCl_2$ yielded the gel with the highest breaking force for both heating conditions ($p<0.05$), in which the breaking force increased by 46 and 33 %, compared with that of the control (without $CaCl_2$ or $MgCl_2$ incorporated). $MgCl_2$ has been reported to dissociate the actomyosin complex (Konno, 1992). Free myosin might undergo aggregation orderly, in which the strong network was developed. Generally, no differences in deformation of gels were observed ($p>0.05$), except for the deformation of gel obtained from two-step heating, which decreased when the concentrations of $CaCl_2$ in the washing media increased ($p<0.05$). Ca^{2+} is required for the activation of endogenous transglutaminase, which induced the formation of ε–γ–glutamyl–lysine linkage isopeptide associated with the stronger network (Kumazawa et al., 1995). This might contribute to the lowered elasticity of gel as $CaCl_2$ added in the washing media increased. Xiong and Brekke (1991) reported the increase in salt-soluble proteins and the improved gel strength for breast and leg chicken muscle when $CaCl_2$ and $MgCl_2$ were added at 5 mM. Furthermore, Solberg et al. (1990) reported that gelling properties of cod surimi were strongly dependent on the type of ion present in the washing media. In the present study, $CaCl_2$ in washing medium had no impact on breaking force of surimi gel, regardless of concentration in washing media used. This might be due to the sufficient Ca^{2+} used for full activation of endogenous tranglutaminase in the mince. As a result, further increase in $CaCl_2$ had no effect on breaking force of resulting surimi gels.
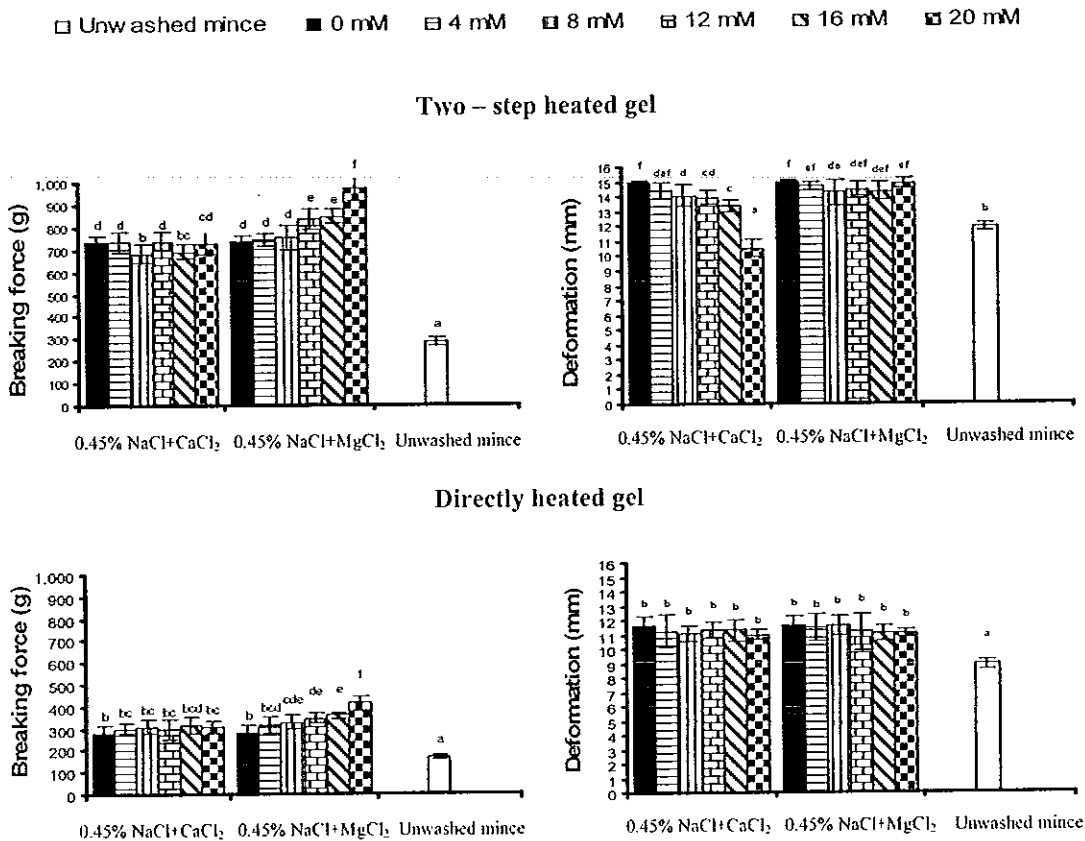
Figure 19. Breaking force and deformation of yellowtail barracuda surimi gels with the third washing media (0.45% NaCl containing $CaCl_2$ or $MgCl_2$ at various levels) prepared with two heating conditions: Directly heated gels were prepared by heating the sol at 90 °C for 20 min. Two–step heated gels were prepared by incubating the sol at 40 °C for 30 min, followed by heating at 90 °C for 20 min. Bars indicate the standard deviation from triplicate determinations. Different letters on the bars indicate the significant differences ($p<0.05$).

3.2.2 Whiteness

Whiteness of surimi gels prepared from mince washed with different washing media using both heating conditions was slightly decreased when the mince was washed with 0.45% NaCl containing higher $MgCl_2$ levels ($p<0.05$) (Table 11), compared with that of the control (without $CaCl_2$ or $MgCl_2$). However, no difference in the whiteness of surimi gels was found when $CaCl_2$ was added in washing media, irrespective of concentrations used. Washing with the higher level of $MgCl_2$ yielded

the washed mince with high sarcoplasmic protein content. This more likely resulted in the decreased whiteness of gel. Regardless of heating condition, the higher whiteness was found in gel prepared by direct heating, compared with those with two- step heating ($p<0.05$). Non–enzymatic browning might take place a higher extent with a longer exposure time used for two–step heating. The effect of metal ions on browning was found to depend on the type of amino acid and heating time, as well as on the type of metal ion. It is known that a transition metal ion catalyzes the Maillard reaction by the oxidative pathway (Morita and Kashimura, 1991). Ca^{2+} ion exhibited the less effect on accelerating Maillard reaction (Morita and Kashimura, 1991). Thus, Ca^{2+} incorporation had the lower negative impact on whiteness of surimi gel in comparison with Mg^{2+}.

3.2.3 Expressible moisture content

The slight decreases in expressible moisture contents of gel with both heating condition were found when the gels were produced from washed mince using the higher $MgCl_2$ levels in the third washing media, compared to the control gel ($p<0.05$) (Table 11). Surimi from mince washed with media containing $CaCl_2$, had the similar expressible moisture contents when gels were prepared by direct heating ($p>0.05$), whereas the slightly higher expressible moisture contents of gel was obtained when two- step heating was implemented ($p<0.05$), Regardless of heating condition, the higher expressible moisture content was found in gel from surimi prepared using media containing $CaCl_2$ compared to those with $MgCl_2$. Therefore, the results indicated that not only type and concentration of ions in washing media but also heating conditions affected the expressible moisture contents of gel. Terrell *et al.* (1981) indicated that magnesium was the best additive for decreasing moisture losses in the raw and cooked beef. This response may be due to the effect of magnesium on increasing myosin extractability (Nayak *et al.*, 1996). Xiong and Brekke (1991b) reported that moisture loss of chicken breast and leg gels was minimize when $CaCl_2$ and $MgCl_2$ at concentration less than 5 mM was used but increased at greater concentrations of both salts. Both divalent cations affected gelation by changing the extraction and protein-protein interaction of salt-soluble protein. Therefore, divalent ions in washing media showed the impact on water holding capacity of gel in conjunction with heating condition.

Table 11. Effect of different washing media (0.45% NaCl) containing $CaCl_2$ or $MgCl_2$ at various levels on the whiteness of yellowtail barracuda surimi gels prepared with two heating conditions.

Samples	Whiteness		Expressible moisture content (%)	
	40/90°C	90°C	40/90°C	90°C
Unwashed mince	69.98 ± 0.94 [a]	70.04 ± 0.41 [a]	8.52 ± 1.32 [d]	9.56 ± 0.52 [c]
Mince washed with 0.45% NaCl (Control)	78.24 ± 0.46 [d]	79.90 ± 0.77 [ef]	6.61 ± 1.27 [abc]	6.41 ± 0.72 [ab]
Mince washed with 0.45% NaCl + 4 mM $CaCl_2$	77.63 ± 0.56 [c]	79.54 ± 0.40 [cd]	7.56 ± 1.10 [cd]	7.03 ± 0.42 [b]
Mince washed with 0.45% NaCl + 8 mM $CaCl_2$	78.44 ± 0.40 [de]	78.70 ± 1.26 [bc]	6.70 ± 0.51 [abc]	6.19 ± 0.42 [ab]
Mince washed with 0.45% NaCl + 12 mM $CaCl_2$	78.32 ± 0.67 [d]	79.52 ± 0.86 [cd]	7.06 ± 0.39 [bc]	7.11 ± 0.68 [b]
Mince washed with 0.45% NaCl + 16 mM $CaCl_2$	79.02 ± 0.57 [f]	80.13 ± 0.55 [ef]	7.64 ± 0.96 [cd]	6.29 ± 0.84 [ab]
Mince washed with 0.45% NaCl + 20 mM $CaCl_2$	78.93 ± 0.50 [ef]	80.70 ± 0.25 [f]	7.78 ± 0.76 [cd]	6.24 ± 0.46 [ab]
Mince washed with 0.45% NaCl + 4 mM $MgCl_2$	77.66 ± 0.41 [c]	79.54 ± 0.35 [cd]	6.29 ± 0.39 [ab]	6.64 ± 0.40 [ab]
Mince washed with 0.45% NaCl + 8 mM $MgCl_2$	77.51 ± 0.53 [c]	79.50 ± 0.52 [cd]	6.55 ± 1.02 [abc]	6.91 ± 0.90 [ab]
Mince washed with 0.45% NaCl + 12 mM $MgCl_2$	76.47 ± 0.60 [b]	78.69 ± 0.42 [bc]	6.18 ± 0.25 [ab]	6.12 ± 0.92 [ab]
Mince washed with 0.45% NaCl + 16 mM $MgCl_2$	77.28 ± 0.63 [c]	78.50 ± 0.29 [b]	5.97 ± 1.08 [ab]	5.90 ± 1.09 [a]
Mince washed with 0.45% NaCl + 20 mM $MgCl_2$	76.58 ± 0.45 [b]	78.58 ± 0.47 [b]	5.87 ± 0.79 [a]	5.89 ± 0.78 [a]

Values are means ± SD from triplicate determinations.

Different superscripts within the same column indicate significant differences ($p < 0.05$).

3.2.4 Microstructure

Microstructures of gels from yellowtail barracuda mince washed with 0.45% NaCl containing without and with 8 and 20 mM $CaCl_2$ or 8 and 20 mM $MgCl_2$ in the third washing media are illustrated in Figure 20. No marked differences in microstructure of gels produced from mince washed with 0.45% NaCl containing 8 mM $CaCl_2$ or $MgCl_2$. However, it was noted that gel from surimi with 0.45%-NaCl containing 20 mM $MgCl_2$ as washing medium had the finer gel network and less number of voids, as compared to the control gel. This ordered gel network correlated with the highest breaking force and deformation as well as the lowest expressible moisture content. On the other hand, surimi with 0.45% NaCl containing 20 mM $CaCl_2$ as washing medium had the fine network but a large size of voids. The result suggested that the appropriate type and concentration of ions in the third washing media had the pronounced impact on the structure of surimi gel.

| 0.45% NaCl (Control) | 0.45% NaCl + 8 mM $CaCl_2$ | 0.45% NaCl + 20 mM $CaCl_2$ |

| 0.45% NaCl + 8 mM $MgCl_2$ | 0.45% NaCl + 20 mM $MgCl_2$ |
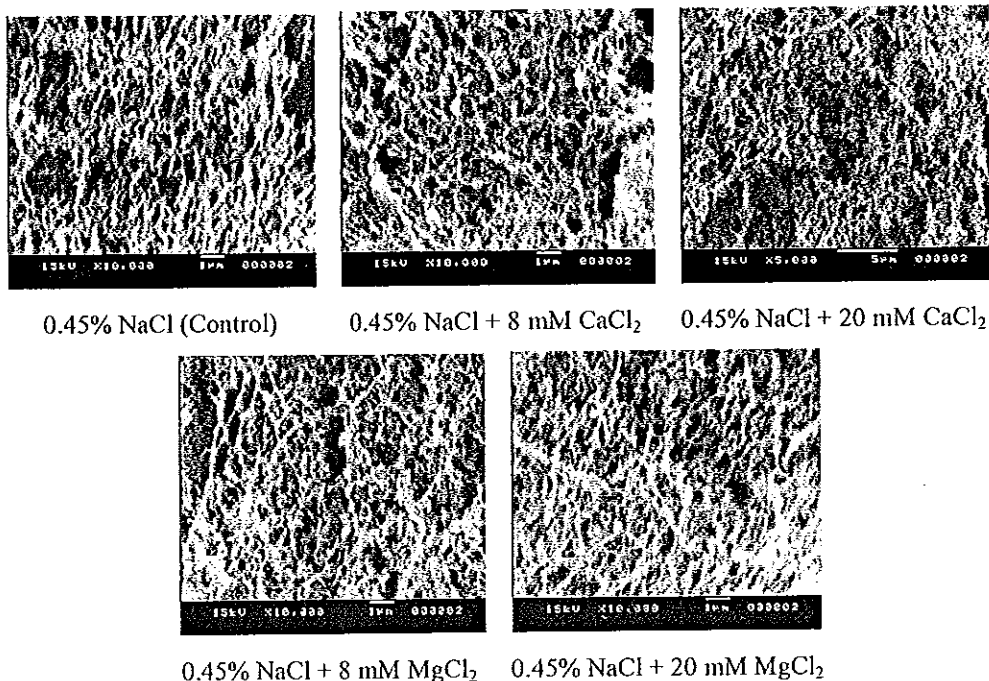
Figure 20. Microstructures of yellowtail barracuda surimi gels with different third washing media (0.45% NaCl containing $CaCl_2$ or $MgCl_2$ at various levels) prepared with two–step heating process. Gels were prepared by incubating the sol at 40°C for 30 min, followed by heating at 90°C for 20 min.

4. Effect of different washing processes on the compositions of washed mince and properties of surimi

4.1 Compositions and properties of washed mince

Different washing processes including different washing media in several steps were used. The obtained washed mince was determined for composition and properties of surimi were also studied.

4.1.1 pH

pH of mince washed with different washing processes is shown in Table 12. When comparing with mince washed by typical process (T1), mince washed with washing medium having 20 mM $MgCl_2$ as the third washing medium (T2) had a slightly lower pH. On the other hand, washing with 0.2% $NaHCO_3$ as the first washing medium caused an increase in pH of washed mince. There was no difference in pH between mince washed with washing processes T5 and T6 ($p>0.05$). In general, the use of 0.3% NaCl in conjunction with 0.2% $NaHCO_3$ as the first washing medium resulted in the slightly lower pH of washed mince as compared to the use of 0.15% NaCl and 0.2% $NaHCO_3$. Therefore, pH of washing media mainly had the impact on the pH of resulting washed mince. Bertram *et al.* (2008) reported that different pH of washing media: pH 6.62 for 5% (w/w) NaCl, pH 8.32 for 3% (w/w) $NaHCO_3$, and pH 8.07 for 5% (w/w) NaCl + 3% (w/w) $NaHCO_3$. NaCl might have influence on pH of washed mince by helping in leaching basic compounds in the mince, leading to the lower pH value.

Table 12. Effect of different washing processes on pH, chemical composition and whiteness of washed mince

Treatments	pH	Salt (% dry wt. basis)	Moisture (%)	Fat (% dry wt. basis)	Sarcoplasmic protein (% dry wt. basis)	Whiteness
T1	7.07 ± 0.02 [b]	1.72 ± 0.21 [a]	84.08 ± 0.08 [c]	3.58 ± 0.07 [a]	7.51 ± 0.14 [bc]	67.31 ± 0.64 [a]
T2	6.91 ± 0.02 [a]	2.02 ± 0.12 [abc]	82.44 ± 0.15 [a]	3.94 ± 0.05 [b]	9.23 ± 0.30 [a]	65.66 ± 1.17 [a]
T3	7.37 ± 0.03 [e]	1.90 ± 0.01 [abc]	85.23 ± 0.28 [d]	4.24 ± 0.06 [c]	8.01 ± 0.37 [b]	65.83 ± 0.95 [a]
T4	7.26 ± 0.01 [d]	2.08 ± 0.03 [bc]	82.85 ± 0.25 [ab]	4.40 ± 0.01 [f]	7.60 ± 0.02 [bc]	66.14 ± 1.08 [a]
T5	7.17 ± 0.01 [c]	1.77 ± 0.02 [ab]	86.14 ± 0.37 [e]	4.27 ± 0.06 [d]	7.91 ± 0.17 [bc]	66.61 ± 0.82 [a]
T6	7.18 ± 0.01 [c]	2.21 ± 0.21 [c]	83.32 ± 0.22 [b]	4.33 ± 0.02 [e]	7.41 ± 0.08 [c]	66.99 ± 0.92 [a]

Values are means ± SD from triplicate determinations.

Different superscripts within the same column indicate significant differences ($p<0.05$).

4.1.2 Salt content

Salt contents of washed mince prepared by different washing processes are shown in Table 12. In general, washing media for all processes had no marked impact on salt content in resulting washed mince. However, washed mince prepared by processes T4 and T6 had higher salt content, compared with that obtained from the typical process (T1) ($p<0.05$). It was noted that $MgCl_2$ presented in the third washing medium might induce the dissociation of actomyosin. This led to the increased absorption of NaCl in the mince. For processes T4 and T6, NaCl presented in the first washing medium more likely contributed to the higher accumulation of NaCl in the mince. Cl^- ions can bind to the filaments of protein and increase the electrostatic repulsive force between them. At a critical salt concentration, one or more transverse structural constraints in the myofibril (probably crossbridges, the M-line or the Z-line) are loosen, allowing the filament lattice to expand (Offer and Trinick, 1983). It was suggested that the use of NaCl in conjunction with $MgCl_2$ at some level brought about the swelling of protein and uptaking of salt into the mince.

4.1.3 Moisture content

The difference in moisture contents of washed mince varied (Table 12), depending upon washing processes, which contained different types and levels of salt. The decrease in moisture content was obtained in mince washed with washing media containing 20 mM $MgCl_2$, compared to those without 20 mM $MgCl_2$ ($p<0.05$). This result indicated that 20 mM $MgCl_2$ increased the efficiency in dewatering mince. Moreover, washing with media containing $NaHCO_3$ in the first washing step resulted in the highest moisture content of washed mince when the third washing media contained no $MgCl_2$. This was evidenced by the high moisture content in washed mince obtained from process T3 and T5. The results indicated that alkaline saline solution (NaCl + $NaHCO_3$) might result in the increased water absorption of mince, more likely associated with a higher water uptaken of swollen myofibrillar proteins mediated by alkaline condition. Within the meat protein structures, water molecules are bound by noncovalent bonds (e.g., hydrogen bonds and electrostatic forces) (Ling, 1962). Individual protein molecules have a variety of electric dipoles, which easily form hydrogen bonds with water molecules. Water molecules in heterogeneous biological systems have been proposed to form polarized multilayer by hydrogen

bonds over extended protein surfaces (Ling, 1962). More negatively charged protein after washing with $NaHCO_3$ might cause the repulsion of proteins, leading to more looser structure, which could absorb more water. The potential mechanism of alkaline saline solution was to expose surfaces of proteins, thereby creating increased interactions between water and the individual meat proteins. For the last washing step, the dewatering was lowered when the media did not contain $MgCl_2$. Thus $MgCl_2$ was required in the last washing media, especially when $NaHCO_3$ was used in the first washing medium.

4.1.4 Fat content

Differences in fat content of mince washed with various washing processes are shown in Table 12. Generally, the higher fat content of washed mince was obtained when the mince was washed with 0.45% NaCl + 20 mM $MgCl_2$ as compared to 0.45% NaCl for all samples, regardless of the first washing media used. Mg^{2+}, a divalent ion, might interact with polar head of phospholipids via ionic interaction. This led to the lowered ability of phospholipids to leach out during washing. Lipid-protein interactions include any specific electrostatic or hydrogen-bonding interaction between the hydrophilic head groups of lipids and the exposed amino acid residues of proteins. This resulted in a preferential clustering of specific lipids around the protein and those lipids can be packed most easily around the proteins or specifically interact with proteins (Meste and Davidou, 1995). When NaCl + $NaHCO_3$ were used as washing media, the amount of lipids could be adsorbed at higher level when the lipids and proteins carry opposite charges. Under alkaline condition, protein turned to be more negatively charged, salt bridge between those proteins and phospholipids might be enhanced by Mg^{2+}.

4.1.5 Sarcoplasmic protein content

When comparing sarcoplasmic protein content in mince washed with 0.45% NaCl and 0.45% NaCl + 20 mM $MgCl_2$ as washing media in the last step, similar sarcoplasmic protein content was found, except for sample washed with tap water for the first two steps and 0.45% NaCl + 20 mM $MgCl_2$ as the last steps (Table 12). Co-precipitation of sarcoplasmic proteins with myofibrillar proteins induced by Mg^{2+} might lead to the increased sarcoplasmic protein content in this sample. Nevertheless, washing with alkaline media in the first washing step might effectively

remove sarcoplasmic proteins in the mince, leading to the lower sarcoplasmic protein content of washed mince. Alkaline saline solution induced swelling of the myofibrillar structures (Bertram *et al.*, 2008), thereby facilitating the solubility of sarcoplasmic proteins during washing. Shahidi *et al.* (1992) reported that the total content of hemoprotein pigments in mechanically deboned chicken meat (MDCM) after the first and second washings with water at pH 6.9 and 7.2 decreased by 43.6 and 48.2 %, respectively. Washing with water and then 0.5% sodium bicarbonate solution was most effective in removing hemoproteins from MDCM and their content was decreased by 75.5%. Therefore, washing processes did not show the pronounced influence on the sarcoplasmic protein content in washed mince.

4.1.6 Whiteness

No difference in whiteness of mince washed with different washing media was observed ($p > 0.05$) (Table 12). Whiteness of washed mince ranged from 65.66 to 67.31. The similar whiteness of washed mince was in agreement with similar content of sarcoplasmic proteins including pigment proteins such as haemoglobin and myoglobin. Dawson *et al.* (1988) reported that washing MDCM with 0.5% sodium bicarbonate solution improved the color of the product better than washing with water. Shahidi *et al.* (1992) suggested that MDCM after washing with 0.5% sodium bicarbonate solution at pH 7.8 gave higher L and lower a value than samples washed with water or salt solutions.

4.1.7 Thermal property

Thermal property expressed as T_{max} and ΔH of washed mince shown in Table 13. Two transition peaks were obtained at temperatures of 50.67-51.83 °C and 68.17-71.33 °C represented myosin heavy chain and actin, respectively. Various washing processes affected the thermal property of washed mince differently ($p < 0.05$). When the third washing media containing 0.45% NaCl and 20 mM $MgCl_2$ was used, the decreases in T_{max} of both peaks were observed, when compared with washing media containing only 0.45% NaCl. This was indicated by the shift of T_{max} to the lower temperatures, except for process T6, which had the higher T_{max} of second peak than that of process T5. Generally, thermal stability of both MHC and actin in mince washed with alkaline process had the lower T_{max} of both peaks. Alkaline pH of washing media containing $NaHCO_3$ might induce the conformational change of both

MHC and actin to some degree. ΔH was also decreased when alkaline washing for the first step was implemented. The results also indicated that $MgCl_2$ in the last washing media might contribute to the less stability of proteins. The attachment of Mg^{2+} with proteins might cause the alteration of proteins. The removal of water by Mg^{2+} was also associated with the higher susceptability of protein to heat. Salts that stabilize proteins enhance hydration of proteins and bind weakly, whereas salts that destabilize proteins decrease proteins hydration and bind strongly (Arakawa and Timasheff, 1984). The denaturing effect of chaotropic salts might be related to destabilization of hydrophobic interactions in proteins (Damodaran, 1996).

Table 13. Effect of different washing media on thermal properties of washed mince

Treatments	Peak I		Peak II	
	T_{max} (°C)	Δ H (J/g)	T_{max} (°C)	Δ H (J/g)
T1	51.83 ± 0.35 [a]	1.68 ± 0.07 [a]	71.33 ± 0.00 [a]	0.41 ± 0.13 [a]
T2	51.00 ± 0.24 [d]	1.50 ± 0.07 [c]	71.17 ± 0.47 [b]	0.38 ± 0.06 [b]
T3	51.33 ± 0.24 [c]	0.90 ± 0.05 [f]	68.83 ± 0.12 [c]	0.03 ± 0.04 [f]
T4	50.67 ± 0.47 [f]	1.62 ± 0.06 [b]	68.67 ± 0.35 [d]	0.34 ± 0.03 [c]
T5	51.50 ± 0.24 [b]	1.38 ± 0.03 [d]	68.17 ± 0.24 [e]	0.31 ± 0.00 [d]
T6	50.83 ± 0.12 [e]	1.23 ± 0.18 [e]	69.83 ± 0.47 [c]	0.27 ± 0.04 [e]

Values are means $\pm$ SD from triplicate determinations. Different superscripts within the same column indicate significant differences ($p < 0.05$).

4.1.8 Protein pattern

Different washing processes had no impact on protein patterns of washed mince (Figure 21). This was coincidental with the similar sarcoplasmic protein content in all samples (Table 12). For all samples, MHC was found to be the predominant protein, followed by actin. MHC has been reported to play a crucial role in gel formation (Samejima et al. 1981; Ishioroshi et al., 1982).
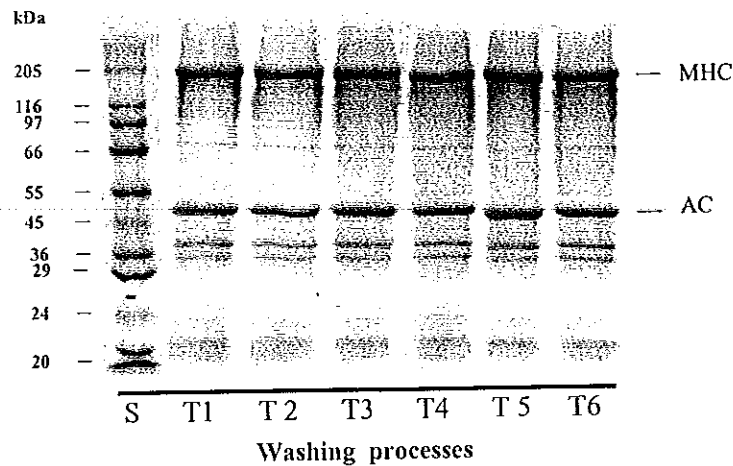
Figure 21. Effect of different washing processes on protein patterns of yellowtail barracuda washed mince. Number denote the washing processes.

4.2 Properties of surimi gel

4.2.1 Breaking force and deformation

Breaking force was obtained among all gels of surimi prepared by different washing processes ($p < 0.05$), while no marked difference in deformation of gels was found. The highest breaking force of gel was obtained when washing media including by 0.3% NaCl + 0.2% NaHCO$_3$ was used in the first step, followed by tap water and 0.45% NaCl + 20 mM MgCl$_2$, respectively (T6). Breaking force increased by 33%, compared with that of gel of surimi prepared by typical process (T1). Alkaline washing process has been reported to increase breaking force of surimi (Tokunaga, 1982). Alkaline condition could modify the charge of protein by inducing deprotonation. The net charge of the protein molecule is modified by attractive and repulsive forces, affecting protein-protein and protein-solvent interactions (Phillips *et al.*, 1994). In the presence of salt in washing media, salt at low concentration might facilitate the removal sarcplasmic proteins, which has been known to show the negative effect on gelation. The highest breaking force of surimi produced by process T6 was plausibly due to the ease of unfolding of proteins during gelation. As a result, the extended protein could undergo aggregation easily. Additionally, Mg^{2+} could induce the dissociation of proteins, in which monomers could form the fine structure with the improved gel strength. From the result, surimi gel from processes T2, T3 and T4 had the similar breaking force ($p > 0.05$). Surimi gel from processes T1 and T5 had

the similar breaking force ($p>0.05$). Thus, washing process had the influence on the gel forming ability of resulting surimi.
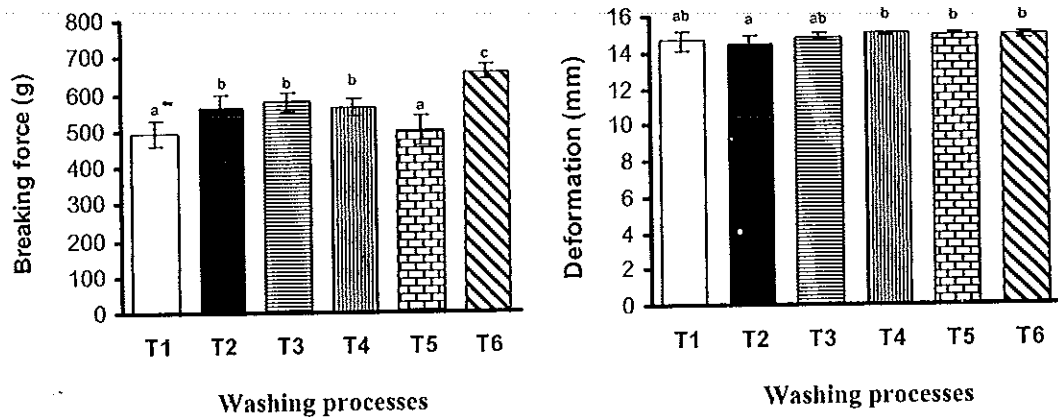
Figure 22. Breaking force and deformation of gels from yellowtail barracuda surimi prepared from different washing processes. Gels were prepared by two–step heating (40°C for 30 min, followed by heating at 90°C for 20 min). Bars represent the standard deviation from triplicate determinations. Different letters on the bars indicate the significant differences ($p<0.05$).

4.2.2 Whiteness

Whiteness of surimi gels prepared from mince washed with different washing processes is shown in Table 14. Slight decrease in whiteness of gel from surimi prepared using all processes was found, compared with typical process (T1), except for surimi prepared using process T5, which had the similar whiteness to surimi from typical process ($p>0.05$). This result was in accordance with the whiteness of mince washed with different washing processes. Whiteness is another important quality index for surimi gel.

4.2.3 Expressible moisture content

Generally, no difference in expressible moisture content of all surimi gels produced from mince using various washing processes were observed (Table 14) ($p>0.05$). Non-significantly lowest expressible moisture content of gel was obtained in gel of surimi prepared from process T6. The lower expressible moisture content of surimi gel was in accordance with the highest breaking force of resulting surimi gel (Figure 22). When the optimum washing condition was used, the better gelation was

achieved. The cross-linking of proteins in the yellowtail barracuda surimi gel could be enhanced. This resulted in the formation of stronger network with greater water holding capacity.

Table 14. Effect of different washing processes on whiteness and expressible moisture content of surimi gels.

Treatments	Whiteness	Expressible moisture content (%)
T1	77.91 ± 0.48 [a]	6.20 ± 0.90 [a]
T2	76.85 ± 0.88 [b]	6.17 ± 0.58 [a]
T3	77.31 ± 0.35 [b]	5.93 ± 0.57 [a]
T4	76.99 ± 0.88 [b]	6.12 ± 0.61 [a]
T5	78.39 ± 0.29 [a]	6.25 ± 0.25 [a]
T6	77.06 ± 0.50 [b]	5.76 ± 0.28 [a]

Values are means $\pm$ SD from triplicate determinations. Different superscripts within the same column indicate significant differences ($p < 0.05$).

5. Effect of selected washing process on chemical compositions of washed mince and properties of gels prepared from yellowtail barracuda stored in ice

Selected washing process involving 1) 1st washing: 0.30% NaCl + 0.20% $NaHCO_3$; 2) 2nd washing: tap water and 3rd washing: 0.45% NaCl + 20 mM $MgCl_2$ (T6) was used to prepare washed mince and surimi from yellowtail barracuda stored in ice for different time. Typical washing process was used as the control processes using tap water for the 1st and 2nd step and 0.45% NaCl as the third step (T1).

5.1 Chemical compositions and properties of washed mince

5.1.1 K-value, TMA, TVB and TBARS

K-values of unwashed yellowtail barracuda mince from whole fish were 42.17 ± 0.48, 61.63 ± 0.63 and 72.17 ± 0.19, when fish were stored in ice for 0, 7 and 14 days, respectively. K-value of unwashed mince increased markedly when whole fish was stored in ice for a longer time ($p < 0.05$). Aubourg *et al.* (2005) reported that a

gradual increase in K-value of turbot was observed during 19 days of iced storage. This result was similar to that of mackerel (Ryder *et al.*, 1984), sardine (Gomez *et al.*, 2001) and seabream (Huidobro *et al.*, 2001). K-value greater than 60% was obtained after the whole fish was stored in ice for 7 day. K-value of 60% is considered as the rejection limit for customer (Ehira, 1976). This indicated that the degradation of nucleotides by nucleotide-degrading enzymes.

TMA content of unwashed mince from whole fish stored in ice for various times is shown in Table 15. TMA in unwashed mince was detected at day 14 (6.86 mg N/100 g sample). After washing, much lower TMA content was obtained, regardless of washing media. Mince washed using typical process and selected process contained TMA of 0.28 and 0.19 mg N/100 g sample, respectively. Both washing processes might remove some decomposition products of nitrogeneous compounds generated during post-mortem handing and storage.

TVB content of unwashed mince increased significantly as whole fish was stored in ice with extended time (Table 15). The result indicated that the spoilage took place to a higher content when the storage time increased. TVB content of mince washed with typical process was 0.23 and 2.43 mg N/100 g sample when whole fish were stored for 7 and 14 days, respectively. When the selected washing process was used, TVB content of washed mince from fish stored for 14 days was 2.47 mg N/100 g. Thus, washing process could remove the degradation or decomposition products in fish mucle, mainly caused by microbial spoilage. TVB content has been used to indicate the spoilage in fish muscle mediated by microorganisms (Lobben and Lee, 1968).

TBARS value of unwashed and washed mince from fish stored in ice for different times is shown in Table 15. Generally, TBARS value of unwashed and washed mince obtained from both washing processes decreased markedly when storage time of fish increased. The higher TBARS at 0 day might be caused by acceleration of lipid oxidation after capture and during handling. During handling, pH reduction might lead to enhanced autoxidation mediated by hemoglobin (Tsuruga *et al.*, 1998). Gutteridge (1987) reported that human hemoglobin stimulated peroxidation of linoleic acid only slightly at pH 7.4, but the rate of oxidation increased considaerably at pH 6.5. The decrease in TBARS value in mince obtained from fish

stored for a longer time might be due to the loss in volatile oxidative compounds during extended storage (Phatcharat, 2006). Chaijan et al. (2005) reported that sardine myoglobin extractability decreased during extended iced storage. This might be associated with increased lipid oxidation. The occurrence of oxidation products, such as aldehydes, is associated with protein cross-linking (Tironi et al., 2002; Li and King, 1999). When comparing TBARS value in washed mince using two different washing processes, mince washed with selected processes had the lower TBARS value than that washed with typical process ($p<0.05$). Selected washing process might remove lipid oxidation products including aldehyde, ketone, etc. more effectively than typical washing process.

Table 15. Effect of selected washing process on TMA, TVB contents and TBARS of mince from yellowtail barracuda stored in ice for different times.

Chemical composition	Washing processes	Storage time (days)		
		0	7	14
TMA	Unwashed mince	ND	ND	6.86±0.50
(mg N/100 g sample)	Typical process (T1)	ND	ND	0.28±0.00
	Selected process (T6)	ND	ND	0.19±0.08
TVB	Unwashed mince	1.87±0.00	7.00±0.70	22.45±0.63
(mg N/100 g sample)	Typical process (T1)	ND	0.23 ± 0.40	2.43±0.08
	Selected process (T6)	ND	ND	2.47±0.08
TBARS	Unwashed mince	43.69±1.50 [cB]	8.89±0.23 [bA]	7.98±0.36 [bA]
(mg/malondialdehyde	Typical process (T1)	20.35±0.65 [bB]	4.88±0.79 [aA]	4.00±0.04 [aA]
/kg sample)	Selected process (T6)	10.48±0.22 [aC]	4.60±0.18 [aB]	3.66±0.27 [aA]

Values are means ± SD from triplicate determinations. The different lowercase superscripts in the same column within the same parameter indicate the significant differences ($p<0.05$). Different uppercase superscripts in the same row indicate the significant differences ($p<0.05$).

5.1.2 pH

Changes in pH of unwashed mince and mince washed with various washing processes prepared from fish stored in ice for 0, 7 and 14 day are shown in

Figure 23. Generally, pH of unwashed and washed mince increased as the storage time increased. This was coincidental with the increase in total volatile bases, as indicated by the increase in TVB content (Table 15). Thus, the washing process could not remove all volatile basic compounds formed during the extended storage. Selected washing process yielded the washed mince with the higher pH than typical washing process. The first washing medium containing $NaHCO_3$ might neutralize acidic compounds such as lactic acid. Furthermore, alkaline pH of this washing medium directly contributed to alkaline pH of washed mince. The pH of mince and mince washed with typical process and selected process from fish stored in ice for 14 days were 7.14, 7.21 and 7.23, respectively.

Figure 23. Effect of selected washing process on pH of mince from yellowtail barracuda stored in ice for different times. Bars represent standard deviation (n=3). Different letters on the bars within the same storage time indicate significant difference (p<0.05). Different capital letters on the bars within the same washing process indicate the significant differences ($p<0.05$).

5.1.3 Moisture content

Moisture content of unwashed mince increased as the storage time in ice increased ($p<0.05$) (Figure 24). At day 0, after washing, the increase in moisture content was found ($p<0.05$). Washing with different washing processes caused the increase in moisture content of washed mince differently. Selected washing process yielded washed mince with the lower moisture content than typical washing process

(p<0.05). Toyoda *et al.* (1992) reported that water-retention properties tended to increase concomitantly with decreasing freshness. The uptake of water by the muscle during leaching is determined by the salts content (ionic strength) and the pH of wash water and muscle proteins (Toyoda *et al.*, 1992). Damodaran (1996) reported that the water binding capacities in various groups of proteins are dependent on type and amount of amino acid residues. Amino acids with charged groups bind about 6 mol water/mol residue; the uncharged polar residues bind amount 2 mol/mol residue; and nonpolar groups bind amount 1 mol/mol residues (Damodaran, 1996). The lowering of raw material quality might cause the loosen structure of muscle, in which hydrophilic amino acids having negatively charged group, especially when glutamic acid (Glu^-) and aspartic acid (Asp^-) were exposed. These amino acids combind with Mg^{2+} presented in the last washing medium of selected washing process, leading to interaction between two protein molecules. As a result, the water was more repelled. Therefore, the use of 0.45% NaCl containing 20 mM $MgCl_2$ as the third washing media in washing process had the efficacy in dewatering of washed mince although the freshness of fish decreased.

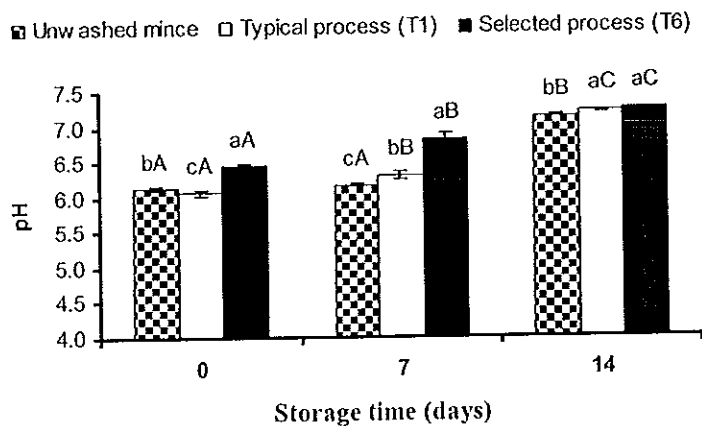Figure 24. Effect of selected washing process on moisture content of mince from yellowtail barracuda stored in ice for different times. Bars represent standard deviation (n=3). Different letters on the bars within the same storage time indicate significant difference ($p<0.05$). Different capital letters on the bars within the same washing process indicate the significant differences ($p<0.05$).

5.1.4 Whiteness

Whiteness of unwashed mince and washed mince using two washing processes prepared from fish stored in ice for 0, 7 and 14 day is shown in Table 16. Slight increases in whiteness of all samples prepared from fish stored in ice for 7 day were observed, whereas the decrease in whiteness of all samples were found when fish stored in ice for 14 days ($p<0.05$). The increase in whiteness of mince and washed mince of fish stored for 7 days might be due to the release of free water from the muscle. This resulted in the higher light scattering of mince and washed mince. Color changes in meat are mainly due to the reaction of myoglobin with other muscle components, particularly myofibrillar proteins (Hanan and Shaklai, 1995). The increase in metmyoglobin content was associated with the darkening of meats caused by the oxidation of myoglobin (Chaijan et al., 2005). Whiteness of washed mince decreased as the storage time of fish increased. This might be associated with the higher oxidation of heme proteins as well as increased Maillard reaction products, formed during the extended storage. In general, both washing processes were able to improve the whiteness of mince markedly. Selected washing process yielded washed mince with higher whiteness than typical washing process, regardless of storage time of fish ($p<0.05$). This related with the decrease sarcoplasmic protein content in the mince when the mince was washed with the selected washing process. Alkaline saline solution (0.3% NaCl + 0.2% $NaHCO_3$) used as the first washing medium of selected washing process might contribute to the swelling of myofibrillar proteins, leading to the increased solubility of sarcoplasmic proteins associated with myofibrillar proteins in the mince.

5.1.5 Sarcoplasmic protein content

Sarcoplasmic proteins content of washed mince prepared from fish stored in ice for 0, 7 and 14 day with different washing processes increased as the fish used for mince preparation were stored in ice for a longer time (Table 17). Generally, sarcoplasmic proteins in fresh fish can be removed during the washing process, leading to the higher concentration of myofibrillar proteins. However, some proteins such as heme proteins become less soluble as the fish undergo deterioration. Chen (2003) reported that ice or frozen storage decreased the myoglobin extracting efficiency in washed milkfish due to the insolubility of myoglobin caused by the

oxidation of myoglobin to form metmyoglobin. As a consequence, the surimi produced from the unfresh fish is more likely to be discolored. Hemoglobin is lost rather easily during handing and storage, while myoglobin is retained by the muscle intracellular structure (Livingston and Brown, 1981). The higher degradation of muscle proteins with increasing storage time might contribute to the increase in water soluble proteins, as indicated by the increased sarcoplasmic protein content. Selected washing process yielded washed mince with slightly lower sarcoplasmic protein content than typical washing process at all iced storage time. Sarcoplasmic proteins were removed to a greater extent with increasing washing cycle (Chaijan *et al.*, 2005). The higher amount of myoglobin removed was found in sample washed with NaCl solution, compared with that washed with distilled water (Chaijan *et al.*, 2005).

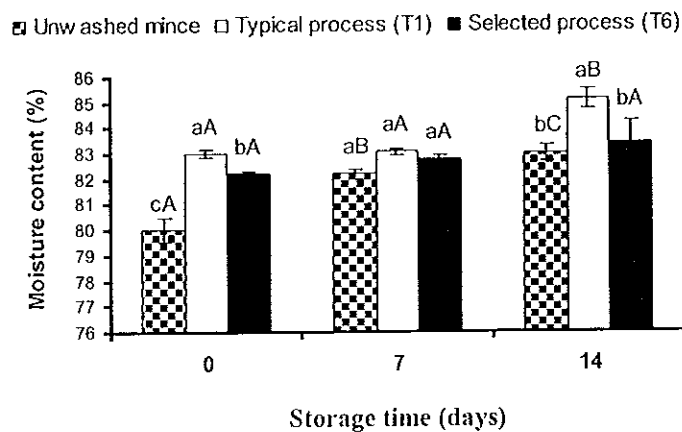**Table 16.** Effect of selected washing process on whiteness of mince from yellowtail barracuda stored in ice for different times.

Washing processes	Storage time (days)		
	0	7	14
Unwashed mince	50.73 ± 0.32^{cA}	52.97 ± 0.90^{cB}	51.43 ± 0.19^{cA}
Typical process (T1)	65.56 ± 0.85^{bB}	67.42 ± 0.64^{bC}	63.21 ± 0.74^{bA}
Selected process (T6)	67.50 ± 0.67^{aB}	68.72 ± 0.12^{aC}	65.80 ± 0.35^{aA}

Values are means $\pm$ SD from triplicate determinations. The different lowercase superscripts in the same column indicate the significant differences ($p<0.05$). Different uppercase superscripts in the same row indicate the significant differences ($p<0.05$).

5.1.6 Salt content

Salt content in washed mince prepared from fish stored in ice for 0, 7 and 14 day varied with washing processes used (Table 17). Generally, mince washed with selected process had the higher salt content of washed mince than typical washing process. Washing with typical process caused the decrease in salt content of washed mince when the fish were stored in ice for a longer time. However, washing with selected process resulted in the slight increase in salt content, when fish used for mince preparation were stored for a longer time. Those might be caused by NaCl present in washing media in the first and third washing step. NaCl in washing media

was more likely responsible for the higher retained NaCl in the washed mince. During extended storage, degradation of muscle proteins occurred. Degraded muscle might have the lower ability in binding with NaCl. As a consequence, the salt could be removed to a higher extent from the muscle during the last step of washing.

5.1.7 Fat content

Fat content of mince prepared from fish stored in ice for 0, 7 and 14 days using different washing processes is shown in Table 17. The higher fat content of washed mince was obtained at 7 and 14 day of storage when typical washing process was used ($p < 0.05$). This suggested that typical washing had the lower capacity of leaching fats or lipids, especially when the storage time increased. When lipid oxidation took place, the adduction between lipid oxidation products and protein might lead to the cross-linking of proteins or lipids in the mince. As a result, lowered leaching was presumed. Nevertheless, the gradual decrease in fat content of washed mince was observed when selected washing process was used as the fish were stored in ice for an extended period. Washing using alkaline solution was able to leach out the decomposed lipids including free fatty acids (Toyomizu et al., 1981). Therefore, different washing process affected markedly the lipid content of washed mince and the iced storage time also showed the impact on lipids retained in washed mince.

5.1.8 Thermal properties

Differential scanning colorimetric (DSC) study of washed mince from fish stored in ice for various times with different washing processes was performed to determine thermal properties of washed mince (Table 18). Two peaks were found with the maximum transition temperatures (T_{max}) of 49.50-51.83 °C and 66.66-71.33 °C, representing MHC and actin, respectively. T_{max} and enthalpy (ΔH) of myosin and actin peaks varied with washing process as well as the storage time of fish. Mince prepared from fish stored in ice for a longer time and washed with typical process had no change in T_{max} of the first peak ($p < 0.05$). However, the decrease in ΔH was observed. For the second peak, the decrease in T_{max} was found for both washing processes. The result suggested that selected washing process using $MgCl_2$ in the third washing medium resulted in the lower stability of both myosin and actin. The instability was more pronounced when the fish was kept for the extended storage time. Typical washing process using NaCl in the third washing medium caused only the lowered

stability of actin. Tseng *et al.* (2002) reported that T_{max} of red claw fish for myosin head (50.2°C) and actin (72.6°C) showed a significant decrease after 7 days of iced storage to 46.3 and 69.7°C and dropped to 39.4 and 60.3°C after 14 days of iced storage The protein conformation changes during iced storage were probably caused by proteolysis as well as chemical reaction. Lipid oxidation products also affected the stability of muscle proteins (Badii and Howell, 2002). Therefore, washing process and iced storage time of fish used for mince preparation had the influence on thermal property of muscle proteins from yellowtail barracuda.

Table 17. Effect of selected washing process on sarcoplasmic protein, salt and fat contents of washed mince from yellowtail barracuda stored in ice for different times.

Chemical composition	Washing processes	Storage time (days)		
		0	7	14
Sarcoplasmic protein	Typical process (T1)	7.27±0.72 [aA]	8.04±0.28 [aA]	11.64±0.12 [aB]
content (% dry wt. basis)	Selected process (T6)	6.37±0.02 [aA]	7.36±0.16 [aB]	10.24±0.01 [bC]
Salt content	Typical process (T1)	1.23±0.11 [aB]	1.17±0.12 [aB]	0.49±0.11 [aA]
(% dry wt. basis)	Selected process (T6)	1.86±0.09 [bA]	2.48±0.04 [bB]	2.11±0.12 [bA]
Fat content	Typical process (T1)	3.08±0.10 [aA]	3.80±0.14 [aC]	3.32±0.11 [aB]
(% dry wt. basis)	Selected process (T6)	4.17±0.27 [bC]	3.08±0.10 [bB]	2.62±0.04 [bA]

Values are means ± SD from triplicate determinations. The different lowercase superscripts in the same column within the same parameter indicate the significant differences ($p<0.05$). Different uppercase superscripts in the same row indicate the significant differences ($p<0.05$).

Table 18. Effect of selected washing process on thermal properties of washed mince from yellowtail barracuda stored in ice for different times.

Days	Washing processes	Peak I		Peak II	
		T_{max} (°C)	ΔH (J/g)	T_{max} (°C)	ΔH (J/g)
0	Typical process (T1)	51.83±0.35 [aA]	1.68±0.07 [aB]	71.33±0.00 [aC]	0.41±0.13 [aC]
	Selected process (T6)	50.83±0.24 [bB]	1.23±0.07 [bA]	69.83±0.47 [bC]	0.27±0.06 [bB]
7	Typical process (T1)	51.83±0.24 [aA]	1.52±0.05 [aA]	69.16±0.12 [aB]	0.19±0.04 [aA]
	Selected process (T6)	49.50±0.24 [bA]	1.53±0.03 [aC]	67.00±0.24 [bB]	0.31±0.00 [bC]
14	Typical process (T1)	51.83±0.47 [aA]	1.48±0.06 [aA]	68.00±0.35 [aA]	0.26±0.03 [aB]
	Selected process (T6)	49.50±0.12 [bA]	1.40±0.18 [bB]	66.66±0.47 [bA]	0.19±0.04 [bA]

Values are means ± SD from triplicate determinations. The different lowercase superscripts in the same column within the same storage time indicate the significant differences ($p<0.05$). Different uppercase superscripts in the same row within the same parameter indicate the significant differences ($p<0.05$).

5.1.9 Protein pattern

Protein patterns of mince and washed mince from yellowtail barracuda stored in ice for various times using different washing processes are presented in Figure 25. Washing can remove some soluble proteins, especially protein having MW of 36 and 55 kDa. Generally, MHC band intensity of unwashed and washed mince slightly decreased, while actin band intensity seemed to be unchanged, suggesting the greater resistance to proteolysis of actin. Yarnpakdee (2009) reported that mince from goatfish stored in ice for 9 days had the lower MHC band intensity. This result revealed that MHC was more degraded during iced storage. Benjakul *et al.* (1997) reported that MHC was more prone to proteolytic degradation than other proteins, i.e. actin, troponin and tropomyosin. Many endogenous proteases involve in proteolysis of fish muscle (Kolodziejska and Sikorski, 1996).
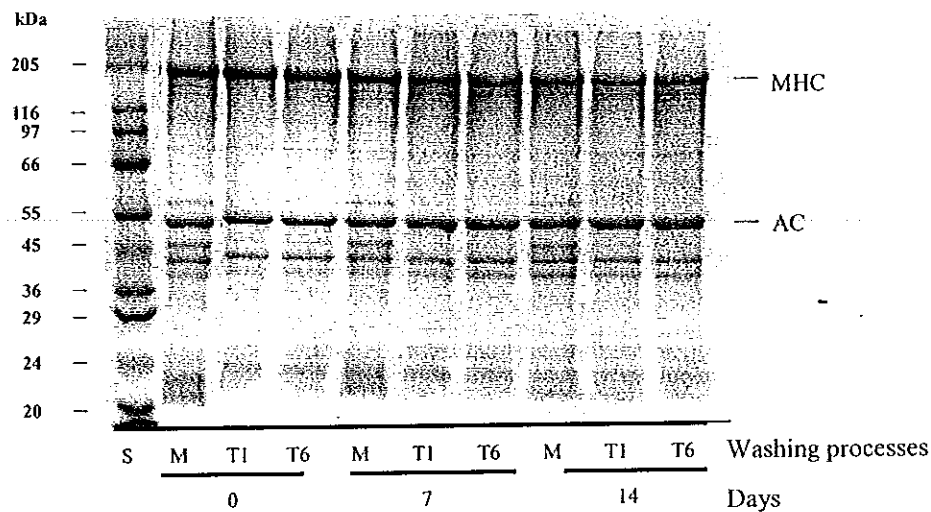
Figure 25. Effect of selected washing process on protein pattern of mince from yellowtail barracuda stored in ice for different times. M: mince; T1: Typical washing process; T6: Selected washing process.

5.2 Properties of surimi gel

5.2.1 Breaking force and deformation

Breaking force and deformation of surimi gel prepared from mince of yellowtail barracuda stored in ice for different times and washed with various washing processes are shown in Figure 26. The decrease in both breaking force and deformation of gels produced from mince washed with both washing processes was obtained when fish were stored in ice for a longer time ($p<0.05$). At the same storage time, the higher breaking force and deformation were obtained in gel prepared from mince washed with the selected washing process. Breaking force of surimi gel prepared by typical washing process decreased from 372.69 g at day 0 to 135.86 g at 14 day. Deformation decreased from 11.68 mm at day 0 to 6.64 mm at day 14. For surimi prepared by selected washing process, breaking force decreased from 437.16 g at day 0 to 262.17 g at day 14 and deformation decreased from 13.75 mm at day 0 to 9.43 mm at day 14. Benjakul et al. (2002) reported that when the storage time increased, breaking force of surimi produced from both whole and headed/eviscerated bigeye snapper decreased up to 15 days of storage. This was coincidental with the increase in protein denaturation and degradation with increasing storage time (Benjakul et al., 2002).
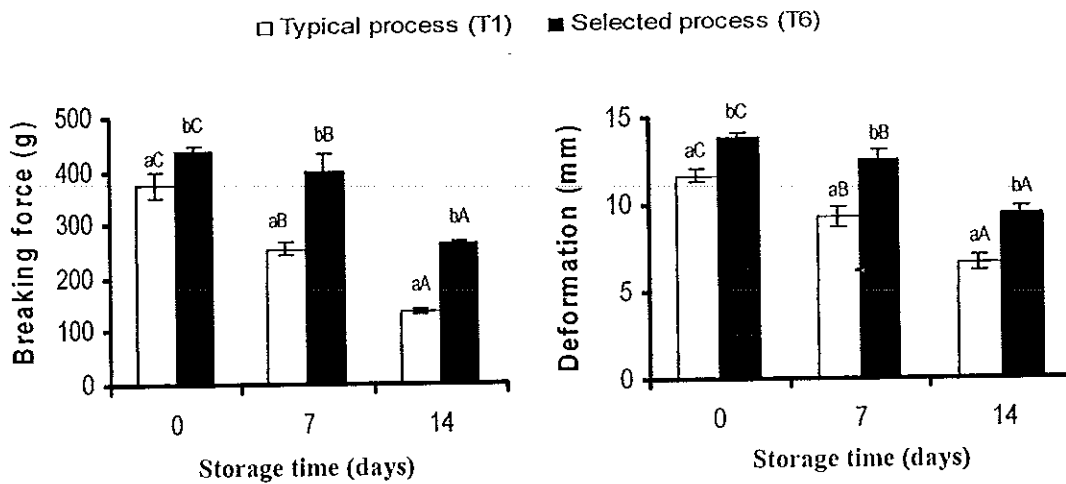
Figure 26. Effect of selected washing process on breaking force and deformation of surimi gel prepared from yellowtail barracuda stored in ice for different times. Bars represent standard deviation (n=7). Different letters on the bars within the same storage time indicate significant difference (p<0.05). Different capital letters on the bars within the same washing process indicate the significant differences ($p<0.05$).

5.2.2 Whiteness

Whiteness of surimi gels prepared from washed mince with different washing processes decreased as the storage time of fish in ice increased ($p<0.05$) (Table 19). When surimi were produced from fish stored in ice for 7 and 14 days, gels prepared from washed mince using selected process had lower whiteness than those from typical washing process. $MgCl_2$ as the third washing medium in selected washing process might induce aggregation of proteins, in which more sarcoplasmic protein including heme proteins were more retained, leading to the decrease in whiteness of gel. Benjakul *et al.* (2003) reported that whiteness of lizardfish surimi gel markedly decreased as storage time increased. During iced storage, the oxidation of pigments in fish muscle, particularly myoglobin or hemoglobin occurred (Saeed and Howell, 2002). These oxidized products possibly bound tightly with muscle proteins, especially in the presence of formaldehyde and could not be removed by washing. During extended storage, blood and liquid from internal organs in whole samples could penetrate through the muscle, especially when autolysis proceeded and caused a looser muscle structure (Benjakul *et al.*, 2003). Therefore, washing processes and

storage time of fish used as raw material production more likely contributed to whiteness of surimi gel.

5.2.3 Expressible moisture content

Expressible moisture content of surimi gels produced from fish stored in ice for different times with various washing processes is shown in Table 19. When surimi gels were produced from fish stored in ice for a longer time, the slight increase in expressible moisture content of gel was obtained when typical washing process was used, while selected washing process caused the slight decrease in expressible moisture content of gel. The increased expressible moisture content was related with the poor gel property. The ordered and strong gel was able to imbibe water in the gel matrix (Julavittayanukul, 2006). Protein degradation took place with increasing storage time might cause the decrease in expressible moisture content of gel (Benjakul *et al.*, 2002).

Table 19. Effect of selected washing process on whiteness and expressible moisture content of mince from yellowtail barracuda stored in ice for different times.

Determination	Washing processes	Storage time (days)		
		0	7	14
Whiteness	Typical process (T1)	77.16±0.44 ^{aB}	77.29±0.28 ^{aB}	75.64±0.51 ^{aA}
	Selected process (T6)	77.27±0.65 ^{aC}	76.37±0.92 ^{bB}	74.49±0.61 ^{bA}
Expressible moisture content (%)	Typical process (T1)	6.72±0.79 ^{aA}	6.93±0.83 ^{aA}	6.92±0.43 ^{aA}
	Selected process (T6)	6.39±0.96 ^{aA}	6.33±0.45 ^{aA}	5.83±0.34 ^{bA}

Values are means ± SD from triplicate determinations. The different lowercase supercripts in the same column within the same parameter indicate the significant differences ($p<0.05$). Different uppercase superscripts in the same row indicate the significant differences ($p<0.05$).

6. Effect of selected washing processes on physicochemical properties and gel-forming ability of surimi produced from yellowtail barracuda during frozen storage

Surimi prepared using different washing processes including 1) control washing processes (TC): using tap water for all three washing steps. 2) typical washing process (T1): using tap water for the 1st and 2nd step and 0.45% NaCl as the 3rd step. 3) selected washing process I (T2): using tap water for the 1st and 2nd steps and 0.45% NaCl + 20 mM $MgCl_2$ for the 3rd step and 4) selected washing process II (T6): 0.30% NaCl + 0.20% $NaHCO_3$ for the 1st step, tap water for the 2nd step and 0.45% NaCl + 20 mM $MgCl_2$ for the 3rd step.

6.1 Changes in physicochemical properties

6.1.1 Ca^{2+}-ATPase activity

No change in Ca^{2+}-ATPase activity of prepared from yellowtail barracuda mince prepared using various washing processes was obtained during storage of 12 weeks of storage at -20°C (Figure 27). During frozen storage, myofibrillar proteins, especially myosin, are susceptible to denaturation as indicated by a decrease in Ca^{2+}-ATPase activity (Benjakul and Bauer, 2000). However, loss of ATPase activity is not necessarily synonymous with aggregation because it is possible to have no aggregation and complete loss in activity if the activity site denatures (Jaczynki et al., 2006). Jiang et al. (1985) reported a decrease in Ca^{2+}-ATPase activity of mackerel and amberfish actomyosin during frozen storage. The loss in ATPase activity is due to the tertiary structural changes caused by ice crystal and an increase in the ionic strength of the system (Benjakul and Bauer, 2000). Additionally, the rearrangement of protein via protein–protein interactions also contributes to the loss in ATPase activity (Benjaukl and Bauer, 2000). However, washing of minced fish muscle prior to incorporating with cryoprotectants was also necessary to prevent denaturation during frozen storage (Fukumi, 1987). For the present study, 4% sugar and 4% sorbitol were used as cryoprtectants to prevent freeze-induced denaturation. Due to unchanged Ca^{2+}-ATPase activity, it was implied that cryoprotectants added effectively prevented the denaturation of muscle proteins in surimi during 12 weeks of storage, regardless of washing processes.

Figure 27. Ca^{2+}-ATPase activity of yellowtail barracuda surimi prepared by different washing processes during 12 weeks of frozen storage. Bars represent the standard deviation (n=3).

6.1.2 Total sulfhydryl content and disulfide bond content

Total sulfhydryl and disulfide bond contents of surimi prepared using different washing processes during 12 weeks of frozen storage are shown in Figure 28. In general, no change in total sulfhydryl content of surimi prepared from various washing processes was obtained after 3 weeks of storage ($p>0.05$). The decrease was observed at week 6 and no marked changes in sulfhydryl group were found during week 6-12 ($p>0.05$). The decrease in sulfhydryl group content during weeks 3-6 was coincidental with the increase in disulfide bond content. At week 0 of storage, T1 and T2 samples had the higher sulfhydryl group content than others ($p<0.05$), but there was no difference during week 6 and 12 of storage ($p>0.05$). The higher sulfhydryl content coincided with the lower disulfide bond content. Thus, the washing processes using different chemicals or pH might affect the induction of disulfide bond formation during washing. The oxidation of sulfhydryl groups located in the head portion of myosin occurred in fish muscle during frozen storage (Benjakul et al., 1997). Disulfide bridges occurring during frozen storage of muscle protein are associated with protein aggregation. Ramirez et al. (2000) suggested that the frozen storage of myosin in suspension resulted in aggregation involving the side-to-side interactions of the rod with a low formation of disulfide bonds. On the other hand, head-to-head interaction with a higher formation of disulfide bonds was involved in aggregation when myosin was solubilized prior to frozen storage.

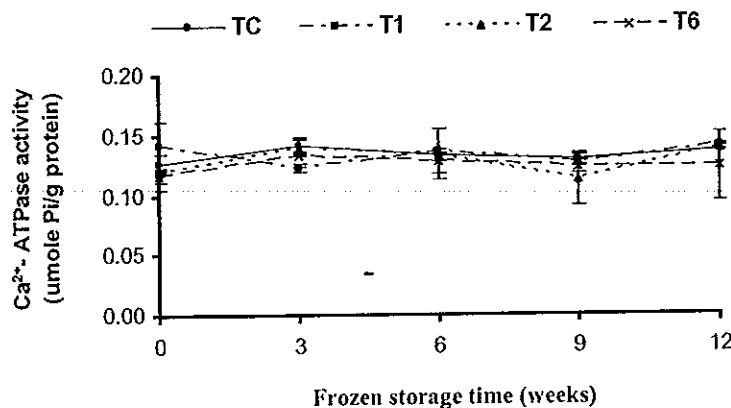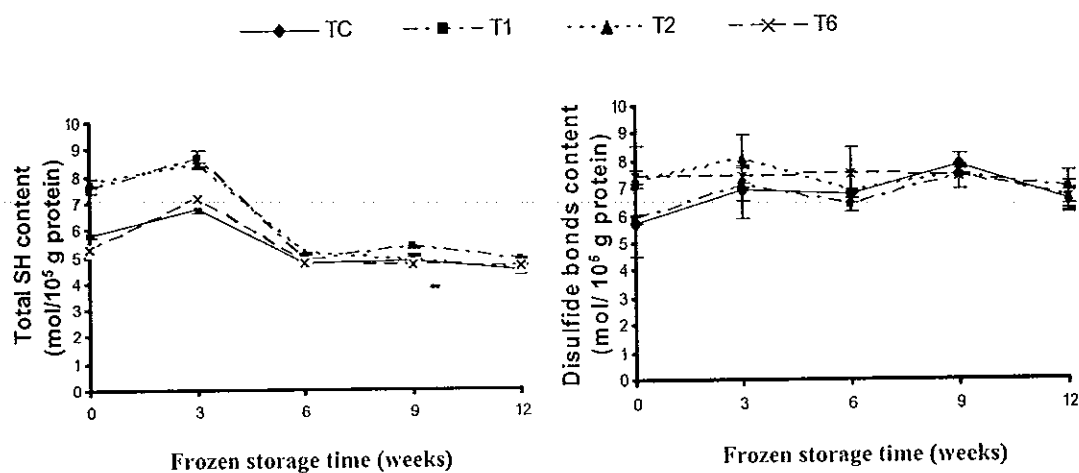**Figure 28.** Total sulfhydryl and disulfide bond contents of yellowtail barracuda surimi prepared by different washing processes during 12 weeks of frozen storage. Bars represent the standard deviation (n=3).

6.1.3 Surface hydrophobicity

Washing processes affected surface hydrophobicity of surimi to some degrees (Figure 29). Higher surface hydrophobicity of surimi produced from process T2 was observed, compared to others ($p<0.05$). The lowest surface hydrophobicity was found in TC sample ($p<0.05$). The chemical used in washing media might cause the conformational change in the way which hydrophobic domains were exposed. The increase in surface hydrophobicity was noticeable after 3 weeks of storage ($p<0.05$) for all surimi samples. The disappearance of liquid water is a major factor affecting protein changes during freezing, particularly the breakdown or change of the structure of the so-called "ordered water" in the vicinity of the hydrophobic groups of the protein molecules. This leads to a change of the native protein structure. Such conformational changes may be supported by the concomitant influence of various factors such as electrolyte concentrations (Jaczynki et al., 2006). Hydrophobic interactions are generally weakened as the temperature decreases. Hence, lowering the temperature to subfreezing temperatures will destabilize proteins whose native structures are critically maintained by hydrophobic forces (Privalov et al., 1986; Franks, 1995). As freezing progresses, proteins are exposed to increased ionic strength in the nonfrozen aqueous phase, which then leads to extensive modification of the native protein structure (Park et al., 1987; Lin and Park, 1998). The effect of salts on

the secondary forces (i.e., noncovalent), which stabilize the tertiary and quaternary conformation of the protein molecule, could be responsible for protein denaturation (Park, 1994). After 3 weeks of storage, the decrease in surface hydrophobicity was obtained, suggesting the interaction of proteins via hydrophobic interaction or other bondings. The aggregation of protein could imbed the hydrophobic domain in the aggregate. It was noted that there was on marked changes in surface hydrophobicity in all surimi samples during 6-12 weeks of storage. Furthermore, TC sample tended to have the lower surface hydrophobicity throughout the storage of 12 weeks. Thus, washing process affected the conformation of muscle proteins to some degrees as indicated by the differences in surface hydrophobicity.

Figure 29. Surface hydrophobicity of yellowtail barracuda surimi prepared by different washing processes during 12 weeks of frozen storage. Bars represent the standard deviation (n=3).

6.1.4 TBARS

Both washing processes and storage time affected TBARS value of frozen surimi (Figure 30). The higher TBARS value was found in surimi produced by process T2 throughout 12 weeks of storage, compared to that of surimi produced by other washing processes ($p<0.05$). It was suggested that process T2 using 0.45% NaCl contaning 20 mM $MgCl_2$ as the third washing medium could enhance lipid oxidation. Thanonkaew (2006) reported that the rate of TBARS formation varied, depending on concentration, type and valency of metal ion. No changes in TBARS value were found

during the first 3 weeks of storage ($p>0.05$). When frozen storage time increased, TBARS value of surimi produced from all washing processes increased ($p<0.05$). Lipid degradation products, such as aldehydes, etc. are capable of cross-linking polypeptides and are responsible for the generation of insoluble protein aggregates (Buttkus, 1970). Free fatty acid (FFA) in frozen muscle is mostly derived from enzymatic hydrolysis of lipids, particularly the phospholipids, since many lipases remain active in frozen muscle tissue (Olley et $al.$, 1962). FFA is able to bind to proteins and cause an increase in the hydrophobicity of proteins (Xiong, 1997a). Both oleic (C18:1) and myristic (C14:0) acids could bind to the hydrophobic regions of fish actomyosin during frozen storage, resulting in major losses of ATPase activity, solubility and viscosity of the protein (Careche and Tejada, 1994). In addition, the carbonyl groups of oxidized lipids participate in covalent bonding, leading to the formation of stable protein–lipid aggregates (Saeed and Howell, 2002). The reaction of proteins with lipid oxidation products results in the formation of protein-centered radicals (Saeed et $al.$, 1999). Therefore, washing processes had an impact on lipid oxidation taken place in resulting surimi during the frozen storage.
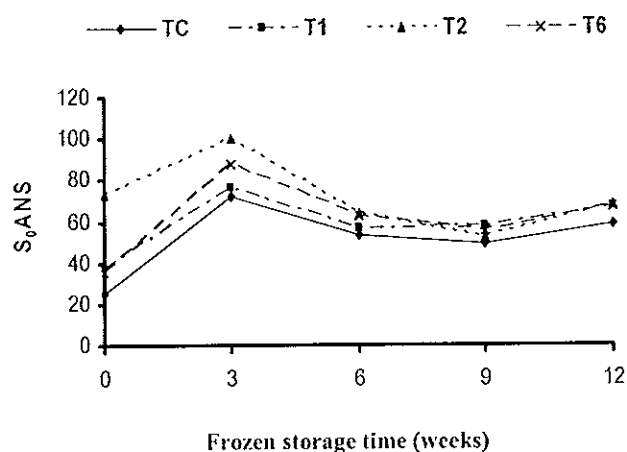

Figure 30. TBARS of yellowtail barracuda surimi prepared by different washing processes during 12 weeks of frozen storage. Bars represent the standard deviation (n=3).

6.1.5 Solubility

The solubility of surimi prepared by various washing processes during frozen storage is shown in Figure 31. Among various surimi, the highest solubility was

observed in solubility of surimi prepared by process T2 and the lowest solubility was found in surimi prepared using process T6 ($p<0.05$). Decrease in solubility of all samples was noticeable as frozen storage time increased, however the degree of decrease varied. The lowest change in solubility was observed in surimi prepared using process T6, while the largest change was found in surimi prepared by process T2. The results suggested that extractability of fish muscle proteins with 0.6 M KCl decreased when frozen storage time increased. Tamoto (1971) reported that the type of salts affected the stability of actomyosin in surimi during frozen storage at -30°C. The highest denaturation rate was reported in surimi washed with $CaCl_2$ and the lowest rate was found in surimi with no salts. As ice crystallization takes place, extracellular salt becomes concentrated, leading to differences in osmotic pressure gradient across cell membrane (Xiong, 1997a). Due to such differences, intracellular moisture flows outward. This causes the dehydration of muscle cell and an increase in ionic strength of the cell. The increased ionic strength in unfrozen phase is associated with the disruption of electrostatic interaction in protein molecules, leading to the denaturation or dissociation of protein molecules (Benjakul and Visessanguan, 2011). Washing with salts might cause the increased ionic strength of cell, contributing to the denaturation and aggregration of muscle proteins. This resulted in the decreased solubility of surimi.

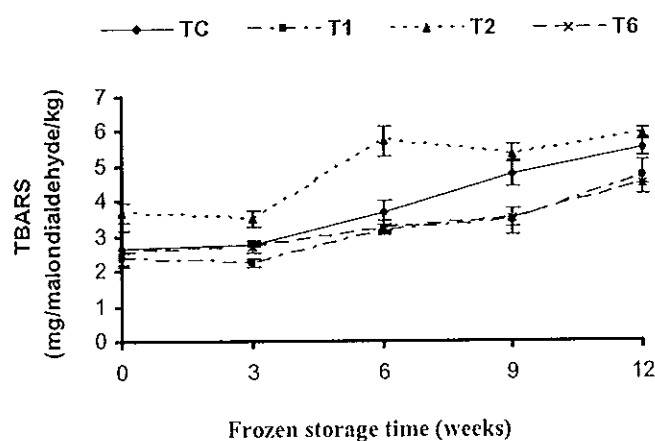Figure 31. Solubility of yellowtail barracuda surimi prepared by different washing processes during 12 weeks of frozen storage. Bars represent the standard deviation (n=3).

6.2 Properties of surimi gel

6.2.1 Breaking force and deformation

Breaking force and deformation of gels from surimi prepared using different washing processes during frozen storage of 12 weeks are shown in Figure 32. Among all samples, surimi prepared using process T6 showed the higher breaking force throughout the storage of 12 weeks ($p<0.05$). No marked changes in breaking force were observed within the first 6 weeks of storage for all samples ($p>0.05$). Thereafter, a slight decrease in breaking force was observed up to 12 weeks of storage. Similar results were found for deformation. After 12 weeks of storage, breaking force of surimi gel decreased by 17.03, 25.42, 20.24 and 15.29 % and deformation of surimi gel decreased by 5.89, 9.16, 5.36 and 7.86 %, for surimi prepared using TC, T1, T2 and T6 processes, respectively. The loss in textural quality of surimi gel might relate to the increased denaturation of proteins as well as the increase in TBARS value of surimi. This might result in the aggregation of muscle protein as evidenced by the decreased solubility (Figure 31). Crystallization could disrupt the water-mediated hydrophobic interactions, which contribute to protein stabilization (Sikorski and Kolakowska, 1990). Polymerization and protein aggregation resulting from oxidized lipids in lipid–protein systems also contributes to decreased protein solubility (Karel, 1973; Karel et al., 1975). Solubility of protein is prerequisite for gelation, in which the solubilized proteins can undergo the aggregation to form ordered network.
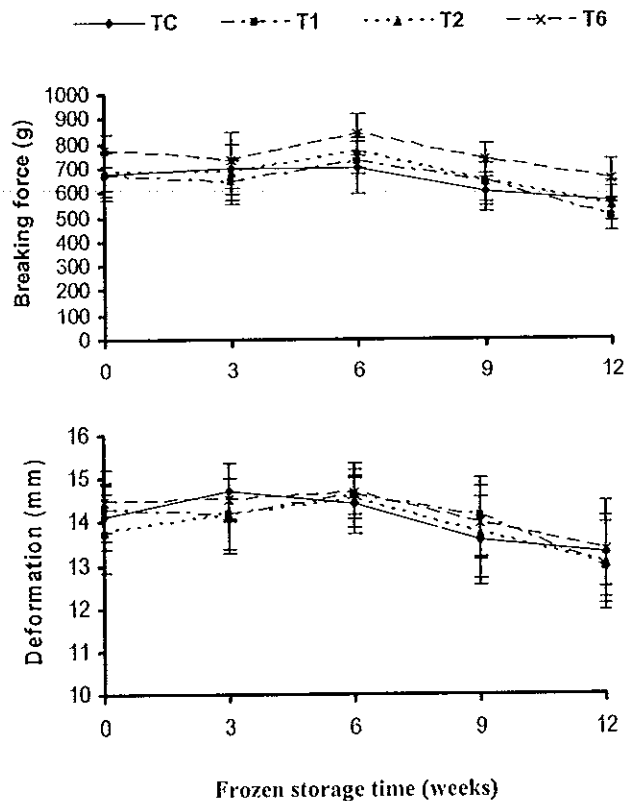
Figure 32. Breaking force and deformation of gels from yellowtail barracuda surimi prepared by different washing processes during frozen storage of 12 weeks.

6.2.2 Whiteness

There was no change in whiteness of surimi gel produced using various washing processes during a prolonged frozen storage (Figure 33). Suvanich *et al.* (2000) reported that washed mince was lighter in color, less redness and yellowness than unwashed mince. Color of both mince did not change during storage. Nakayama and Yamamoto (1977) studied the color stability of deboned flesh from several underutilized fish species during 6 months of storage at -20°C. The deboned flesh of pollock became less dark, whereas the flesh of the shortspine thornyhead, turbot and dogfish revealed a gradual shift to more yellowness. Chemical groups affecting fish flesh color are hemes, carotenoids and melanins (Pearson and Dutson, 1994). Color of fish mince is affected by different constituents in whole fish. Greyness arises from melanins and red or red/brown from blood and dark muscles (Hutching, 1994). Pigments in darker meat are especially vulnerable to oxidation, which causes deep

yellow or brown discoloration during handling, chilling, and frozen storage (Pearson and Dutson, 1994). High myoglobin content in dark muscle darkens from oxidation and turns green from reaction of trimethylamineoxide with cysteine (Govindarajan, 1973; Hutching, 1994). Thus, unwashed mince can be more susceptible to oxidation with higher discoloration compared with washed mince. When washing processes were implemented, those pigments as well as other enhancing factor for color changes were removed. Washing minced flesh has a beneficial effect on color by increasing lightness and decreasing redness (Nakayama and Yamamoto, 1977; Jahncke et al., 1992). As a consequence, no obvious changes in whiteness were found during the storage of 12 weeks.
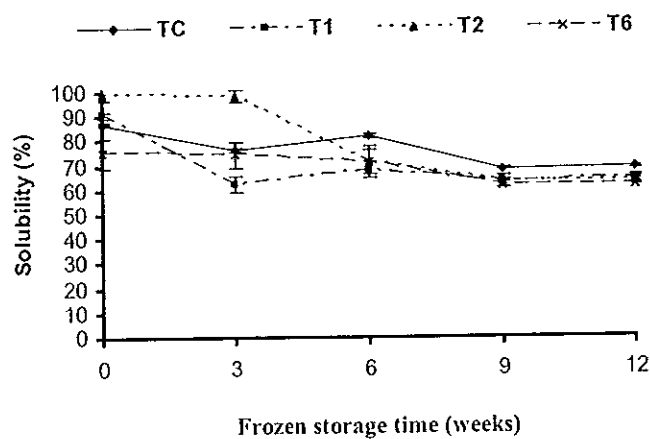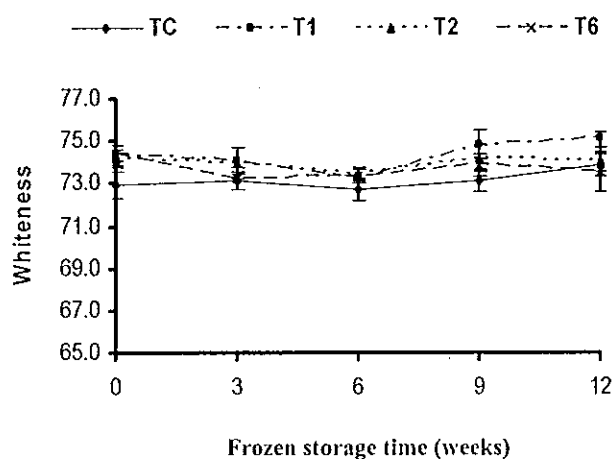

Figure 33. Whiteness of gels from yellowtail barracuda surimi prepared by different washing processes during 12 weeks of frozen storage. Bars represent the standard deviation (n=3).

6.2.3 Expressible moisture content

During frozen storage, surimi with various washing processes had slight changes in expressible moisture content (Table 20). At 0 week of storage, surimi with TC washing process tended to yield the gel with the highest expressible moisture content, compared to that of other washing processes. The result indicated that the presence of sodium chloride in the last washing medium might improve the water holding capacity of surimi gel. Sodium chloride in the last washing medium was able to remove other proteins such as sarcoplasmic proteins, which exhibited the interfering effect on gelation of myofibrillar proteins. In general, when storage time increased, the

higher expressible moisture content of surimi gel was observed for surimi with various washing processes up to week 6. Tejada *et al.* (1995) reported that brittleness of hake muscle during frozen storage coincided with two separate events, loss of salt-soluble protein and water holding capacity, both of which relate to protein denaturation. Formation of disulfide bonds played an important role in textural deterioration of frozen red hake (Lian *et al.*, 2000). In the present study, disulfide bonds were formed, especially in the first 6 weeks. This might contribute to the protein cross-linking, which was associated with the loss in water holding capacity. Nevertheless, the lowered expressible moisture content was found in all surimi gel after 6 weeks of frozen storage. The decreased expressible moisture content of surimi gel during that peroid of frozen storage might be caused by the lowered hydrophobic domains, in which more water could be bound to a higher extent to the proteins. In general, washing process had no marked effect on the water holding capacity of gel from surimi stored for a longer time at -20°C.

6.2.4 Microstructure

Microstructures of gels from surimi of yellowtail barracuda prepared using various washing processes at week 0 and 12 of storage at -20°C are illustrated in Figure 34. At week 0, surimi from processes T2 and T6 yielded more ordered and finer network of surimi gel than those prepared using processes TC and T1. It was noted that T6 surimi gel had the larger strand of network, suggesting the stronger gel. The use of alkaline washing process (T6) might facilitate the removal of other proteins from surimi. The concentrated myofibrillar proteins underwent the aggregation more effectively and the ordered network with the stronger and larger strand was developed correlated to the highest breaking force in this surimi gel. After 12 weeks of storage, the irregular networks with the larger voids were observed for all gels. This was coincidental with the decrease in breaking force and deformation (Figure 32) as well as the increased expressible moisture content of gel. Factors possibly causing freeze-denaturation are ions and ice crystals, chemical interactions of proteins, and binding of fatty acids and lipid oxidation products (Sikorski and Sun Pan, 1994). The rate of moisture migration between different regions in a complex food, and the accretion of ice crystals, is controlled by the diffusivity of water (Le Meste *et al.*, 1995), which may vary with moisture content, solute concentration or structural integrity. The extent

of diffusion of water molecules from myofibrillar proteins determines both loss of water holding ability and protein aggregation, resulting in changes in gel structure and stability during frozen storage. Freeze drip and development of rubbery texture are the main objectionable textural changes found in frozen surimi-based products (Lee and Chung, 1989)

Table 20. Expressible moisture content (%) of gels from yellowtail barracuda surimi prepared by different washing processes during 12 weeks of frozen storage.

Storage times	Washing processes			
(weeks)	TC	T1	T2	T6
0	5.63 ± 0.31 [a]	5.25 ± 0.58 [a]	5.18 ± 0.98 [a]	5.17 ± 0.33 [a]
3	6.03 ± 0.79 [a]	6.36 ± 1.07 [a]	6.68 ± 1.11 [a]	6.28 ± 1.21 [a]
6	7.62 ± 0.76 [a]	7.85 ± 0.85 [a]	7.80 ± 0.76 [a]	7.37 ± 0.82 [a]
9	6.01 ± 0.65 [a]	6.30 ± 0.93 [a]	6.62 ± 0.65 [a]	6.49 ± 0.76 [a]
12	6.61 ± 1.33 [a]	6.18 ± 0.80 [a]	6.25 ± 0.44 [a]	5.78 ± 0.90 [a]

Values are means $\pm$ SD (n=3).

Different superscripts within the same row indicate significant differences ($p < 0.05$).

Week 0 Weeks 12

TC

T1

T2

T6
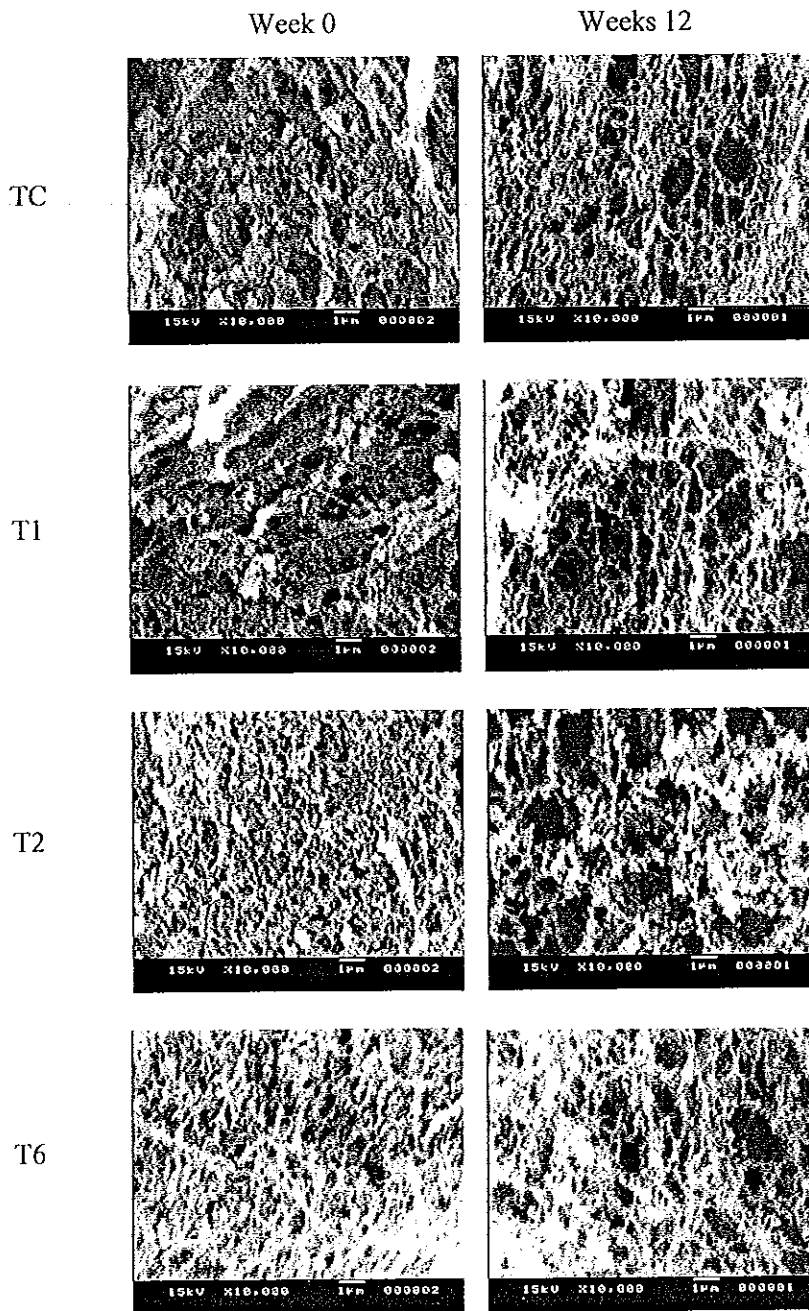
Figure 34. Microstructure of gels from yellowtail barracuda surimi prepared by different washing processes at week 0 and 12 of frozen storage. Magnification: 10,000x.

CHAPTER 4

CONCLUSIONS

1. Yellowtail barracuda mince was good source of proteins, especially myofibrillar protein. However, excessive moisture content of washed mince was still obtained after dewatering process. Typical washing process using 0.45% NaCl as the third washing media could improve dewatering of washed mince and gel-forming ability of surimi gel to some degree.

2. To enhance the dewatering of washed mince and properties of surimi gel, washing with 0.45% NaCl containing 20 mM $MgCl_2$ as the third washing media yielded the gel with the highest breaking force for both direct and two-step heating, in which the breaking force increased by 46 and 33%, respectively, compared to that of typical washing process.

3. Washing mince with the selected washing process involving 0.30% NaCl + 0.20% $NaHCO_3$/tap water/0.45% NaCl + 20 mM $MgCl_2$ yielded surimi with the increase in breaking force by 33%, compared to that of surimi gel prepared by typical washing process.

4. Selected washing process could enhance dewatering and improve breaking force and deformation of gel prepared from yellowtail barracuda, regardless of iced storage time.

5. During frozen storage at -20°C, surimi prepared using selected washing process showed the higher breaking force and deformation throughout the storage of 12 weeks, compared to that of typical washing process.

Future Research

1. Effect of appropriate mixture including NaCl, $MgCl_2$ and $CaCl_2$ as the third washing media on dewatering and gel-forming ability of yellowtail barracuda should be further studied.

2. The use of $NaHCO_3$ incorporated with polyphosphate compounds as the first washing medium on chemical compositions and properties of gel prepared from yellowtail barracuda should be further investigated.

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APPENDIX

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 by Kjeldahl method (AOAC, 1999)

Reagents

- Kjeldahl catalyst: Mix 9 parts of potassium sulphate (K_2SO_4) with 1 part of copper sulphate (Cu_2SO_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 = conversion 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

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

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 filter paper 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°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

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

5. Quantitation of protein (Lowry *et al.*, 1951)

Reagent

- A: 2% sodium carbonate in 0.1 N NaOH
- B: 0.5% $CuSO_4.5H_2O$ in 1% sodium citrate

- C: 2 N Folin phenol reagent diluted with deionized water (1:1 v/v)
- D: 50 ml reagent A + 1 ml reagent B
- Standard reagent : Bovine serum albumin (BSA) at concentration of 1 mg/ml

Method

1. Add 2 ml of reagent D to 200 µl of the standards and unknown and vortex immediately.
2. Incubate precisely for 10 min at room temperature.
3. Add 0.2 ml of reagent C (previously dilute 1: 1 with distilled water) and vortex immediately.
4. Incubate the mixture for 30 min at room temperature not longer than 60 min.
5. Read the absorbance at 750 nm (glass cuvate).
6. Plot standard curve and calculate the unknown.

Standard

- Bovine serum albumin (BSA) at concentration of 1 mg/ml (used to determine protein concentration).
- Tyrosine at a concentration of 1 mM
- Standard volumes (µl) : 0, 20, 40, 60, 100, 140, and 200

Standard curve of Lowry's procedure

Std. volume (µl)	Tyrosine (µl)	Distilled water (µl)
0	0	200
20	20	180
40	40	160
60	60	140
100	100	100
140	140	600
200	200	0

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

Reagent

- protein molecular weight standards

- 30% Acryamide - 0.8% bis Acrylamide

- Sample buffer: Mix 30 ml of 10% of SDS, 10 ml of glycerol, 5 ml of β-Mercaptoethanol. 12.5 ml 0f 50 mM Tris – HCl, pH 6.8, and 5-10 mg Bromophenol blue (enough to give dark blue color to the solution). Bring the volume to 100 ml with distilled water. Divide into 1 ml aliquot and store at -20°C

- 2% (w/v) Ammonium persulfate

- 1% (w/v) SDS

- TEMED (*N, N, N'N'-* tetramethylenediamine)

- 0.5 M Tris- HCl, pH 6.8

- 1.5 M Tris- HCl, pH 8.8

- 0.1 M EDTA

- Electrode buffer: Dissolve 3 g of Tris, 14.4 g of glycine and 1 g of SDS in distilled water. Adjust to pH 8.3. Add distilled water to 1.1.

- Staining solution: Disslove 0.04 g of Coomassie blue R-250 in 100 ml of methanol. Add 15 ml of glacoial acetic acid and 85 ml of distilled water.

- Destaining solution I: 50% methanol- 7.5% glacial acetic acid.

- Destaining solution II: 5% methanol- 7.5% glacial acetic acid.

Method

Pouring the separating gel:

1. Assemble the minigel apparatus according to the manufacture's detailed instructions. Make sure that the glass and other components are rigorously clean and dry before assembly.

2. Mix the separating gel solution as shown in Table.

3. Transfer the separating gel solution by using a Pasture pipettes to the center of

4. sandwich, which is about 1.5 to 2 cm from the top of the shorter (front) glass plates.

5. Cover the top of the gel with a layer of water by gently squirting the water against the edge of one of the spacers. Allow the resolving gel to polymerized fully (usually 30-60 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 comb into the layer of stacking gel solution by placing one corner of the comb into the gel and slowly lowering the other corner in. Allow the stacking gel solution to polymerize 30 to 45 min at room temperature.

Reagents	10%running gel	4% stacking gel
30% Acrylamide-bis	1.167 ml	0.4 ml
1.5 M Tris-HCl buffer, pH 8.8	0.875 ml	-
0.5 M Tris-HCl buffer, pH 6.8	-	1.0 ml
10%SDS	0.35 ml	0.3 ml
Distilled water	0.7585 ml	0.9 ml
0.1M EDTA	-	0.8 ml
2% Ammonium persulfate	0.35 ml	0.4 ml
TEMED	6 μl	5 μl

Sample preparation:

1. Weigh 3 g of sample and homogenize with 5% (w/v) SDS in final volume of 30 ml.

2. Incubate the mixture at 85°C for 1 h.

3. Centrifuge at 3,500×g for 5 min at ambient temperature and collect the supernatant.

Loading the gel:

1. Dillute the protein with sample buffer at a ratio of 1:4 (v/v) in microcentrifuge tube and boil for 1 min at 100°C

2. Remove the comb without tearing the edge of polyacrylamide wells.

3. Fill the wells with electrode buffer.

4. Place the upper chamber over 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 syring with a flate-tipped needle, load the protein sample into the wells by carefully applying the sample as a thin layer at the bottom of well.

7. Fill the remainder of the upper buffer chamber with additional electrode buffer.

Runing the gel:

1. Connect the power supply to the anode and cathode of the gel apparatus and run at 50 V and 150 V.

2. After the bromophenol blue tracking dye has reached the bottom of the separating gel, disconnect the power supply.

Dissembling 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 apacers out from the edge of the sandwich along its entire length.

3. Insert a spectula between the glass plates at one corner where the spacer is, 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 or 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 1 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 about 15 min.

3. Pour off the destaining solution I and cover the destaining solution II. Discard destaining solution and replace with fresh solution. Repeat unit the gel is clear except for the protein bands.

7. Quantitation of protein (Copeland *et al.*, 1994)

Reagent

- 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 plastic bottle for storage.

- Distilled water

- Standard reagent: 10 mg/ml bovine serum albumin (BSA)

Method

1. Standard volumes (0, 100, 200, 300, 400 and 500 µl) were prepared.

2. For the unknown protein samples, add 500 µl of the samples and mix the contents of each tube well.

3. Add 2.0 ml of the biuret reagent to each tube and mix well.

4. Incubate the mixture at room temperature for 30-45 min, then read the absorbance of each tube at 540 nm.

5. Using the average absorbance for the three samples of unknown, read the concentration of sample from the plot.

Preparation of standard curve of the Biuret's assay

Tube number	Water (µl)	10 mg/ml BSA (µl)	Effective 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

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

Reagents

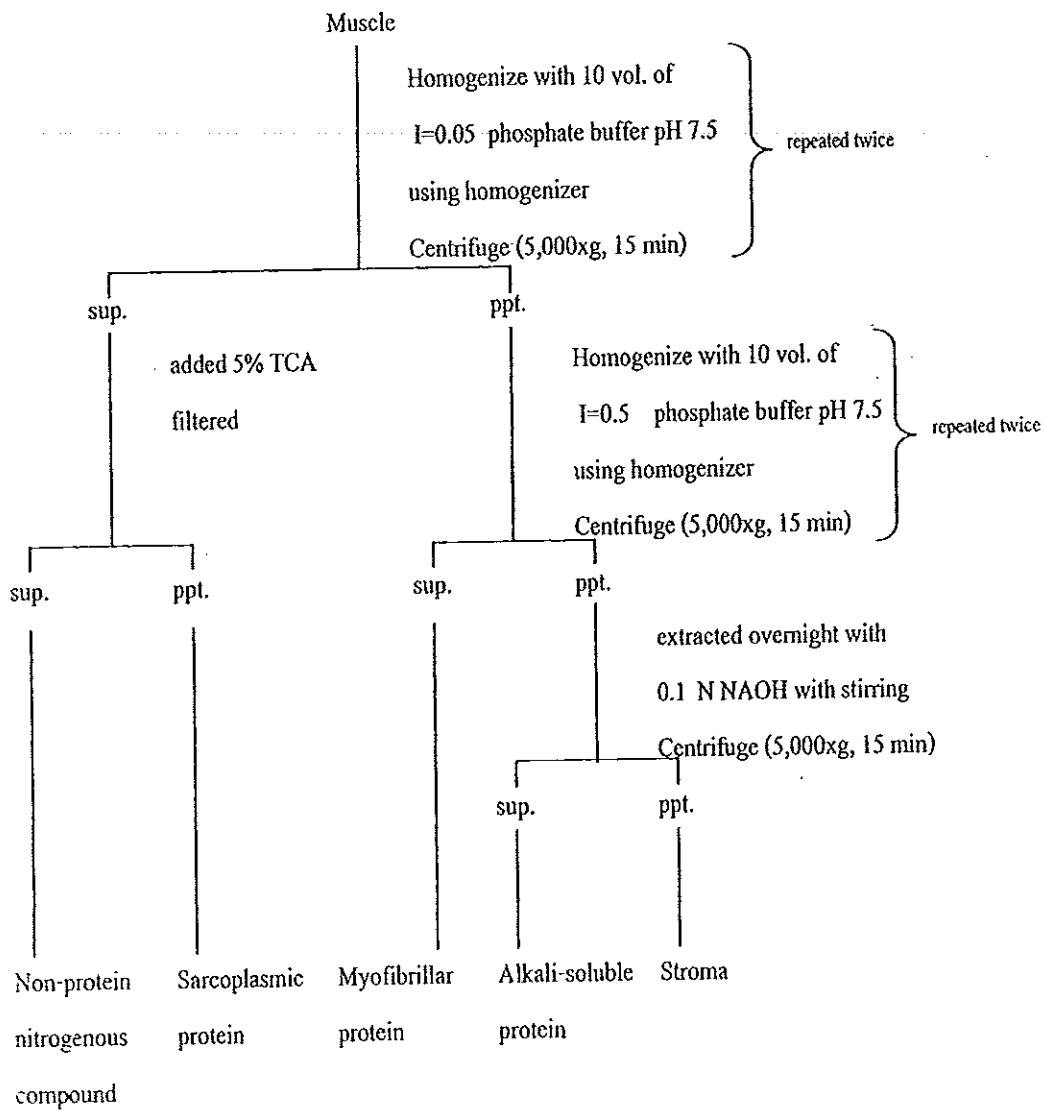- 0.6 M KCl, pH 7
- Distilled water

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.

9. Fractionaion of muscle proteins (Hashimoto *et al.*, 1979)

Reagents

- 0.05 M phosphate buffer, pH 7.5
- 0. 5 M KCl phosphate buffer, pH 7.5
- Trichloroacetic acid
- 0.1 N NaOH

Method

Muscle

Homogenize with 10 vol. of

I=0.05 phosphate buffer pH 7.5

using homogenizer

Centrifuge (5,000xg, 15 min) ⎫ repeated twice

sup.   ppt.

added 5% TCA

filtered

Homogenize with 10 vol. of

I=0.5 phosphate buffer pH 7.5

using homogenizer

Centrifuge (5,000xg, 15 min) ⎫ repeated twice

sup.   ppt.   sup.   ppt.

extracted overnight with

0.1 N NAOH with stirring

Centrifuge (5,000xg, 15 min)

sup.   ppt.

Non-protein   Sarcoplasmic   Myofibrillar   Alkali-soluble   Stroma

nitrogenous   protein   protein   protein

compound

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List of Proceedings

Lertwittayanon, K. and Benjakul, S. 2010. Effect of type of salts in washing media on dewatering and properties of yellowtail barracuda surimi. Research Conference. Prince of Songkla University, Pattani campus. July 3.