Effects of Some Hydrocolloids on the Properties and the Changes during Frozen and Refrigerated storage of Gels from Bigeye Snapper Mince and Surimi

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ชื่อวิทยานิพนธ์	ผลของไฮโดรคอลลอยด์บางชนิดต่อสมบัติและการเปลี่ยนแปลงระหว่างการเก็บรักษาในสภาพแช่แข็งและการเก็บรักษาที่อุณหภูมิต่ำของเจลเนื้อปลาบดและซูริมิจากปลาตาหวาน
ผู้เขียน	นางสาวศศิกัญญา ทวนเกรียงไกร
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ปีการศึกษา	2550

บทคัดย่อ

จากการศึกษาผลของไฮโดรคอลลอยด์ชนิดต่างๆ (แซนแทนกัม โลกัสบีน กัม ไอโอตา คาราจีแนน และแป้งดัดแปร (Gelpro HC30, Gelpro HC 715 และ Bestesk) ที่ระดับต่างๆ (ร้อยละ 0, 2 และ 5 น้ำหนัก/น้ำหนัก) ต่อสมบัติของเจลเนื้อปลาตาหวานหนังหนาบดที่ผ่านการแช่แข็งทำละลายจำนวน 1, 3 และ 5 รอบ พบว่า ค่าแรงเจาะทะลุและระยะทางก่อนเจาะทะลุและปริมาณของเหลวจากการบีบอัดเพิ่มขึ้นเมื่อจำนวนการแช่แข็งทำละลายเพิ่มขึ้น การเติมไอโอตาคาราจีแนน และ ไฮดรอกซีโพรพิเลท ไดสตาร์ชฟอสเฟต (Gelpro HC30) ที่ระดับร้อยละ 2 สามารถชะลอการเปลี่ยนแปลงสมบัติของเจลระหว่างการแช่แข็งทำละลายได้

จากการศึกษาผลของ Gelpro HC30 ที่ระดับต่างๆ ร้อยละ 0, 1, 2 และ 3 (น้ำหนักต่อน้ำหนัก) ต่อสมบัติทางกายภาพ เคมี และทางประสาทสัมผัสของเจลเนื้อปลาบดและซูริมิที่ผ่านการแช่แข็งทำละลาย 1, 3 และ 5 รอบ พบว่า ค่าแรงเจาะทะลุและระยะทางก่อนเจาะทะลุของเจลที่ไม่เติม Gelpro HC30 เพิ่มขึ้นเมื่อจำนวนรอบของการแช่แข็งทำละลายเพิ่มขึ้น เจลที่เติม Gelpro HC30 ที่ระดับร้อยละ 1 มีการเปลี่ยนแปลงของค่าแรงเจาะทะลุและระยะทางก่อนเจาะทะลุและปริมาณของเหลวจากการบีบอัดน้อยที่สุด เมื่อศึกษาโครงสร้างทางจุลภาคของเจลจากเนื้อปลาบดและซูริมิที่มีการเติม Gelpro HC30 ที่ระดับร้อยละ 1 พบว่า เจลมีโครงข่ายที่เป็นเส้นใยซึ่งมีการจัดเรียงตัวเป็นระเบียบ เมื่อเปรียบเทียบกับเจลที่ไม่มีการเติม Gelpro HC30 ภายหลังผ่านการแช่แข็งทำละลายสำหรับทุกจำนวนรอบ จากการศึกษารูปแบบโปรตีนโดยใช้ SDS-PAGE และการละลาย พบว่า พันธะไดซัลไฟด์เป็นพันธะที่มีบทบาทสำคัญต่อการจับรวมตัวของโปรตีนระหว่างการแช่แข็งทำละลาย

การศึกษาการเปลี่ยนแปลงทางเคมี-กายภาพของเจลเนื้อปลาบดและซูริมิในระหว่างการเก็บรักษาที่อุณหภูมิ-18 องศาเซลเซียสเป็นเวลานาน 10 สัปดาห์ พบว่า ค่าแรงเจาะทะลุและระยะทางก่อนเจาะทะลุของเจลเนื้อปลาบดและซูริมิเพิ่มขึ้นเมื่อเก็บรักษานานขึ้น ($p<0.05$) ซึ่ง

สัมพันธ์กับการลดลงของความสามารถในการละลายโปรตีนในสารละลายโซเดียมโดเดซิลซัลเฟต ร้อยละ 2 และการเพิ่มขึ้นของความสามารถในการละลายของโปรตีนในสารละลายโซเดียมโดเดซิล ซัลเฟตร้อยละ1 ยูเรีย 8โมลาร์ และเมอร์แคปโตเอทธานอลร้อยละ2 ปฏิกิริยาออกซิเดชันของไขมัน ซึ่งวัดด้วยปริมาณ TBARS มีแนวโน้มเพิ่มขึ้นเมื่อการเก็บรักษานานขึ้น เมื่อพิจารณาโครงสร้างทาง จุลภาคของเจลซูริมิที่มีการเติม Gelpro HC30 พบว่าเจลซูริมิมีลักษณะละเอียดมากกว่าและมีช่องว่าง ขนาดเล็กกว่าเมื่อเปรียบเทียบกับเจลเนื้อปลาบดที่มีการเติม Gelpro HC30 ที่ระดับเดียวกัน การเติม Gelpro HC30 ที่ระดับร้อยละ 1 สามารถรักษาโครงสร้างเจลได้ซึ่งสอดคล้องกับค่าการยอมรับทาง ประสาทสัมผัสที่สูงกว่า

เมื่อพิจารณาคุณภาพของเจลเนื้อปลาบดที่เก็บรักษาภายใต้การดัดแปลงบรรยากาศ ต่างๆ (คาร์บอนไดออกไซด์ ร้อยละ 60, ไนโตรเจนร้อยละ 35 และ ออกซิเจน ร้อยละ 5; คาร์บอนไดออกไซด์ ร้อยละ 60, ไนโตรเจนร้อยละ 30 และ ออกซิเจน ร้อยละ 10; คาร์บอนไดออกไซด์ ร้อยละ 80, ไนโตรเจนร้อยละ 15 และ ออกซิเจน ร้อยละ 5; คาร์บอนไดออกไซด์ ร้อยละ 80, ไนโตรเจนร้อยละ 10 และ ออกซิเจน ร้อยละ 10) ระหว่างการเก็บ รักษาที่อุณหภูมิ 4 องศาเซลเซียส พบว่าการยับยั้งการเจริญของแบคทีเรียเป็นสัดส่วนโดยตรงกับ ความเข้มข้นของก๊าซคาร์บอนไดออกไซด์ ปริมาณของจุลินทรีย์ในชุดที่เก็บรักษาภายใต้การ ดัดแปลงบรรยากาศมีค่าต่ำกว่าตัวอย่างที่เก็บภายใต้บรรยากาศปกติ เจลเนื้อปลาบดที่เก็บภายใต้การ ดัดแปลงบรรยากาศมีปริมาณของด่างระเหยที่ระเหยได้ทั้งหมดต่ำกว่า อย่างไรก็ตามปฏิกิริยา ออกซิเดชันของไขมันซึ่งวัดค่าด้วยTBARS มีปริมาณสูงกว่าเมื่อเปรียบเทียบกับตัวอย่างที่เก็บภายใต้ บรรยากาศปกติ ตัวอย่างที่ไม่มีการเติม Gelpro HC30 และเก็บภายใต้การดัดแปลงบรรยากาศมี ปริมาณน้ำอิสระเพิ่มขึ้น อย่างไรก็ตามการเติม Gelpro HC30 ที่ระดับร้อยละ 1 สามารถลดปริมาณ น้ำอิสระ ดังนั้นเจลเนื้อปลาบดที่เติม Gelpro HC30 ร้อยละ 1 และเก็บภายใต้การดัดแปลงบรรยากาศ ที่มีคาร์บอนไดออกไซด์ร้อยละ 60 หรือร้อยละ 80 ที่อุณหภูมิ 4 องศาเซลเซียสมีอายุการเก็บรักษา นาน 15 วัน

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ABTRACT

Effects of different hydrocolloids (xanthan gum, locust bean gum, iota carrageenan and modified starches including Gelpro HC30, Gelpro HC715 and Bestek at various levels (0, 2 and 5% (w/w)) on the properties of gel from bigeye snapper mince subjected to different freeze-thaw cycles (0, 1, 3 and 5) were investigated. Breaking force, deformation and expressible moisture content increased with increasing freeze-thaw cycles. Addition of 2% iota carrageenan or 2% hydroxypropylated distarch phosphate (Gelpro HC30) could retard the changes in gel properties induced by freeze-thawing.

Effects of Gelpro HC30 at various levels (0, 1, 2 and 3 % w/w) on the chemical, physical, textural and sensory properties of mince and surimi gels subjected to different freeze–thaw cycles (0, 1, 3 and 5) were elucidated. Breaking force and deformation of control gel increased with increasing freeze-thaw cycles. Gels added with 1% Gelpro HC30 showed the least changes in breaking force, deformation and expressible moisture content. Scanning electron microscopic study revealed that gel from both mince and surimi added with 1% Gelpro HC30 had the finer matrix with smaller strands, compared with the control gel at all freeze-thaw cycles used. From the SDS-PAGE and solubility study, disulfide bond played an important role in protein aggregation during freeze-thawing process.

Physicochemical changes of mince and surimi gels during storage at -18°C were investigated for up to 10 weeks. Breaking force and deformation of mince and surimi gels increased as storage time increased ($p<0.05$). This was concomitant with the decrease in solubility in 2% SDS and the increase in 1% SDS+8M urea+2%βME. Lipid oxidation as monitored by thiobarbituric acid reaction substance (TBARS) increased as storage time increased. Scanning

electron microscopic study revealed that surimi gels added with 1% Gelpro HC30 was finer with a smaller void, compared with that of mince gels added with the same level of Gelpro HC30. Addition of 1% Gelpro HC30 could maintain the gel structure to some extent, which was in accordance with higher likeness tested by 9-point hedonic scale.

Quality of mince gels stored under different modified atmosphere packaging (MAP) (60%CO_2, 35%N_2, 5%O_2; 60%CO_2, 30%N_2, 10%O_2; 80%CO_2, 15%N_2, 5%O_2 and 80%CO_2, 10%N_2, 10%O_2) during storage at 4°C was monitored. Inhibitory effect on bacterial growth increased proportionally to the CO_2 concentration. Lower bacterial count was found in all samples, compared with the sample kept in air. Mince gels stored in CO_2 enriched atmosphere had lower TVB content. Nevertheless, lipid oxidation as monitored by thiobarbituric acid reaction substance (TBARS) was enhanced in samples kept under MAP, compared with those stored in air. Slight increases in exudate loss were observed for sample without Gelpro HC30 packaged in CO_2 enriched atmosphere. Nonetheless, the negative effect of CO_2 on exudate could be minimized with the addition of 1% Gelpro HC30. Thus, atmosphere containing 60% or 80%CO_2 could extend the shelf-life of mince gels containing 1% Gelpro HC30 for up to 15 days at 4°C.

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Sasikunya Tuankriangkrai

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CHAPTER 1

INTRODUCTION

The prime concern in surimi manufacturing is to maintain functionality of the myofibrillar protein mainly involved in gel formation. Frozen storage is widely used in the surimi industries for processing and long-term preservation. Nevertheless, the biochemical changes during frozen storage is inevitably associated with the reduction of the gelation properties of surimi and such reduction is attributable to the denaturation of myofibrillar protein (Noguchi and Matsumoto, 1970; Matsumoto, 1979). To prevent such an unwanted change or to improve the functionality of frozen meat, a variety of denaturation-inhibiting substances, such as protein hydrolysates, amino acids, beef plasma protein, soybean protein, sucrose, polyphosphate, sorbitol, and starch have been applied for surimi processing (Hossain *et al*., 2003; Park, 1994). The protective mechanisms of such additives have been explained by the hydration and stabilization of protein molecular structure (Hanafusa, 1973). Freeze-thawing process can also promote protein denaturation and lipid oxidation, which may affect the texture of fish muscle (Benjakul and Bauer, 2000). Deterioration of muscle proteins during frozen storage depends on many factors including species, storage temperature, time and enzymatic degradation (Hsieh and Regenstein, 1989). Application of low temperature, both refrigeration and freezing, allows extension of the shelf-life of many foods by slowing the rate of chemical reactions and inhibiting microbial growth (Cilla *et al*., 2006). The use of vacuum or modified atmospheres in seafoods and seafood products can affect the quality of the product throughout the storage period in different ways (Church and Parsons, 1995; Reddy *et al.,* 1992; Stammen *et al*., 1990). Spoilage is due to microbial activity, which can be related to production of the characteristic spoilage odors and flavors, and also to the activity of autolytic enzymes in the fish tissue that have a major impact on textural deterioration (Truelstrup *et al*., 1996).

A large variety of seafood analogues have been developed by modifying the functional and textural properties of surimi proteins through the addition of biopolymers with gel-forming or water holding capacity (Okada, 1963). Food hydrocolloids could modify the gelling capacity of myofibrillar proteins. This influences the formation of the continuous matrix by

interacting with muscle components (mainly water and proteins) during the formation of the paste or during the heat-induced formation of the continuous matrix. Some additives are considered to interact with the proteins to form a more structured system, while other acts as filler, just binding water and modifying the viscosity of the system (Lee *et al*., 1992). Hydrocolloids have the advantage of improving gel strength at low concentrations, producing negligible increase in caloric content and sweetness, and reducing fat absorption during frying (de-Ponte *et al*. 1987). Furthermore, some hydrocolloids, particularly the modified starch, can be used to improve the stability of gel product, particularly during the extended frozen storage. However, a little information regarding the impact of hydrocolloids on the gel properties of mince or surimi from tropical fish as well as the stability during refrigerated and frozen storage has been reported. The information gained can be applied to improve the quality and stability of gel produced from mince and surimi. Thus, the shelf-life of gel and gel products can be maximized.

Literature Review

1. Chemical composition of fish

The main constituents of fresh fish are water (65-80%), protein (15-24%), fat (0.1-22%), carbohydrate (1-3%) and inorganic substances (0.8-2%) (Suzuki, 1981a). The relative amounts of these components are generally within the range found in mammals (Mackie, 1994). Protein is a major composition of fish muscle ranging from 15 to 20% (wet weight), but protein content is reduced in spawning period (Almas, 1981). Protein compositions of fish vary, depending upon muscle type, feeding period and spawning, etc. (Suzuki, 1981a).

Muscle proteins can be divided into the following categories (Kijowski, 2001):

- location in the structure of muscle and of muscle fiber
- physicochemical properties, e.g., solubility
- functionality in regard to further processing of meat.

Muscle fiber consists of thick and thin filaments, M-line, Z-line, and N-line (Figure 1). Muscle proteins form three large groups of proteins of different positions in the muscle and different solubilities, i.e., 1) myofibrillar proteins, 2) connective tissue or stromal proteins, and 3) sarcoplasmic proteins (Kijowski, 2001).

1.1. Myofibrillar protein

Myofibrillar proteins form the largest group, accounting for 55-60% of the total quantity of muscle protein. Myofibrils constituted 80% of fiber volume (Xiong, 1997b). The majority of myofibrillar proteins can be isolated from comminuted muscle tissue using a salt solution of $\geq$ 0.6 ionic strength, but they are not soluble at low ionic strength.

Based on physiological and structural role of proteins in the muscle of a live animal, they can be further divided as follows:

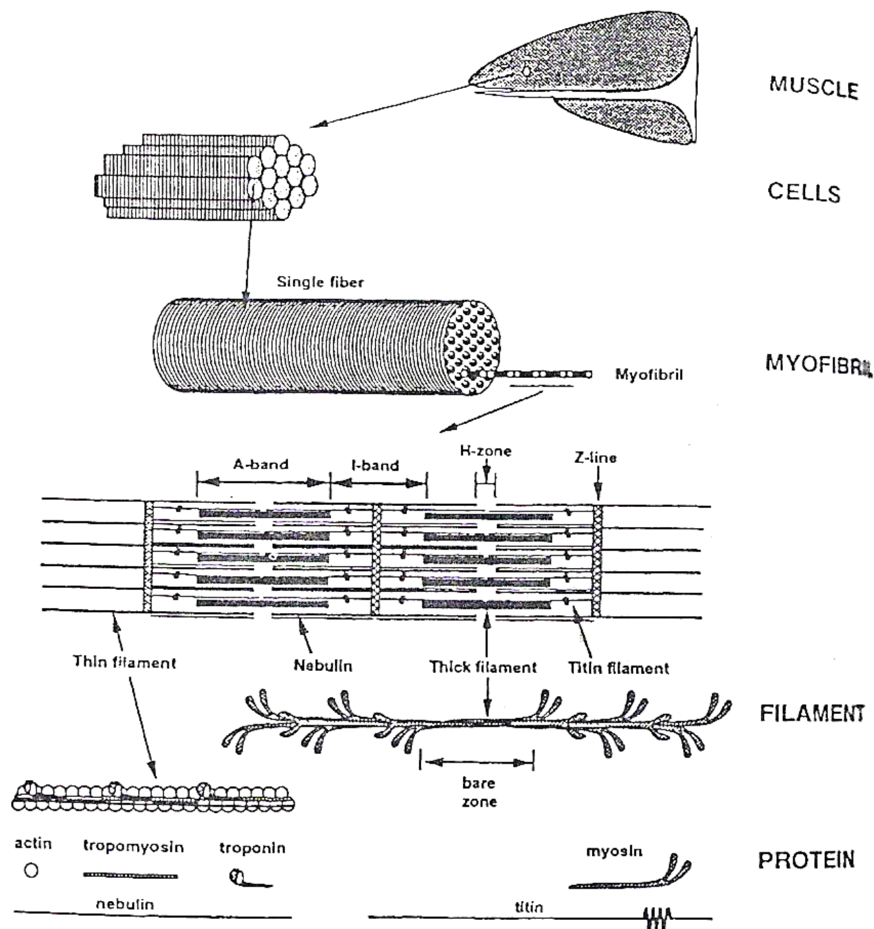


Figure 1. Myofibrillar protein structure

Source: Greaser (1997)

- Myosin

Myosin can be extracted with KCl solution of concentration higher than 0.16 mol/dm^3 from myofibrillar muscle structure after previous removal of water-soluble protein. Simultaneous extraction of actin can be prevented by $MgCl_2$ and ATP or pyrophosphate addition (Kijowski, 2001). Myosin is susceptible to aggregation due to oxidation of thiol groups. The addition of ethylenediaminetetraacetic acid (EDTA) and β-mercaptoethanol can prevent its aggregation. In the presence of sodium dodecylsulfate (SDS), myosin dissociates into subunits of high and low molecular weight (Kijowski, 2001).

Myosin is large molecule of fiber-like form with molecular weight of 500 kDa. In the meat of mammals, bird, and fish, it predominates and accounts for around 45% of myofibrillar proteins (Maruyama, 1985; Robinson, 1995). When myosin is digested by trypsin or

chymotrypsin for a short period, myosin is divided into two components, a rapid sediment component called H-meromyosin (HMM), and a slow sediment called L-meromyosin (LMM). When HMM is treated with papain, it is divided into a head and a neck part. A head is called S-1 and the neck part is S-2 (Suzuki, 1981a) (Figure 2). The myosin head contains the actin binding site, ATP site, alkali light chain site, and DTNB [(5,5-dithiobis)-2-(nitrobenzoic acid)] light chain site. The light chains bind to the alpha-helical regions of the heavy chain. The tail portion of the heavy chain molecule is responsible for its association into thick filaments (Foegeding *et al*., 1996).

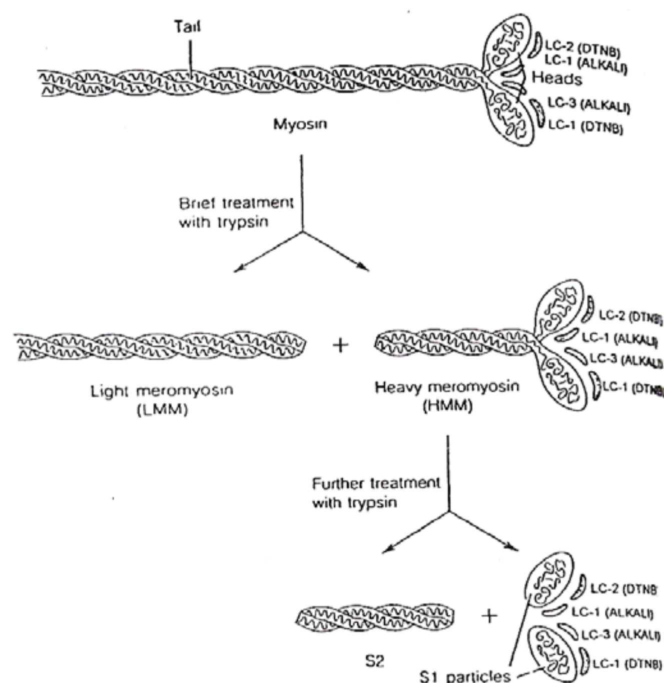


Figure 2. Myosin molecule and the generation of LMM, HMM, S1, S2 subfragments

Source: Kijowski (2001)

- Actin

Actin is the second major myofibrillar proteins and accounts for above 20% of their weight. It belongs to the contractile proteins of the muscle fiber. Actin is present in all eukaryotes and is a component of thin filaments. It occurs in the muscle in a polymerized F-actin fibrillar form. In solutions of low ionic strength, actin is present as a monomer of 42 kDa in the

so-called G-actin globular form. Actin also possesses ATP-ase activity, however it does not cause muscle contraction but participates in polymerization and depolymerization of filaments (Stryer, 1995). Actin molecule contains 376 amino acid residues with the high content of proline and glycine. The high content of those two amino acids is presumably responsible for the α-helix structure and globular shape of the molecule. The isoelectic point of that protein is 4.8. The thin filament in the muscle is composed of 400 actin molecules. In the muscle, actin is naturally bound to tropomyosin and troponin complex as shown in Figure 3. Actin also has a binding site to myosin, facilitating formation of complexes during muscle contraction and the rigor mortis phase (Kijowski, 2001).

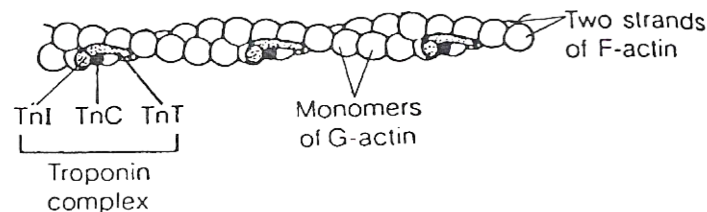


Figure 3. Protein of thin filament

Source: Kijowski (2001)

- Tropomyosin

Tropomyosin molecule has asymmetrical structure. It consists of two subunits, α and β, with molecular weight of 34 and 36 kDa, respectively, and demonstrates α-helical structure twisted into a superhelix (Kijowski, 2001). Tropomyosin accounts for around 5% of myofibril. It contains many acidic and basic amino acid residues and a low proline level. In physiological conditions, it binds to F-actin at 1:7 stoichiometric ratio (G-actin), binds to troponin at 1:1 ratio, and regulates the activity of myosin ATP-ase. The shift of tropomyosin in the actin folds due to binding or releasing calcium ion by troponin results in masking or exposing the actin active center that participates in myosin binding (Kijowski, 2001). Formation of actomyosin complex is not feasible under masking of the center. Tropomyosin demonstrates a tendency toward head-to-tail polymerization of the molecules, and it shows a high resistance to denaturation (Kijowski, 2001).

- Troponin

Troponin accounting for 8-10 % of myofibrillar proteins consists of three subunits such as troponin C, which is a calcium binding protein and confers calcium regulation to the contraction process via the thin filament; troponin I, which strongly inhibits ATPase activity of actomyosin; and troponin T, which provides a strong association site for binding of tropomyosin (Foegeding *et al*., 1996).

1.2 Sarcoplasmic protein

Sarcoplasmic proteins, or myogen, are soluble in water or low molarity saline (50<mM) solutions. These proteins constitute 30-35% of the total muscle protein. They include a large number of proteins such as myoglobin, enzymes and other albumins. The content of sarcoplasmic proteins is generally higher in pelagic fish species as compared with demersal fish (Suzuki, 1981a). Sarcoplasmic enzymes are responsible for quality deterioration of fish after death. These include glycolytic and hydrolytic enzymes (Shahidi, 1994).

1.3 Stroma protein

Stroma is the protein, which forms connective tissue, representing approximately 3 % of total protein content of fish muscle. It cannot be extracted by water, acid, or alkali solution and neutral salt solution of 0.01-0.1 M concentration. The component of stroma is collagen, elastin or both (Suzuki, 1981a). Elastin is very resistant to moist heat and cooking. Normally, it is a reflection of the different structural arrangements of muscle cells in fish, compared to mammals (Mackie, 1994).

2. Functional properties of fish protein

2.1 Gel-forming ability

Fish myofibrillar protein has excellent functional characteristics such as emulsifying properties, gel-forming ability, and water holding capacity (Tanabe and Saeki, 2001; Lin and Park, 1996). Generally, fish myofibrillar protein is thermally and chemically less stable

than that of other vertebrates (Yamashita *et al*., 1978; Hashimoto *et al*., 1982) and its functional properties are generally lowered when protein denaturation occurs (Regenstein *et al*., 1983). The functional property of muscle protein varies with the product, processing method, and stage of processing (Xiong, 1997b; Smith, 1988). Additionally, the functional properties of proteins are governed by primary, secondary, tertiary and quaternary structural components of the molecules (Pomeranz, 1991).

2.2 Water holding capacity

The ability of proteins to bind water and fat and retain them during heating and storage is essential in the manufacture of processed meats. These water and fat binding properties largely determine the cooking yield, but also have a considerable influence on the sensorial properties which represent an important aspect of the final product quality and thus of consumer acceptance (Nakai and Li-Chan, 1988). Together with tenderness, juiciness of meat is considered to be the most important quality attribute of meat products by the consumers (Xiong, 2005). The water present in meat can be divided into bound and free water. Bound water is associated with the hydrophilic groups of proteins and accounts for 8 to 10% of the total water (Nakai and Li-Chan, 1988). Free water on the other hand is held by capillary forces and surface tension and largely depends on protein structure. The three-dimensional filament network in myofibrils provides voids that are suited for water to be immobilized (Zayas, 1997). Factors that can change the interfilamental spacing directly influence the water holding capacity of meat. Increasing the electrostatic charges, which can be done by adding salt or moving the pH further away from the protein isoelectric point, enlarges the repulsive forces between myofilaments and induces myofibril swelling, thus resulting in an increased water retention (Samejima *et al*., 1985; Xiong, 1997b). Cross-linking of myosin and actin on the other hand is thought to constrain myofibril swelling and thus reduces the water holding capacity of meat (Offer and Trinick, 1983). Similarly, some regulatory and cytoskeletalproteins, presenting structural myofibril constraints, may limit the immobilization of free water in meat. Heating of meat can cause important structural changes like denaturation and subsequent aggregation of myofibrillar proteins, shrinkage of the myofibrils, and as a consequence a tightening of the microstructure (Zayas,

1997). The extent of this tightening and the decrease in water holding capacity depends on the temperature and duration of the applied heat treatment.

2.3 Solubility

Most functional properties of myofibrillar proteins are closely related to protein solubility. Protein solubility is a prerequisite step for emulsification, gelation, and water retention (Xiong, 1994). It can be defined as the percentage of total protein that goes into solution under specified conditions and that is non-sedimentable by a specified centrifugal force (Zayas, 1997). Myofibrillar proteins are often referred to as salt-soluble meat proteins due to the fact that salt solutions with a minimal ionic strength of 0.5 M are required for their extraction (Offer and Trinick, 1983; Xiong, 1993). As these conditions are usually met in processed meats, protein solubilization and extraction take place and result in the desired product functionalities. The presence of a tiny amount of salt was found to be sufficient to increase the protein surface charges, leading to increased protein-water interactions (Stanley *et al*., 1994; Stefansson and Hultin, 1994; Stone and Stanley, 1994). The main factors determining the solubility and extractability of myofibrillar proteins appear to be certain constraints that prevent the myofibrils from dissociating (Xiong, 1997b). Protein extraction can be achieved by disrupting these physical constraints, thus allowing the dissocation of myofibrils. In meat processing, disruption of the myofibrillar structure is usually achieved by the addition of salt and phosphates, an increase in pH, and a prolonged mixing and tumbling time (Samejima *et al*., 1985; Wang and Smith, 1992; Xiong, 1992, 1997b; Zayas, 1997). The solubility of a protein under a given set of environmental conditions is the thermodynamic manifestation of the equilibrium between protein-protein and protein-solvent interactions (Lawrence *et al*., 1986). It is related to the net free energy change arising from the interactions of hydrophobic and hydrophilic residues of the protein with the surrounding aqueous solvent. The lower the average hydrophobicity, the higher the solubility is obtained (Damodaran, 1996). Decrease in solubility during frozen storage and loss of ATPase activity was described for myosin in frozen fish (Li-Chen *et al*., 1985). Since the solubility in high ionic strength media of the protein is impaired with the progress of protein denaturation, the solubility of muscle proteins in salt solutions has been used to assess the quality of fish meat

(Regenstein *et al*., 1983; Akahane *et al*., 1984). The textural properties depend largely on protein solubility, especially in the case of fish (Colmenero and Borderias, 1983).

3. Surimi and its gelation

Surimi is concentrated myofibrillar protein from fish muscle produced by continuous processing steps of heading, deboning, mincing, washing and dewatering, and then mixing with cryoprotectants, such as sugar, sorbitol, and phosphate to improve frozen shelf-life (Park and Lin, 2005). The washing and dewatering process refines fish myofibrillar proteins from the sarcoplasmic proteins and undesirable materials such as fat, blood, pigments, and odorous substances. Therefore, high quality surimi is naturally odorless and colorless (Pipatsattayanuwong *et al*., 1995). Fish muscle proteins tend to lose their gel-forming ability during frozen storage. High quality surimi can only be made from fish whose myofibrillar proteins are not denatured (Matsumoto, 1979; Suzuki, 1981b; Acton *et al*., 1983; MacDonald *et al*., 1990). Fresh surimi from Pacific whiting was found to have higher gel strength than frozen surimi (Pipatsattayanuwong *et al*., 1995). Additionally, gel-forming ability of surimi made from fresh fish in good condition does not change significantly for up to one year when held at a constant temperature below -20°C (Iwata *et al*., 1971).

A gel consists of a tree-dimensional lattice of macromolecules or their aggregates that immobilize within its structure the solvent, different solutes, and the filling material. Three-dimensional structure is responsible for the elasticity and the textural strength of the gel. In a proteinaceous food gel, the lattice is formed by different type of macromolecules. The interactions of macromolecules of different proteins or proteins and polysaccharides may, in some cases, decrease the lattice forming ability in the system. Some of the component may behave only like a filling material (Sikorski, 2001).

Generally, gelation is a two-step process. The first step is usually a dissociation of the quaternary structure of the protein and unfolding of the molecule. Gelation of myofibrillar proteins requires ionic strengths greater than 0.5 which is typically achieved upon addition of 2-3% salt. As solubility is the key factor governing gelation, the environmental conditions have to assure that myofibrillar proteins are dissolved (Xiong, 1994). Salt induces myofibril dissociation and produces an extract that is composed of sarcoplasmatic proteins and myofibrillar components

(Xiong and Brekke, 1990). Myofibrillar protein gelation is a process that requires heat since thermal denaturation and subsequent aggregation is a prerequisite for the formation of a three-dimensional gel network (Smith *et al*., 1988; Xiong and Blanchard, 1994; Zayas, 1997). There are mainly four types of bonds which contribute to the building of a network structure during the gelation of a surimi paste: salt linkages, hydrogen bond, disulfide bond, and hydrophobic interactions (Niwa, 1992). A generalized scheme for thermal gelation is shown in Figure 4.

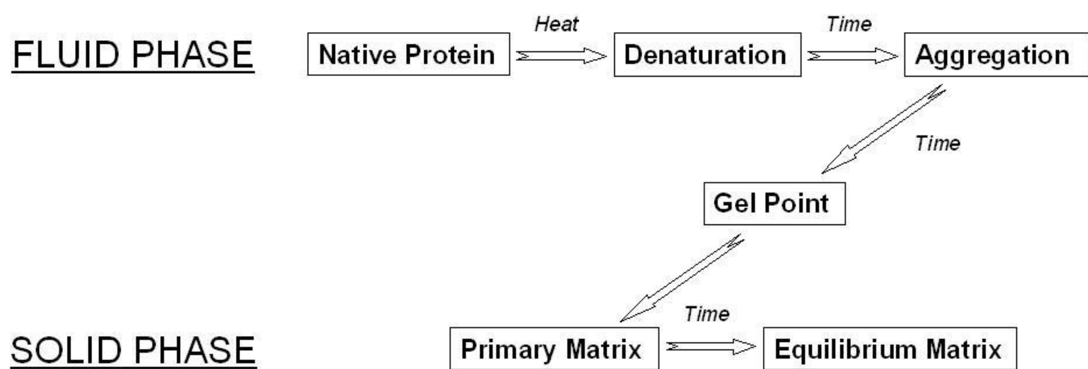


Figure 4. Thermal gelation process of myofibrillar proteins

Source: Foegeding and Hamann (1992)

As described by Ziegler and Acton (1984), the different myofibrillar proteins undergo major conformational changes upon heating. Around 38°C, F-actin, that has a double-stranded helical structure, dissociates into single chains. The myosin light chains disconnect from the heavy chains at approximately 40°C, inducing conformational changes in the head and hinge region of the myosin molecules (Ziegler and Acton, 1984). Around 50°C, the actomyosin complex dissociates, followed by a helix-coil transition of the light meromyosin chains in the temperature range between 50 and 55°C (Ziegler and Acton, 1984).

During heating of surimi pastes, the proteins unfold, exposing reactive surfaces of neighboring protein molecules, which then interact to form intermolecular bonds (Lanier, 2000). Chan *et al*. (1992) reported that thermal aggregation of fish myosin was coincidental with an increase in the surface hydrophobicity of the unfold domains of myosin molecules and was affected by the temperature at which these domains unraveled. Extent of aggregation for fish myosin seems to depend on the amount of hydrophobic surface exposed on the head portion (Chan *et al*., 1992; Wicker *et al*., 1986). 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. Covalent bonds such as disulfide bonds are dominant when heating at high temperatures (>40°C) (Lanier, 2000). However, a variety of crosslinks, including ε-(γ-glutamyl)lysine [ε-(γ-Glu)Lys] crosslinks and crosslinks through aldol condensation, are believed to be closely related to texture (Sakamoto *et al*., 1995). The formation of ε-(γ-Glu)Lys crosslinks by the catalytic action of transglutaminase (TGase) have been reported (Ikura *et al*., 1980; Motoki and Nio, 1983; Kurth and Rogers, 1984; Kato *et al*., 1991).

3.1 Salt linkages and hydrogen bonds

The intermolecular salt linkages which occur between charged amino acids of the protein chain are thought to play an important role in the stabilization of the network structure of various food gels. Additionally, there are many amino acids capable of forming intermolecular hydrogen bonds. Tyrosine, serine, hydroxyproline, and threonine residues contain a hydroxyl group, and the proline and hydroxyproline residues contain an imino group, both of which act as proton donors and acceptors. Glutamine and asparagine residues both contain a large number of carbonyl groups in the polypeptide chain (Niwa, 1992). A large amount of water molecules is hydrogen bonded to the polar amino acid residues, which are abundantly exposed on the molecular surface of the proteins (Niwa, 1992).

3.2 Disulfide bonds

Disulfide interchange occurs during heating of the surimi paste. Intramolecular S-S bonds are converted to intermolecular S-S bonds. This is because surimi gels are strengthened not only by addition of oxidant prior to heating, but also by the addition of reducing agents such as ascorbic acid and cysteine (Itoh *et al*., 1980). The aggregation of head portions of the myosin molecules, in which the sulfhydryl groups are located, is mainly through disulfide bond formation. This is followed by cross-linking of the rod portion of myosin molecules which accompanies the conversion of the α-helix to a random coil. Such a contribution of the sulfhydryl groups to gelation was suggested from the finding that the thermally induced increase in rigidity and turbidity of myosin subfragment S1 and heavy meromyosin (HMM) solution was suppressed upon the addition of dithiothreitol, and that SH content was remarkably decreased

upon heating the S1 fragments (Samejima *et al*., 1981). The formation of S-S bonds is more intensive for carp (Itoh and Ikeda, 1979) and Atlantic croaker actomyosin (Liu *et al*., 1983) at the higher temperatures of cooking (80°C or above) than at the lower temperatures at which setting occurs. Benjakul *et al*. (2001b) reported that disulfide bonds were found in bigeye snapper actomyosin during thermal gelation process.

3.3 Hydrophobic interactions

About 25 % of amino acids that constitute the myosin molecule are hydrophobic amino acids such as alanine, valine, leucine, isoleucine, proline, tryptophan, and phenylalanine (Tsuchiya and Matsumoto, 1975). If these hydrophobic residues are in contact with water molecules, a so-called “clathrate” or “iceberg” is formed, whereby a large number of water molecules are hydrogen-bonded to one another around the residue (hydrophobic hydration). However, such an ordering of water molecules in this way is not stable thermodynamically. If the temperature rises, hydrogen bonds become less stable and hydrophobic hydration becomes favored. Thus the hydrophobic amino acid residues become more exposed and subsequent hydrophobic interaction occurs.

During the slow-setting upon incubation near 40°C, hydrophobic groups are introduced onto their molecular surface and hydrophobic interactions proceed and play an important role in the setting phenomenon (Niwa *et al*., 1981). Benjakul *et al*. (2001b) reported the increase in surface hydrophobicity of actomyosin from bigeye snapper during thermal gelation, suggesting that hydrophobic interaction involved in gelation.

3.4 Other bondings

Covalent cross-linking reactions other than disulfide bonding occur in surimi gel (Kamath *et al*., 1990). Appearance of higher molecular weight polymers of Alaska pollack MHC corresponded to its disappearance of MHC during setting. At 25°C, the optimum setting temperature for pollock, the cross-link formation of MHC is the most pronounced. However, cross-link formation is also evident in sols incubated at 4°C and 40°C (Niwa, 1992). Gelation of the surimi sol also occurs during incubation at 50°C; however, such gelation evidently does not involve the non-disulfide cross-linking of myosin (Niwa, 1992). The mechanism of the cross-

linking reaction has been proven to be attributed to the action of transglutaminase (Seki *et al*., 1990). Its activity may be species-specific, but is also likely related to the surface conformation of the substrate, as determined by the heat stability of the particular myosin (Niwa, 1992). It was reported that transglutaminase may require a hydrophobic region on one of the substrate molecules for cross-linking reaction (Niwa, 1992).

4. Denaturation of surimi protein during freezing/ frozen storage

4.1 Change in protein in frozen stored fish

Frozen storage is a long-term preservation method that allows the storage of fish under more controlled conditions. Ideally, there should be no difference between fresh fish and frozen fish after thawing. If appropriate conditions are used, fish in the frozen state can be stored for several months without appreciable changes in quality (Santos-Yap, 1996). However, freezing and frozen storage can adversely affect the quality of a variety of muscle foods including beef, pork, fish and chicken. Quality deterioration during frozen storage is more pronounced in fish muscle than in other muscle foods. During the freezing of fish, the separation of water as pure ice crystals creates an environment that is conducive to protein denaturation, and this phenomenon has been cited as a possible cause for deterioration in frozen fish.

After several months of storage at -20°C, a loss in functional characteristics of the muscle proteins, mainly solubility, water retention, gelling ability, and lipid emulsifying properties is generally observed. Freezing and thawing may result in lysis of mitochondria and lysosomes and a change in distribution of enzymes (Karvinen *et al.,* 1982). A gradual decline in the activities of various muscle enzymes has also been observed during storage at freezing temperatures. The loss of ATPase activity, both in meat homogenates and in protein solutions, may reach 50-80 % (Buttkus, 1967). Total solubility of proteins in neutral 5 % NaCl solution may decrease to about 30%, whereby the main loss regards the contractile proteins, mainly myosin heavy chain, M-proteins, tropomyosin, and troponins I and C in descending order (Owusu *et al*., 1987). Fish muscle proteins, mainly myosin, are more susceptible to abuse conditions of freezing and frozen storage than those from land animals. Factors influencing protein denaturation during freezing and frozen storage include salt concentration, pH, ionic strength, surface tension, and the physical effects of ice and dehydration (Park 1994; Reynolds *et al*., 2002). The sharp decrease in

gel forming ability, water holding capacity, and fat emulsifying capacity reflects the deterioration of proteins during frozen storage. Denaturation of myosin and actomyosin has so far been ascribed to intermolecular aggregation, but some investigations have shown that intramolecular transconformation and the unfolding of polypeptide chains occur in globular proteins and in subunits within the globular structures. Enzymes of sarcoplasmic fluid also undergo denaturation during frozen storage (Matsumoto, 1980).

4.2 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 (Xiong, 1997a). The size and location of ice crystals formed during freezing is greatly influenced by the freezing rate, storage time, and temperature fluctuations (Xiong, 1997a). At a slow freezing rate, the exterior fluid of cells cools more rapidly than the interior fluid. As the supercooled extracellular fluid reaches a critical temperature, water separates from the solute and forms ice crystals. As crystallization proceeds, extracellular salt becomes 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 (Love, 1968). 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 proteins. In contrast, at a fast freezing rate, the interior moisture of the cell is cooled so rapidly and the small ice crystals, usually spearlike and separated by proteins, form inside the cell. Therefore, less dehydration and mechanical damage to proteins occurs (Love, 1968). Recrystallization involving enlargement in size, changes in shape and orientation, and movement of ice crystals usually causes tissue damage and accelerates protein denaturation (Xiong, 1997a).

Under normal commercial freezing regimes, ice nucleates not between the myofibrils but between the muscle cells (Goodband, 2002). As these ice crystals grow, water is abstracted from the myofibrils and the cells become condensed. The effect of this condensation of the muscle fibres on the myofibrillar lattice is shown in Figure 5. As a result of the condensation of the fibres, the myofilaments are in closer proximity to each other. If the fish is thawed soon

after being frozen, the lattice recovers its original dimensions and a large proportion of the water returns to the muscle cells. In these circumstances, the textural quality of the fish is maintained. If however, fish is stored in the frozen state for a period of time, interactions between the myofilaments can occur. As a result, on thawing, the water is unable to return to the cells and remains in the extracellular spaces (Goodband, 2002). Some of this water may be lost as drip and water may be further lost on cooking. This redistribution or loss of moisture can result in the typical characteristics of frozen deteriorated fish. The fish may have a very high initial juiciness, but on further chewing, the fish is tough, fibrous and dry (Goodband, 2002).

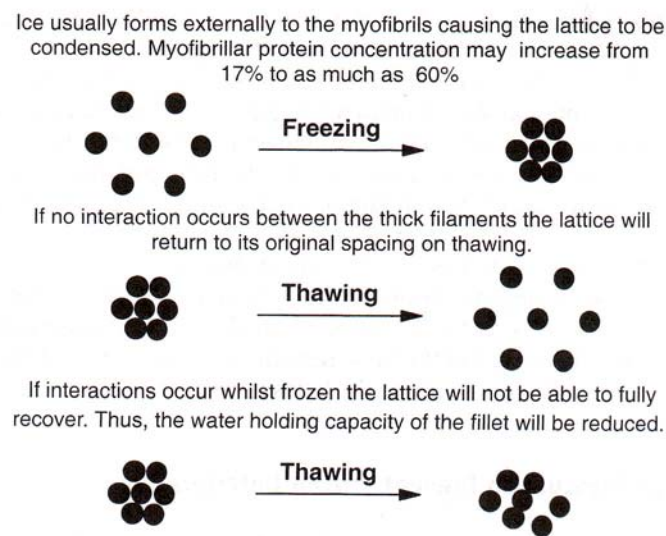


Figure 5. Effect of freezing on the myofibrillar lattice

Source: Goodband (2002)

4.3 Use of cryoprotectants

Extended frozen storage can produce profound effects on the structural and chemical properties of muscle proteins, which can, in turn, significantly influence the quality attributes of muscle food products (Park and Lanier 1989; Reynolds *et al.,* 2002). The addition of cryoprotectants is important to ensure maximum functionality of frozen surimi. Cryoprotectants are uniformly incorporated into the dewatered meat using a kneader or silent cutter before formation of the surimi blocks. Sucrose (4%) and sorbitol (4- 5%) serve as the primary cryoprotectants and polyphosphate (0.2-0.3%) is also added as both a chelating agent by making metal ions in surimi inactive and as a pH enhancing agent. Combining theses ingredients together

protects fish myofibrillar proteins and minimizes protein denaturation during long periods of frozen storage (Pipatsattayanuwong *et al.,* 1995).

5. Hydrocolloids

Hydrocolloids are natural carbohydrate polymers of high molecular weight used in the food industry for obtaining important functional properties, such as thickening, gel formation and texture improvement. Pectin, gellan, agar–agar, kappa-carrageenan and sodium alginate are the most used hydrocolloids among the gelling agents accepted as additives in foods. Besides the application in the manufacture of modified foods, they are used to produce particles for retention of enzymes, microorganisms or aromatic substances (Enrique *et al*., 2003). Hydrocolloids are ordinarily added to myosystems in the form of an unhydrated, dry powder, since water is a limiting factor affecting the texture of the final product. Addition of prehydrated or thermally-activated hydrocolloids may structurally interfere with the cross- linking required for the protein gel network formation, giving rise to gel weakening (Filipi and Lee, 1998). Hydrocolloids have been used in different food products such as blends of locust bean gum and iota carrageenan (Troy *et al*., 1999); k-carrageenan and iotacarrageenan or xanthan gum in low-fat frankfurters (Bloukas *et al*., 1997) in restructured meats; k-carrageenan/locust bean mixture (Nielsen *et al*., 1996) and carboxymethylcellulose (Shand *et al*., 1993) in alginate restructured beef.

5.1 Type of hydrocolloids

Xanthan gum

Xanthan gum is an extracellular polysaccharide secreted by the microorganism, *Xanthomonas campestris*. Xanthan gum is a linear (1⟶4) linked ß-D-glucose backbone with a trisaccharide side chain on every other glucose at C-3, containing a glucuronic acid residue linked (1⟶4) to a terminal mannose unit and (1⟶2) to a second mannose than connects to the backbone (Figure 6) (Sworn, 2000). At high temperatures, xanthan viscosity is temporarily reduced. Xanthan gum recovers its viscosity upon cooling. A xanthan gum solution is highly shear-thinning with a low yield value. This makes it flow readily when poured and once the flow stops, its viscosity is recovered. Additionally, it thins under shear in the mouth, facilitating flavor

release. Xanthan gum solutions form a synergistic gel with locust bean gum but not with guar gum. A small amount of xanthan gum in starch-thickened foods improves freeze-thaw stability (Pomeranz, 1985). Unique properties of water-soluble gums include high or complete solubility in cold water, high viscosity and gel strength after cooking, acting as excellent suspension agents in acid media and for general purposes, good emulsifying properties, high swelling in cold water, good film forming, high milk reactivity, and good water absorption (Pomeranz, 1985).

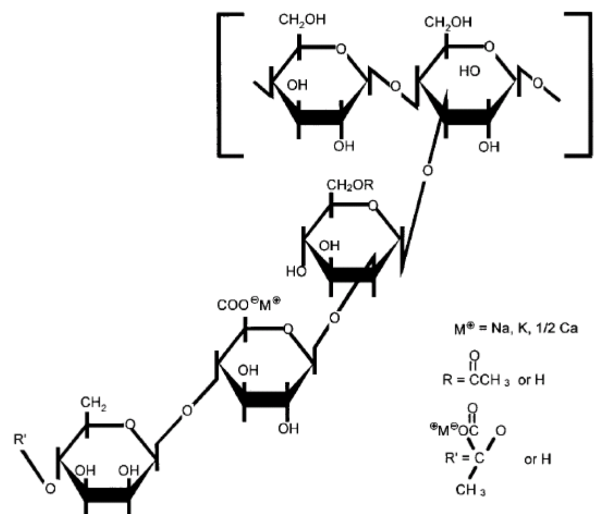


Figure 6. Primary structure of xanthan gum

Source: Sworn (2000)

Locust bean gum

Locust bean gum, another plant seed galactomannan, is derived from the carob seed, *Ceratonia siliqua*. The plant grows predominately in the Near East and Mediterranean areas. It consists of a D-mannopyranosyl backbone with attached D-galactopyranosyl units with a ratio of 4:1 (Figure 7) (Whistler and Bemiller, 1973). However, its D-galactopyranosyl units are not uniformly distributed, leaving long stretches of the mannan chain devoid of D-galactopyranosyl units. This leads to unique synergistic properties, especially with the seaweed polymer carrageenan, where the two cross-links form a gel (Whistler and Daniel, 1994).

Locust bean gum is employed in frozen desserts to bind water and provide body, smoothness and chewiness. In soft cheese manufacture, it speeds curd formation and reduces the

loss of solids. In composite meat products, such as salami, bologna, and sausages, it acts as a binder.

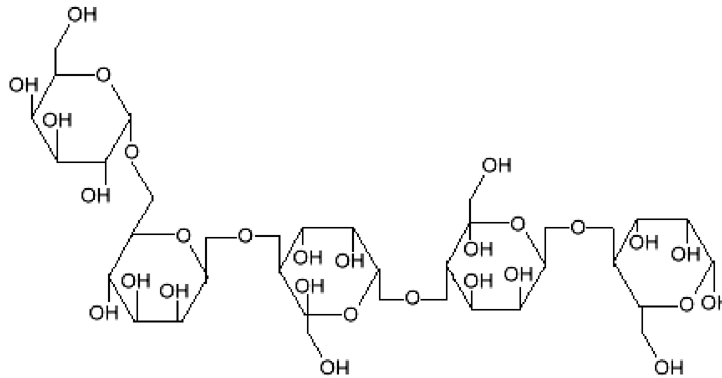


Figure 7. Structure of locust bean gum

Source: Whistler and Bemiller (1973)

Carrageenans

Carrageenans are high molecular weight polysaccharides having the repeating galactose units and 3, 6-anhydrogalactose, both sulfated and non-sulfated, joined by alternating α-(1,3) and β-(1,4) glycosidic linkages (Figure 8). The main carrageenan types are λ, κ and ι-carrageenan and can be prepared in pure form by selective extraction techniques. Mu and nu carrageenans are postulated as the precursor structures which, as a result of the internal rearrangement by alkali treatment, form kappa and iota carrageenans (Imeson, 2000). Different carrageenans differ in their structure. The ester sulfate and 3,6- anhydrogalactose content is 25% and 34% for κ, 32% and 30% for ι, and λ contains 35% ester sulfate with little or no 3,6-anhydrogalactose content (Imeson, 2000).

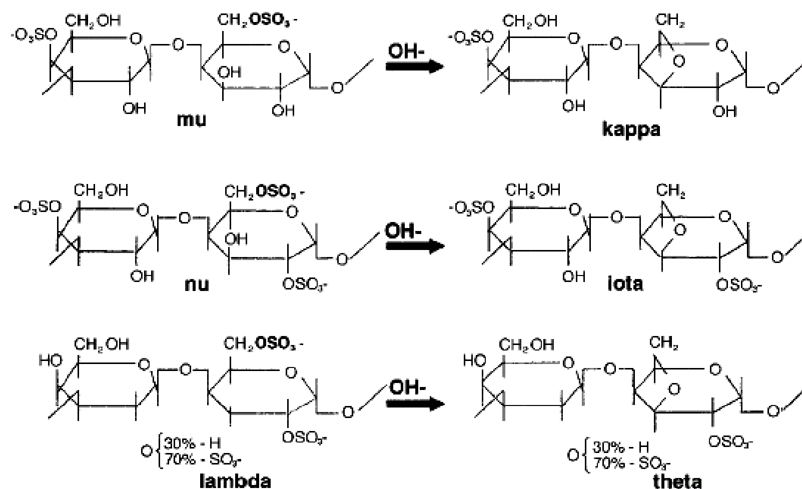


Figure 8. Carrageenan structures (alkali conversion of kappa, iota and lambda)

Source: Imeson (2000)

The distinct carrageenan structures differ in 3,6-anhydrogalactose and ester sulfate content. Variations in these components influence hydration, gel strength and texture, melting and setting temperatures, syneresis and synergism (Anon, 1988). These differences are controlled and created by seaweed selection, processing and blending of different extracts. For food applications, carrageenan is best described as 'extracts from *Rhodophyceae* which contain an ester sulfate content of 20% and above and are alternately α-(1,3) and β-(1,4) glycosidically linked' (Anon, 1988). Commercial kappa carrageenan extracts have the molecular weight between 400 and 560 kDa, while processed *Euchema* seaweed has a slightly higher molecular weight of 615 kDa (Hoffmann *et al*., 1996). All carrageenans contain a fraction (<5%) of material below 100 kDa and this low molecular weight material is believed to be inherent in native algal weed (Imeson, 2000).

Starches

Starch is one of the most abundant plant products and plays a major role in the human diet as a bulk nutrient and energy source (Zobel and Stephen, 1995). It can be derived from (waxy) maize, wheat, potato, rice, tapioca, and many other sources and functions as an energy reservoir in those plants. Starch is extensively used in the food industry for nutritional, technological, sensorial, and even aesthetic purposes (Light, 1990). In order to meet the specific requirements to fulfil a certain role, starches are frequently modified both physically and

chemically (Light, 1990). The resulting compatibility with modern processing technologies and increased functionality makes starch become an attractive ingredient in food products (Murphy, 2000).

Molecular structure

Starch is a biopolymer containing glucose which exists in two molecular forms: the linear amylose and the branched amylopectin (Figure. 9). The relative amounts of both depend on the botanical source, but starch usually contains 18-33% amylose and 67-82% amylopectin. However, some mutant genotypes of common maize contain as much as 70% amylose, whereas other genotypes called waxy maize contain less than 1% (Buleon *et al*., 1998).

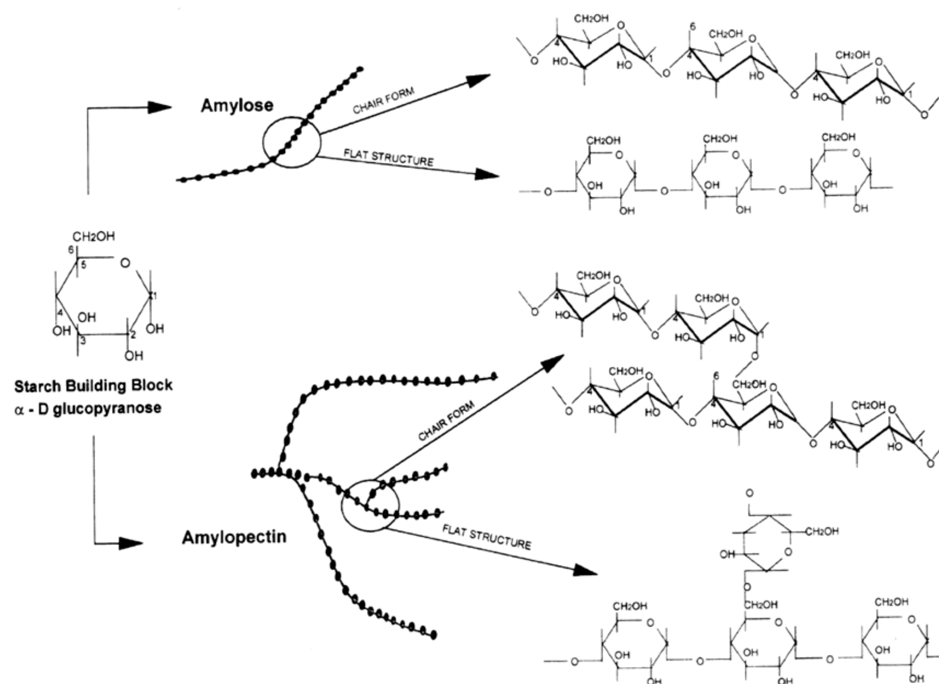


Figure 9. Structure of amylose and amylopectin

Source: Murphy (2000)

Amylose is a flexible, essentially linear molecule consisting of α-D-glucopyranosyl units connected by α-(1,4) linkages (Zobel and Stephen, 1995). Amylose shows a small degree of branching, although the amount of glucose units involved in branching points is less than 1% (Ball *et al*., 1996). The degree of polymerization ranges from a few hundred up to

6000, providing molecular weights from 10^4 to 10^6 Dalton (Rapaille and Vanhemelrijck, 1997). Amylose forms a left-handed helix with a hydrophobic interior, which allows it to form complexes with nonpolar molecules, particularly lipids (BeMiller, 1993; Hill, 2003).

Amylopectin is a highly branched molecule composed of (1,4)-linked α-D-glucopyranosyl units interrupted by α-(1,6)-linked branching points every 20-26 monomer units. Its degree of polymerization may be larger than 10000 yielding molecular weights of 10^7 Dalton and more, which makes amylopectin one of the largest molecules in nature (Rapaille and Vanhemelrijck, 1997).

Modified starches

Native starches from various plant sources have their own unique properties. Native starches, however, lack the versatility to function adequately in the entire range of food products currently available in the marketplace. The diversity of the modern food industry and the enormous variety of food products require starch which tolerates a wide range of processing techniques as well as various distribution, storage, and final preparation conditions. These demands are met by modifying native starches by chemical and physical methods (Whistler *et al*., 1984; Rutenberg and Solarek, 1984).

Chemical modification

Starch modification is the treatment of native starch with small amounts of approved chemical reagents. Chemical modification of starch changes the functionality of the starch. Modification of starch is quite straightforward and involves primary reaction associated with the hydroxyl groups of the starch polymer. Derivatization via ether or ester formation, oxidation of the hydroxyl groups to carbonyl or carboxylic groups, and hydrolysis of glycosidic bonds are some of the major mechanisms of chemical modification (David *et al*., 1998).

- crosslinking

The most common type of chemical modification is crosslinking. The derivatization of starch can be carried out by using a bi- or poly functional chemical reagent that is able to react with two or more different hydroxyl groups on the same or different starch

polymers. Covalently bonded crosslinks act as “spot welds” to reinforce the granular structure (Figure 10)

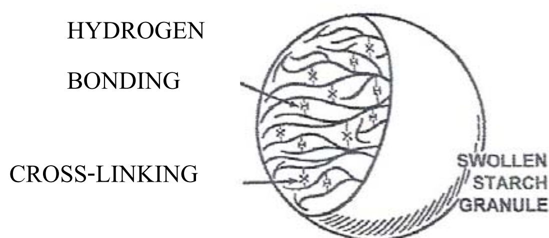


Figure 10. Structure of a crosslinked starch granule. Each X refers to a covalently bonded crosslinking reagent

Source: David *et al*. (1998)

Phosphorus oxychloride is commonly used to produce crosslinked starch esters. The reaction is typically run at a high pH with or without salts (Figure 11). Salts commonly used include sodium chloride and sodium sulfate. Depending upon the extent of crosslinking, the di-starch phosphate has the improved viscosity stability and process tolerance (David *et al*., 1998).

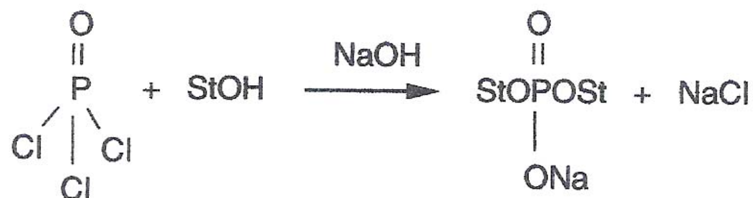


Figure 11. Chemical reaction for phosphorus oxychloride crosslinking of starch. St= starch polymer

Source: David *et al*. (1998)

Hydroxypropylated starch have improved clarity, greater viscosity, reduced syneresis, and freeze-thaw stability (David *et al*., 1998). Crosslinked, hydroxypropylated starches are perhaps the most commonly used modified starh in the food industry. They are typically used in a wide range of food applications, including gravies, dips, sauces, fruit pie fillings, and puddings, where a smooth, viscous, clear thickener is necessary and freeze-thaw stability is required.

Hydroxypropylated starches are prepared by reacting a starch slurry with propylene oxide under highly alkaline conditions at a temperature of approximately 30-50°C (Figure 12) (David *et al*., 1998).

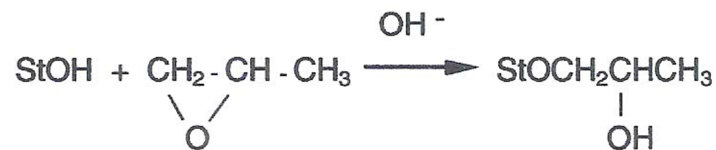


Figure 12. Chemical reaction for hydroxypropyl substitution of starch. St= starch polymer

Source: David *et al*. (1998)

Modified starches generally possess the enhanced rheological characteristics, textural qualities, optical properties, and enhanced stability of the system. It is important to recognize that chemical modification often involves only small amounts of change in the starch structure. A commercially modified starch that has been etherified with propyleneoxide has only about 10 hydroxypropyl substituents per 100 glucose residues in the starch. Each anhydroglucose residue has three hydroxyl groups that, theoretically, can react (Deboer, 1991).

5.2 Gelation of hydrocolloids

Food gels are usually complex systems and the functionality of a mixture of biopolymers is superior to that of the individual components due to the occurrence of molecular interactions (Aguilera and Stanley, 1999; Oakenfull *et al*., 1997). In a solution of two biopolymers, interactions can lead to three different phenomena as shown in Figure 13 (Tolstoguzov, 1991):

• Co-solubility or compatibility: no significant interaction takes place and both biopolymers coexist in a single homogeneous phase; in case of partial compatibility two phases exist, both enriched in one biopolymer.

• Incompatibility: the existence of repulsive forces results in the formation of two separated phases

• Complexation: attraction between the two biopolymers induces complex formation, as a result of which both are present in a single phase or precipitate

polysaccharide
protein
segregation
association
incompatibility
co-solubility
complexation

Figure 13. Overview of different interactions that may occur in a protein/polysaccharide mixture
Source: de Kruif and Tuinier (2001)

Obviously, the behaviour of the polymers upon mixing has a great influence on the gels that may be formed from the mixture. Since gelation involves a transfer of molecules from the solution to the forming network, the excluded volume effect of the polymers decreases, making the dispersion medium a better solvent (Zasypkin *et al*., 1997). This implies that the structure and properties of a mixed gel depend on the nature of the polymers present as well as on the kinetics of gelation. From a mixture of two biopolymers, each capable of forming a three-dimensional network, different gel structures may be formed (Figure 14). In a swollen network, one biopolymer forms a continuous gel network, while the other is trapped in the voids of the network (Figure 14a). If the latter biopolymer also gels, gel fragments are constituted within the spaces of the main network as shown in Figure 14c. The resulting structure is called a phase separated network (Aguilera, 1992; Williams and Phillips, 1995). These types of network can only arise from phase separated solutions and therefore requires thermodynamic incompatibility between the two polymers in solution (Aguilera and Stanley, 1999).

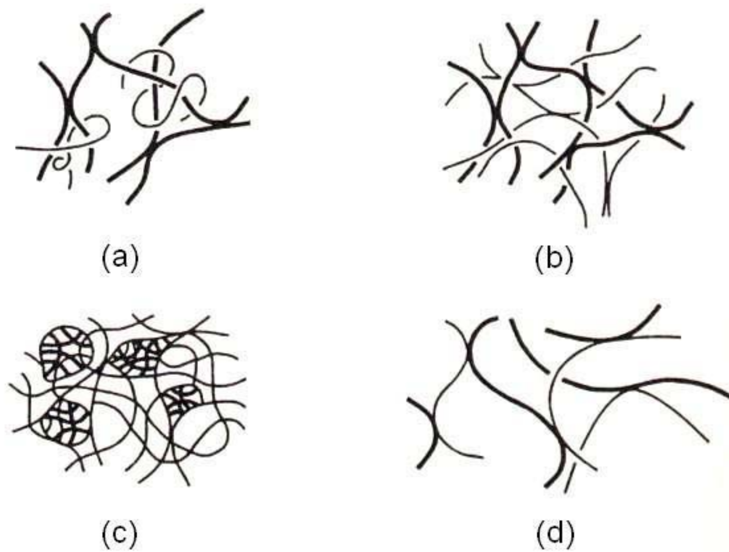


Figure 14. Representation of different mixed gel networks: (a) swollen network; (b) interpenetrating network; (c) phase separated network; (d) coupled network

Source: Williams and Phillips (1995)

An interpenetrating network consists of two individual, entangled networks that stretch out over the entire container volume and originates from a solution of compatible biopolymers (Oakenfull *et al*., 1997). It can also arise from a solution of incompatible polymers that is rapidly gelled upon mixing before phase separation could take place. In a coupled network, chains of both polymers interact directly to form the gel network (Figure 14d). Coupled networks may even be constituted of two components that are not able to gel individually.

5.3 Uses of hydrocolloids in foods

Freezing of foods causes immense changes to structure, due to a combination of both the formation of ice crystals and the freeze-concentration of solutes and dispersed macromolecules in the unfrozen phase (Goff, 1992). Polysaccharide stabilizers (gums, hydrocolloids, eg., guar, locust bean gum, carboxymethyl cellulose, carrageenan) are often used in formulated frozen foods to control ice recrystallization, in addition to textural attributes they may contribute to the food itself (Goff, 1992). Defreitas (1997) reported that 0.5% kappa and iota carrageenan may be useful for increasing freeze/thaw stability of cooked meat products, and these carrageenans improved moisture retention of cooked pork sausage. Addition of 0.5% kappa carrageenan to oven-roasted turkey breasts increased the yield, improved slice ability and rigidity,

and decreased expressible moisture content (Bater *et al*., 1992). According to da Ponte *et al*. (1985), iota-carrageenan has greater water holding capacity than kappa-carrageenan and further prevents syneresis during thawing of frozen fish gels. Although carrageenan may help to improve the water holding properties of meat emulsion, it perhaps does so by holding water in the intersticial spaces of the gel network rather than by true interactions with the proteins in the formation of the network (Bernal *et al*., 1987).

Increased gel forming capacity of Alaska pollack surimi, when carrageenan is added, is due to the interaction of the carrageenan's sulphate groups with myofibrillar proteins (Bullens *et al*. 1990). Montero *et al*. (2000) showed heat induced blue whiting gels with 2% iota carrageenan having small, round cavities and those with kappa-carrageenan having large, elongated cavities. Brigham *et al*. (1994) also observed a homogeneous structure of thin and thick fibres in kappa carrageenan gels, while they were thinner in the case of iota carrageenan. Go´mez-Guille´n *et al*. (1996) suggested that iota-carrageenan forms a fine three-dimensional network with some points of connection with the protein matrix.

Addition of locust bean gum in low fat meatballs resulted in good sensory properties (Hsu and Chung, 1999). Xanthan gum affected textural properties of low fat frankfurters (Mittal and Barbut, 1994) and low-fat sausages (Solheim and Ellekjaer, 1994). Locust bean gum and guar gum can be located inside large round cavities evenly and distributed throughout the protein matrix of blue whiting mince gel (Montero *et al*., 2000). However, xanthan gum caused a decrease in the gel forming capacity of the myofibrillar protein

Starch/modified starches are the major ingredients to improve the texture, water-binding capacity, freeze/thaw stability of surimi and surimi based products (Kim and Lee, 1987; Wu *et al*., 1985c; Lee *et al*., 1992). Potato or wheat starch at a level of 5% or less improved the textural properties of surimi gels. Use of potato starch, corn and tapioca modified starch improved shear stress and shear strain values in freeze-thawed surimi gels (Hernandez *et al*., 1996). Addition of 5% (w/w) waxy maize starch, waxy maize and tapioca modified starch in surimi made from tilapia (*Oreochromis* sp.) resulted in the increased penetration force value (firmness) of gel (Barreto *et al*., 1999). Starch increases gel strength and elasticity through both a composite reinforcing and water binding effect (Lee, 1984). During heating, starch absorbs some water from the fish mince and becomes partially gelatinized and fills the pores of the protein network (Lee *et*

al., 1992). The gel strengthening ability of starch is affected by its water-holding capacity during gelatinization and the viscosity of the gelatinized starch. (Lee, 1984). The use of modified starch in surimi and surimi-based products is mainly for freeze-thaw stability. Modified starches will increase freeze-thaw stability by preventing retrogradation or aggregation of amylase molecules with their cross-linked branches. The aggregation of amylose molecules results in the release of free water or freeze syneresis which is usually observed in unmodified starches. However, sometimes a freeze syneresis is desired to enhance moistness and juiciness of surimi. The recommended starch usage in surimi is half unmodified and half modified starch to access both the gel strengthening effect and freeze-thaw stability (Lee *et al*., 1992).

6. Modified atmosphere packaging

Modified atmosphere packaging (MAP) is widely used as a supplement to ice or refrigeration to delay spoilage and extend the shelf-life of fresh fishery products, while maintaining a high-quality end product. The principle of MAP is the replacement of air in the package with a different gas mixture. CO_2 is the most important gas used in MAP of fish, because of its bacteriostatic and fungistatic properties (Sivertsvik, 2002). It inhibits growth of many spoilage bacteria and the inhibition is increased with increased CO_2-concentration in the atmosphere (Sivertsvik, 2002). Such an effect is influenced by the CO_2 concentration, initial bacterial population, storage temperature and product type (Reddy *et al.,* 1992). Devlieghere *et al.* (1998a, 1998b) demonstrated that the growth inhibition of microorganisms in MAP is determined by the concentration of dissolved CO_2 in the product. After the packaging has been opened, the CO_2 is slowly released by the product and continues to exert a useful preservative effect for a certain period of time, referred to as CO_2 residual effect (Stammen *et al*., 1990). The action of CO_2 on the preservation of foods was originally thought to be caused by displacement of some or all of the O_2 available for bacterial metabolism, thus slowing growth (Daniels *et al*., 1985). The effect of CO_2 on bacterial growth is complex and four activity mechanisms of CO_2 on micro-organisms has been identified (Parkin and Brown, 1982; Daniels *et al*., 1985; Dixon and Kell, 1989; Farber, 1991):

1. Alteration of cell membrane function including effects on nutrient uptake and absorption;

2. Direct inhibition of enzymes or decreases in the rate of enzyme reactions;

3. Penetration of bacterial membranes, leading to intracellular pH changes;

4. Direct changes in the physico-chemical properties of proteins.

A combination of all these activities account for the bacteriostatic effect. MAP fish has a shelf-life extension from a few days to a week or more, compared with air storage, depending on species and temperature. The best effect of MAP storage on shelf-life has been obtained with fish from warm waters and the spoilage flora on some packed fish products was found to be dominated by Gram- positive micro-organisms (Banks *et al*., 1980; Huss, 1995). However, *Clostridium botulinum* type E and non-proteolytic type B strains will grow and produce toxin in low-oxygen atmospheres at refrigerated temperatures. This leads to a great concern for the uses of MAP of seafood (Church, 1994). *Clostridium botulinum* produces a neurotoxin that causes facial paralysis, and is classified into A, B, C, D, E, F and G types (Hintlian and otchkiss, 1986). In general, O_2 may inhibit the growth of exclusively anaerobic bacteria, although anaerobic microorganisms show different sensitivity levels to oxygen (Farber, 1991). The use of O_2 in modified atmosphere packaging for fish reduces exudation in fish during storage (Davis, 1995).

Nitrogen (N_2) is an inert and tasteless gas, and is mostly used as a filler gas in MAP, because of its low solubility in water and fat (Church and Parsons, 1995). N_2 is used to replace O_2 in packages to delay oxidative rancidity and inhibit growth of aerobic microorganisms, as an alternative to vacuum packaging. The use of oxygen in MAP is normally set as low as possible to inhibit the growth of aerobic spoilage bacteria. Its presence is reported to increase oxidative rancidity (Chen *et al*., 1984), although others claim that rancidity caused by presence of O_2 in the atmosphere is negligible (Haard, 1992). However, for some products, oxygen could or should be used. High levels of oxygen are used in red meat and red fish meat (tunas, yellowtails, etc.) to maintain the red color of the meat, to reduce and retard browning caused by formation of metmyoglobin (Oka, 1989). O_2 in MAP packages of fresh fish will also inhibit reduction of TMAO to TMA (Boskou and Debevere, 1997). Originally, oxygen was introduced into the package of selected products to reduce the risk of anaerobic pathogenic bacterial growth, but this has been discredited (Davies, 1995).

Microbial safety of MA packaged fish and fish products

The shelf-life increased as a result of lag-phase extension of several aerobic spoilage bacteria and retardation of enzymatic spoilage. It has been reported that the shelf life of fish under modified atmosphere storage could be extended. Carbon dioxide is used to maintain the fungistatic and bacteriostatic properties, whereby levels of 20–40% of this gas are used in MAP. In general, gas mixtures of 30% O_2 + 40% CO_2 + 30% N_2 and 60% CO_2 + 40% N_2 are recommended for lean fish and fat fish, respectively (Smith *et al*., 1990; Randell *et al*., 1997; Ordonez *et al*., 2000; Gimenez *et al*., 2002; Masyinom *et al*., 2002). Fish and shellfish are highly perishable and their deterioration is primarily because of bacterial action (Colby *et al*., 1993). Typical shelf-life under current icing and refrigerated storage conditions ranges from 2 to 14 days (Stammen *et al*., 1990). Generally, fish stored in aerobic conditions have been spoiled by Gram-negative organisms, primarily *Shewanella putrefaciens*. In CO_2-packed fish, the growth of *S. putrefaciens* is strongly inhibited. In contrast, the Gram negative organism *Photobacterium phosphoreum* has been identified as the organism responsible for spoilage (Erkan *et al*., 2007). No growth and difference in survival of *E. coli*, *S. aureus, V. parahaemolyticus*, and *C. perfringens* was observed in Jack mackerel (*Trachurus japonicus*) during storage under air, 100% N_2 or 40% CO_2±60% N_2 atmospheres at 5 °C (Kimura and Murakami, 1993). Gibson *et al*. (2000) demonstrated that 100% CO_2 could have an inhibitory effect on the growth of *C. botulinum* at chilled temperatures, and an increased inhibitory effect was observed when combining 100% CO_2 with increased NaCl level and decreased pH level. Lyver *et al.* (1998a) and Lyver *et al*. (1998b) showed that growth and toxin production by *C. botulinum* in raw and cooked surimi nuggets could be controlled with competitive inhibition by *Bacillus* species.

The advantages of extended shelf-life and quality as well as the disadvantages including providing conditions conductive to botulinal toxin production of packaging fresh fish with VAC and MAP have been outlined (Wolfe, 1980, Yambrach, 1987 and Cann, 1988). A CO_2 atmosphere extends the lag phase and generation time of aerobic bacteria that in turn decreases the growth rate and extends shelf-life (Finne, 1982; Parkin and Brown, 1982; Genigeorgis, 1985). The inhibition of bacterial growth in food packaged with CO_2 increases as the storage temperature decreases (Reddy *et al*., 1992). A 100% CO_2 atmosphere has been shown to inhibit *C. botulinum* at chilled temperatures (Gibson *et al*., 2000). Detrimental quality effects may also occur including

the lowering of pH, absorption of CO_2 into the flesh causing a deflation of the package, pigmentation changes (Parkin and Brown, 1982) and increased drip from the alteration in the water holding capacity associated with the CO_2 solubility (Davis, 1998).

The Advisory Committee on the Microbiological Safety of Food (ACMSF) in the UK published in 1992 concluded that if the storage temperature and the shelf-life are less than, respectively, 10 °C and 10 days, the risk is low. For products with a shelf-life of longer than 10 days, which are not subjected to a heat treatment that will sufficiently inactivate the spores of *psychrotrophic C. botulinum*, combinations of preservative factors such as pH (<5), water activity (<0.97), and salt (>5%) should be established to prevent the growth of *psychrotrophic C. botulinum* (Gibson and Davis, 1995). Handumrongkul *et al*. (1994) reported a count of 6–7 log CFU g^{-1} for striped bass after 12 days of under MAP refrigeration. Villemure *et al*. (1986) reported that psychrotrophic bacteria count of gutted cod under MAP (25/75: CO_2/N_2) was lower than that in air-packed samples during the storage at 0 °C. Ozogul *et al*. (2000) reported that the amount of mesophilic bacteria count in herring flesh at the time of rejection in MAP (10 days) and in vacuum (8 days) was <6.0 log CFU g^{-1}. Ozogul *et al*. (2004) reported that initial total viable count of iced sardines was 4 log CFU g^{-1}, reaching the limit counts of 10^6–10^7 at day 3 in air, at day 8 for vacuum and at day 10 for MAP. The low initial psychrotrophic bacteria (<3 log CFU g^{-1}) indicate very good fish quality. In samples stored under air, vacuum and MAP conditions, psychrotrophic bacteria counts exceeded the value of 10^5 CFU g^{-1} (5 log CFU g^{-1}), considered as the upper acceptability limit for marine species (Lapa-Guimaraes *et al*., 2002).

OBJECTIVES

1. To study the effect of some hydrocolloids on the properties and freeze-thaw stability of gel from bigeye snapper mince.
2. To study the effect of modified starch at different levels on the properties and freeze-thaw stability of mince and surimi gels.
3. To investigate the changes of chemical and physical properties of mince and surimi gels containing modified starch during frozen storage.
4. To elucidate the effect of modified atmosphere packaging (MAP) on quality of mince gels during storage at 4°C.

CHAPTER 2

MATERIALS AND METHODS

1. Materials

1.1 Fish samples and preparation

Bigeye snapper (*Priacanthus tayenus*) were purchased from the dock in Songkhla, Thailand. The fish, off-loaded approximately 36-48 h after catching, were transported in ice with a fish/ice ratio of 1:2 (w/w) to the Department of Food Technology, Prince of Songkla University, Hat Yai, Thailand within 1 h. The fish were immediately washed, deheaded, eviscerated and filleted. The fillets were minced to uniformity using a mincer with the diameter hole of 5 mm and used as the composite samples.

1.2 Chemicals

Sodium dedocyl sulfate (SDS), β-mercaptoethanol (βME), trichloroacetic acid and thiobarbituric acid (TBA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). *N,N,N′N′*-tetramethylethylenediamine (TEMED), acrylamide, bis-acrylamide, urea and glutaraldehyde were procured from Fluka (Buchs, Switzerland). Peptone water, standard plate count agar (PCA), potato dextrose agar (PDA) and Man rogaso sharpe broth (MRS broth) were purchased from Merck (Darmstadt, Germany). Modified starch (Gelpro HC30), Xanthan and locust bean gum were obtained from General starch Co. (Bangkok, Thailand). Iota carrageenan was purchased from Union Chemical 1986. Co., Ltd (Bangkok, Thailand).

2. Instruments

Instruments	Model	Company/City/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
Water bath	W350	Memmert, Schwabach, Germany
Microcentrifuge	MIKR 020	Zentrifugan, Hettich, Germany

3. Methods

3.1 Determination of chemical composition and property of bigeye snapper mince

1 Proximate analysis

Mince was determined for moisture, protein, ash and fat contents according to AOAC method (AOAC, 2000). The values were expressed as % (dry weight basis).

2. pH determination

pH of samples was determined as described by Benjakul *et al.* (1997). Ground sample was homogenized with 10 volumes of deionized water (w/v) using an IKA labortechnik homogenizer at a speed of 11,000 rpm for 2 min. The pH was then measured using a pH meter (Schott, Mainz, Germany).

3. Determination of TVB and TMA contents

TVB and TMA contents in mince were determined using the Conway microdiffusion method as described by Conway (1950). The sample (2 g) was mixed with 8 ml of 4% trichloroacetic acid (TCA). The mixture was homogenized at 6,500 rpm using a homogenizer (IKA Labortechnik, Selangor, Malasia) for 1 min. The homogenate was filtered through

Whatman No. 41 filter paper and the filtrate was used for analyses. To determine the TMA content, formaldehyde was added to the filtrate to fix ammonia present in the 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 or TMA was calculated.

4. Protein patterns

Protein patterns were analyzed using SDS-PAGE using 10% running gel and 4% stacking gel (Laemmli, 1970). To prepare the protein sample, 27 ml of 5% (w/v) SDS solution heated to 85°C were added to the sample (3g). The mixture was then homogenized for 2 min at a speed of 11,000 rpm using an IKA Labortechnik homogenizer. The homogenate was incubated at 85°C for 1 h to dissolve total proteins. The sample was centrifuged at 8,500xg for 20 min to remove undissolved debris. Protein concentration was determined by the Biuret method (Robinson and Hodgen, 1940) using bovine serum albumin as standard. Sample with the protein content of 20 μg was loaded onto the gel. Electrophoresis was conducted at 15 mA/plate. After separation, proteins were fixed and stained for 5 h in 0.125% Coomassie Brilliant Blue R-250 in 40% methanol and 10% glacial acetic acid. Gels were destained with destaining solution 1 (50% methanol and 7.5% glacial acetic acid) for 15 min and with the destaining solution II (5% methanol and 7.5% glacial acetic acid) for 3 h.

5. Determination of salt content

Salt content in samples was measured by the method of AOAC (2000). Sample (1g) was added with 10 ml of 0.1 N $AgNO_3$ and 10 ml of HNO_3. The mixture was boiled gently on hot plate until all solids except $AgCl_2$ were dissolved (usually 10 min). The mixture was then cooled using running water; 50 ml of distilled water and 5 ml of ferric alum indicator were added. The mixture was titrated with standardized 0.1 N KSCN until solution became permanent brownish-red. The salt content was then calculated and expressed as % NaCl.

3.2 Effects of some hydrocolloids on the properties and freeze-thaw stability of bigeye snapper mince gels

3.2.1 Effect of some hydrocolloids on gel properties

To study the effect of some hydrocolloids (xanthan gum, locust bean gum, iota carrageenan, Gelpro HC30, Gelpro HC715 and Bestek) on the properties of gels from bigeye

snapper mince, hydrocolloids at different levels (0, 2 and 5%, (w/w)) were added as dry powder form into the mince of bigeye snapper. The mixture was adjusted to obtain the moisture content of 80% and mixed thoroughly using a mixer (National Model MKK77, Tokyo, Japan) for 4 min. Temperature was maintained below 10°C during preparation.

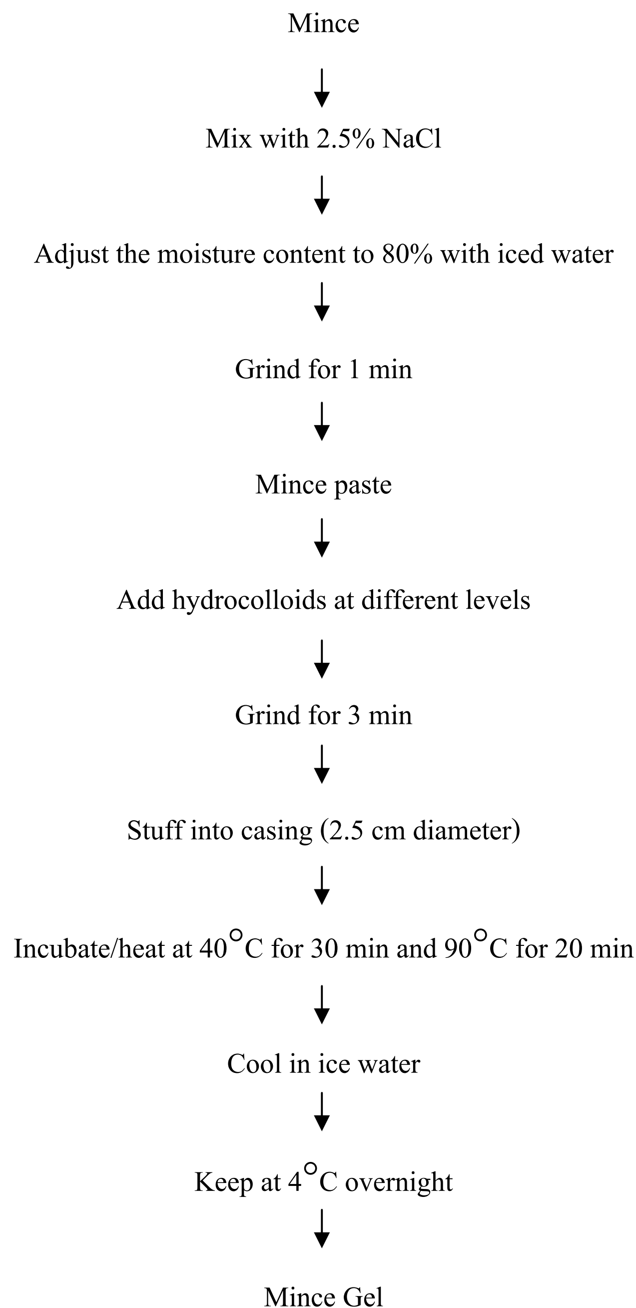


Figure 15. Scheme for preparation of gel from bigeye snapper mince added with different hydrocolloids

Gel were prepared as shown in Figure 15. Mince sol was stuffed into polyvinylidine casing with a diameter of 2.5 cm and both ends of the casing were sealed tightly. The sol were incubated at 40°C for 30 min, following by heating at 90°C for 20 min using a temperature controlled water bath (Memmert, Schwabach, Germany). The gels were cooled in iced water and stored for 24 h at 4°C prior to analyses or freezing. To freeze the gel samples, gels were placed in an air-blast freezer at -18°C for 48 h. The frozen samples were thawed using running water (25–26°C) until the core temperature reached 0–2°C. Freeze-thawing was carried out for 1, 3 and 5 cycles. Mince gel without hydrocolloid was used as the control. Gel obtained were subjected to analyses as follows:

1. Breaking force and deformation

Gels were equilibrated and evaluated at room temperature (28-30°C) before analyses. Five cylinder-shaped samples with a length of 2.5 cm were prepared. Breaking force (gel strength) and deformation (elasticity/deformability) were measured using the texture analyzer equipped with a spherical plunger (diameter 5 mm, depression speed 60 mm min^{-1}).

2. Expressible moisture content

Expressible moisture content was determined according to the method of Benjakul *et al.* (2001a). Gel samples with a thickness of 0.5 cm was weighed and placed between two pieces of filter paper (Whatman, Maidstone, England) at the top and three pieces of the same filter paper at the bottom. The standard weight (5 kg) was placed on the top of the sample and maintained for 2 min. The sample was removed and weighed again. The expressible moisture was calculated based on the difference in weight before and after pressing.

3. Determination of whiteness

Color of mince gel was determined using a colorimeter (HunterLab, ColorFlex Reston, VA, USA). *L** (lightness), *a** (redness/greenness) and *b** (yellowness/blueness) were measured and whiteness was calculated as described by Lanier *et al.* (1991) as follows:

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

4. Sensory evaluation

Fish mince gels, with and without hydrocolloids, subjected to 0 and 5 freeze-thawing cycles were evaluated for acceptance by a 30 panelists. The panelists were

graduate students in the Food Technology program, Faculty of Agro-Industry, Prince of Songkla University. of age ranging from 22 to 35 years. Panelists had sensorial acquaintance with fish mince gel. A nine-point hedonic scale, in which a score of 1 = dislike extremely, 5 = neither like nor dislike and 9 = like extremely, was used for evaluation (Meilgaard *et al.,* 1990). Samples were sliced perpendicular to the long axis to obtain the length of 2.0 cm. Individual samples of each fish mince gel were placed on dishes (3.0 cm diameter). The samples were allowed to stand at room temperature for at least 30 min prior to evaluation. Samples were randomly selected and coded with three-digit random numbers and presented to the panelists at room temperature. During evaluation, the panelists were situated in private booths. Room temperature water was given to rinse the mouth between samples. The panelists evaluated each sample for appearance, color, texture, taste, flavor, juiciness and overall liking.

Mince gel added with hydrocolloids yielding the gel with the high stability after freeze-thawing were used for further study on gel improvement and freeze-thaw stability study.

3.2.2 Effect of iota carrageenan (IC) and modified starch (Gelpro HC30) (MS) at different levels on the properties and freeze-thaw stability of bigeye snapper mince gels

Iota carrageenan and modified starch (Gelpro HC30) at different levels (0, 1, 2, 3, 5, 7 and 10%, (w/w)) were added into the mince gel. Gel preparation was performed as described in section 3.1.1. Gels were subjected to freeze-thawing with different cycles (0, 1, 3 and 5 cycles). Resulting gels were determined as follows:

1. Breaking force and deformation as described in sections 3.2.1.
2. Expressible moisture content as described in sections 3.2.1.
3. Color and whiteness as described in sections 3.2.1.
4. Microstructure

Microstructure of gels from mince gels added with iota carrageenan and modified starch (Gelpro HC 30) at the level yielding the lowest changes in breaking force and deformation after five freeze- thawing cycles were examined for microstructure in comparison with the control gel (without freeze-thawing). Microstructure was determined using SEM as described by Jones and Mandigo (1982). Samples were cut into a cube (4 x 4 x 4 mm) with a

razor blade. The prepared samples were fixed in 2.5% glutaraldehyde in 0.2 M phosphate buffer, pH 7.2 at room temperature for 2 h. All specimens were washed three times with deionized water for 15 min per time and dehydrated with a serial concentration of 50-100% ethanol for 15 min each. All specimens were coated with 100% gold (Sputter coater, SPI-Module, PA, USA). The microstructure was visualized using a scanning electron microscope (JEOL, JSM-5800 LV, Tokyo, Japan).

Mince gel added with iota carrageenan or modified starch at the level yielding the lowest changes induced by repeated freeze-thawing were used for further study.

3.3 Effect of Gelpro HC30 on the stability of mince and surimi gels subjected to multiple freeze-thawing

Surimi was prepared as described in Figure 16. Surimi obtained was subjected to analyses as described in section 3.1.

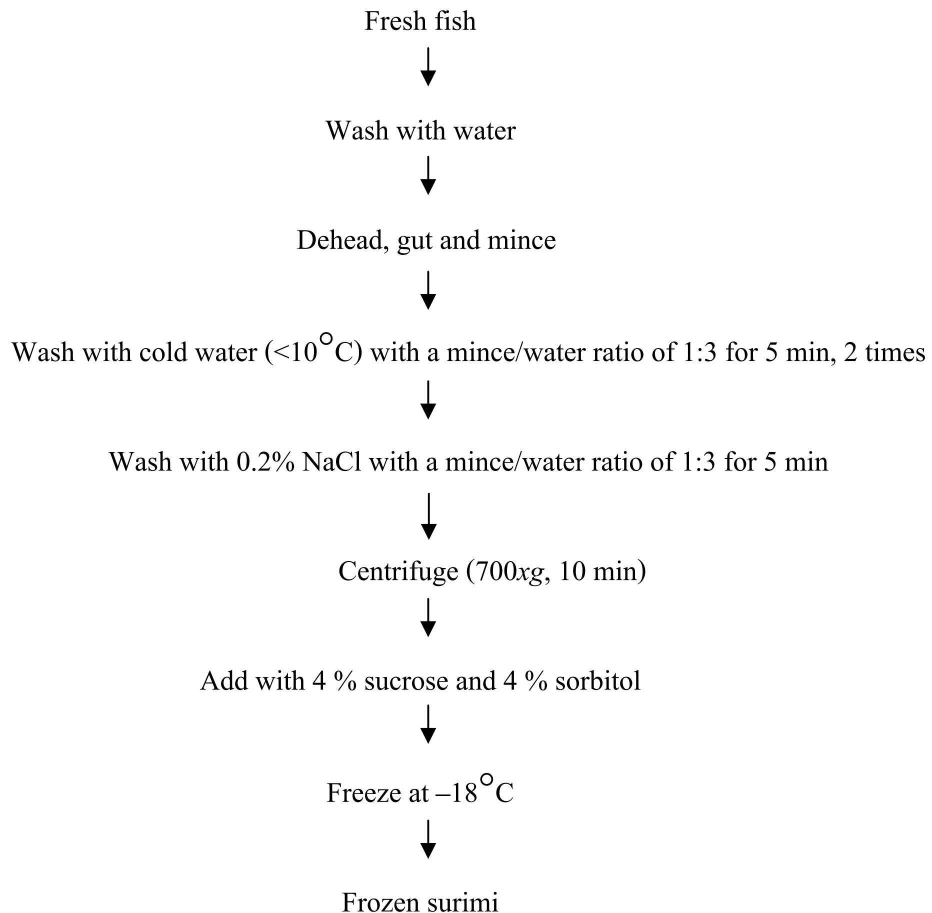


Figure 16. Scheme for surimi preparation from bigeye snapper

To study the effect of Gelpro HC30 on gel properties of mince and surimi, Gelpro HC30 at different levels (0-3%, (w/w)) was added into the mince and surimi gel of bigeye snapper. Gel from mince and surimi were prepared as described in section 3.2.1.

Gel from both mince and surimi were subjected to freeze-thawing for 0, 1, 3 and 5 cycles as described in section 3.1.1. Gels obtained were then determined as follows:

1. Breaking force and deformation as described in sections 3.2.1.
2. Expressible moisture content as described in sections 3.2.1.
3. Color and whiteness as described in sections 3.2.1.
4. Protein solubility

Protein solubility of sample gels, either freshly prepared or freeze-thawed, was determined according to the method of Benjakul and Bauer (2000) with some modifications. To 1.5 g sample, 30 ml of 2% SDS or the mixture of 1 % (w/v) SDS, 8 M urea and 2 % (v/v) β-mercaptoethanol were added and the mixture was homogenized for 1 min at speed of 12,000 rpm using a homogenizer (IKA, T25 basic, Selangor, Malaysia). The homogenate was stirred at 4°C for 4 h, followed by centrifuging at 7,500*xg* for 30 min at 4ºC using a refrigerated centrifuge (Sorvall, RC-5B plus, Norwalk, CT, USA). To 10 ml of supernatant, cold 50% (w/v) trichloroacetic acid was added to obtain the final concentration of 10%. The precipitate was recovered by centrifuging at 7,500*xg* for 30 min. The precipitate was washed with 10% trichloroacetic acid and solubilized in 0.5 M NaOH. The sample was also directly solubilized using 0.5 M NaOH and used for total protein determination. Protein content was determined using the Biuret method (Robinson and Hodgen, 1940) and expressed as the percentage of total protein in the sample.

5. Sensory evaluation

Mince and surimi gels, with and without Gelpro HC30, subjected to multiple freeze-thawing cycles (0, 1, 3 and 5 cycle) were evaluated for sensory property as described in sections 3.2.1.

6. Determination of microstructure by scanning electron microscopy (SEM)

Microstructures of mince and surimi gels without and with 1% Gelpro HC30 and subjected to 0, 1, 3 and 5 freeze-thaw cycles were visualized as described in sections 3.2.2.

3.4 Changes of chemical and physical properties of mince and surimi gels containing Gelpro HC30 during frozen storage

3.4.1 Preparation of frozen mince and surimi gel

Mince and surimi gels added with Gelpro HC30 at a level rendering the high stability after freeze-thawing (section 3.2) were prepared. Gels were placed in a polyethylene bag and sealed, under vacuum. All samples were kept at -20°C for 10 weeks. At definite time (0, 1, 2, 4, 6, 8 and 10 weeks), samples were removed, thawed with running water (26-28°C) to obtain the core temperature of 0-2°C and subjected to analyses.

3.4.2 Analyses

1. Breaking force and deformation as described in section 3.2.1
2. Expressible moisture content as described in section 3.2.1
3. Color and whiteness as described in section 3.2.1
4. Protein solubility as described in section 3.3
5. Thiobarbituric acid reactive substances (Buege and Aust, 1978)

Thiobarbituric acid reactive substances (TBARS) were determined according to the method of Buege and Aust (1978). Ground sample (0.5 g) was dispersed in 10 ml of TBARS solution (0.375 g of thiobarbituric acid, 15 g of trichloroacetic acid, and 0.875 ml of hydrochloric acid in 100 ml of distilled water). The mixture was heated in boiling water for 10 min, followed by cooling in running water. The mixture was centrifuged at 3,600xg for 20 min (MIKRO20, Hettich Zentrifugan, Germany) and the absorbance was measured at 532 nm using UV 1601 spectrophotometer (Shimadzu, Kyoto, Japan). A standard curve was prepared with malonaldehyde bis (dimethyl acetal) (MDA) at concentrations ranging from 0 to 10 ppm. TBARS was calculated and expressed as mg malondialdehyde/ kg sample.

6. Sensory evaluation as described in section 3.2.1.
7. Determination of microstructure by scanning electron microscopy (SEM)

At definite time (0, 2, 6 and 10 weeks), samples without and with 1% Gelpro HC30 were removed, thawed with running water (26-28°C) to obtain the core temperature of 0-2°C and subjected to analyses (section 3.2.2).

8. Protein patterns

At definite time (0, 2, 6 and 10 weeks), mince and surimi gels without and with 1% Gelpro HC30 were subjected to SDS-PAGE under reducing and non-reducing condition and subjected to analyses (section 3.1.4).

3.5 Effect of modified atmosphere packaging (MAP) on quality of mince gel during storage at 4°C

3.5.1 Preparation of bigeye snapper mince gel for MAP storage at 4°C

Mince gels added with 1% Gelpro HC30 were prepared as mentioned in section 3.1.1. Seven gel samples were placed on polystyrene trays. A tray containing gels was inserted in a vacuum bag (7 inch x 11 inch) with gas permeability (O_2 transmission rate of 0.66 g $m^{-2}day^{-1}$ at 23°C, 1 atm pressure) and was packaged with a gel/gas ratio of 1:3 (w/v) using a Henkovac type 1000 (Tecnovac, Italy). Gas mixtures used included 1) 60% CO_2, 35% O_2, 5% N_2; 2) 60% CO_2, 30% O_2, 10% N_2; 3) 80% CO_2, 15% O_2, 5% N_2; 4) 80% CO_2, 10% O_2, 10% N_2. Mince gels packed under MAP with different gas ratios were stored at 4°C. Samples kept in air were used as the control. All samples were taken for chemical, physical and microbiological analyses every 3 days for up to 15 days.

3.5.2 Chemical analyses

1. Determination of TVB contents as described in section 3.1.3
2. Thiobarbituric acid reactive substances as described in section 3.4.2

3.5.3 Physical analyses

1. Breaking force and deformation as described in section 3.2.1
2. Expressible moisture content as described in section 3.2.1
3. Color and whiteness as described in section 3.2.1

3.5.4 Microbiological analyses

1. Total viable count

Mince gels (50 g) were weighed into a stomacher bag containing 450 ml peptone water (2% NaCl). Blending was done in a Stomacher (M400, SEWARD, UK) for 2 min. Thereafter, the sample was diluted using peptone water in serial 10-fold steps and used as

inoculum for the three-tube MPN procedure and aerobic plate counts (APC). APC was done by the spread plate technique on plate count agar (PCA). The plates were incubated at 35°C for 2 days for counting mesophilic bacteria and at 4°C for 7 days for counting psychrophic bacteria (Speck, 1976). Microbial counts were expressed as CFU/g.

2. Lactic acid bacteria count

Mince gels were blended as previously described (section 3.5.4). A series of ten-fold dilutions was made using the saline solution for microbiological analyses. Lactic acid bacteria were counted in double-layer in Man rogaso sharpe (MRS) agar and incubated at 35°C for 3 days according to the method of Ordonez *et al*. (1991). Microbial counts were expressed as CFU/g.

3. Yeast

Mince gels were blended as previously described (section 3.5.4). A series of ten-fold dilutions was made using the saline solution for microbiological analyses. Yeast were counted in PDA agar and incubated at 35°C for 3 days according to the method of Speck (1976). Microbial counts were expressed as CFU/g.

4. Statistical analysis

All experiments were run with triplicate determination and CRD (Completely Randomized Design) was used. For sensory evaluation, twelve determinations were performed and RCBD (Randomized Completely Block Design) was implemented. Analysis of variance (ANOVA) was performed and mean comparisons were carried out by Duncan's multiple range tests (Steel and Torrie, 1980). Analyses were conducted using a SPSS package (SPSS 11.0 for windows, SPSS Inc, Chicago, IL).

CHAPTER 3

RESULTS AND DISCUSSION

1. Chemical composition and property of bigeye snapper mince and surimi

1.1 Proximate composition

Proximate composition of mince and surimi is shown in Table 1 mince and surimi contained high moisture content, accounting for 79.29% and 75.02%, respectively. Protein contents of mince and surimi were 86.86% and 71.66%, respectively. The pH of mince and surimi was 6.06. Trace amounts of ash, fat, sodium chloride and TMA were found. After washing, fat and TVB contents decreased to a high extent. Blood, pigments, odors, enzymes, mucus, and some water-soluble proteins are removed with washing process (Miyauchi *et al*., 1973; Okada *et al*., 1973; Lee, 1984; Babbitt, 1986; Chou, 1993; Hoke, 1993). Low molecular weight nonprotein nitrogen components are water soluble ranging from 9% to 18% of the total nitrogen in fish (Huss, 1988). Those constituents might be removed during washing process. High content of carbohydrate in surimi was most likely from cryoprotectant added. Reduction of fat content might increase the oxidative stability of washed mince or surimi. This might be associated with the less fishy odor in surimi.

Table 1 Proximate composition of mince and surimi from bigeye snapper

Compositions (%)	Mince	Surimi
Moisture	79.29±0.67	75.02±0.66
Protein*	89.86±0.23	71.66±0.33
Fat*	4.92±0.10	0.85±0.12
Ash*	5.21±0.02	3.51±0.05
Carbohydrate*	0.01±0..23	23.98±0.12
NaCl	0.021±0.0038	0.012±0.007
pH	6.06±0.02	6.06±0.04
TVB (mgN/100g sample)	7.00±0.71	0.84±0.71
TMA (mgN/100g sample)	0.56±0.01	0.53±0.01

Values are given as mean ± SD from triplicate determinations.

*dry weight basis

1.2 Protein pattern

Protein patterns of mince and surimi produced from bigeye snapper are shown in Figure 17. MHC and actin band intensity of mince was lowered than that of surimi. During surimi process, washing might remove sarcoplasmic proteins as well as the degradation products, associated with endogenous and microbial proteinases. Since most of sarcoplasmic protein and connective tissue are generally removed during surimi processing, myofibrillar proteins in muscle become concentrated and thus play an essential role in muscle functional properties, especially gelling properties (Foegeding *et al.,* 1996).

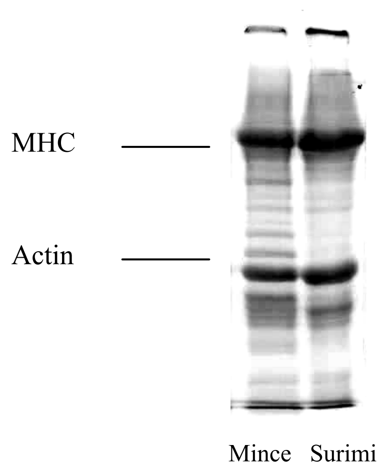


Figure 17. SDS-PAGE patterns of protein in mince and surimi produced from bigeye snapper

MHC: Myosin heavy chain

2. Effect of some hydrocolloids on the properties and freeze-thaw stability of fish mince gels

2.1 Breaking force and deformation

Effects of different hydrocolloids (xanthan gum, locust bean gum, iota carrageenan and modified starches including (Gelpro HC30, Gelpro HC715 and Bestek) at levels of 0, 2 and 5% on properties of bigeye snapper mince gels subjected to multiple freeze-thaw cycles are shown in Tables 2 and 3. Increases in breaking force and deformation were observed in the control mince gel (without hydrocolloids) when the freeze-thaw cycles increased ($p < 0.05$). It indicated that the control gel became more rubbery in texture. This suggested that mechanical damage was more pronounced in the control gel, particularly with repeated freeze-thawing. The freezing process induces muscle tissue changes by the formation and accretion of ice crystals,

dehydration, and increases in solutes (Shenouda, 1980). Dehydration of the proteins favors crystal formation and also protein aggregation, which increases the density of the protein matrix and hence breaking force (Sikorsky and Kolakowska, 1994). The drip loss from ice crystals in the gel is not retained in the gel network. Most of drip in gel is retained and the causes a soggy and spongy texture (Lee, 1984). During multiple freeze-thaw cycles, recrystallization of water or exudates may increase protein denaturation and fiber shrinkage by mechanical damage (Sikorsky, 1976).

Gels added with all hydrocolloids at both levels, had the decreases in breaking force, except those added with 5% iota carrageenan, 2 and 5% Gelpro HC30, which had the increased breaking force. Among all mince gels, those added with 2 and 5% xanthan gum possessed the lowest breaking force ($p<0.05$). The presence of xanthan gum produced a decrease in the gel forming capacity of the myofibrillar protein, which was reflected in poorer resistance to the folding test and lower values of most of the other properties (Montero *et al*., 2000). Anionic hydrocolloids could distribute throughout the protein matrix, probably through interaction with the myofibrillar protein. However, neutral hydrocolloids were dispersed throughout the matrix but did not interact with it (Montero *et al*., 2000). Additionally, the highest deformation was noticeable in gels added with Gelpro HC30 at both levels. Nevertheless, decreases in both breaking force and deformation were found when Gelpro HC715 was added ($p<0.05$). Hydrocolloids are ordinarily added to myosystems in the form of an unhydrated, dry powder, since water is a limiting factor affecting the texture of the final product (Filipi and Lee, 1998). Interfering effect of hydrocolloids on the cross linking required for the protein gel network formation is associated with gel weakening. In addition, there are important differences between myosystems, even though they all consist of myofibrillar proteins (Troy *et al*., 1999). Carrageenans are sulphated polysaccharides (Belton *et al*., 1985, 1986). Kappa-carrageenan solubilizes at 60–70°C, when the myosin has already begun to gel, whereas iota carrageenan solubilizes at 50°C before the myofibrillar protein has gelled and is thus better able to penetrate the protein matrix (Trius *et al*., 1994, 1995). Giant squid gels containing 2% iota carrageenan had particularly high values of adhesiveness and elasticity (Montero *et al*., 2000). The addition of 0.5% kappa carrageenan and iota carrageenan increased hardness of the pork sausage (De Freitas *et al*., 1997). Go´mez-Guille´n *et al* (1996) suggested that iota carrageenan forms a fine three

dimensional network with some points of connection with the protein matrix. In addition, there is considerable continuity of the protein matrix and the hydrocolloid all over the cavity surface. However, Go´mez-Guille´n and Montero (1997) found a decrease in breaking deformation of gels in giant squid (*Dosidicus gigas*) gels containing carrageenan. On the other hand, Nakayama *et al*. (1988) reported the increased penetration in sardine mince gels added with iota carrageenan. Perez-Mateos and Montero (2000) reported that higher percentages of hydrocolloids (locust bean gum, guar gum, xanthan gun, carboxymethylcellulose) significantly reduced the rheological properties of blue whiting (*Micromesistius poutassou*) gels. The addition of certain hydrocolloids resulted in differing gel properties of mince, depending on the functional properties of the mince (Gomez-Guillen and Montero, 1996).

With increasing freeze-thaw cycles, the increases in both breaking and deformation were found. In the presence of hydrocolloids, the rate of increase was lowered. However, the lowering of the increases in both breaking force and deformation varied with hydrocolloids. Among all hydrocolloids tested, Gelpro HC30 yielded the gel with no much changes in breaking force and deformation as freeze-thaw cycles increased. As starch granules absorb water from the surroundings during heating, the expanded starch granules exert pressure to the gel matrix, resulting in increased gel strength (Lee *et al*., 1992). Modified starch (Cross-linking) inhibit granule swelling on gelatinization and gives increased stability to acid, heat treatment, and shear force. Stabilized starches resist retrogradation and provide excellent freeze stability (Tuschhoff, 1986; Thosmas and Atwell, 1999; Jang, 1999). Peremans (1997) showed that hydroxypropyl crosslinked starches improved pie filling structure and texture after freezing and thawing. No syneresis was found and no differences were noted in terms of texture, structure and transparency. Hydroxypropyl distarch phosphate completely inhibits recrystallization of starch after ten freeze-thaw cycles. Those starches that were most stable to freeze-thawing are also most stable to refrigerated storage (White *et al*., 1989).

Table 2 Breaking force of mince gels from bigeye snapper added with various hydrocolloids at different levels and subjected to different freeze-thaw cycles

Hydrocolloids	Concentration (%)	Freeze-thaw cycle			
		0	1	3	5
Control		483.48±6.94c,B	544.15±22.28b,C	490.00±18.63b,C	1021.25±20.01a,A
Xanthan	2	202.10±4.62g,C	222.08±5.26f,B	324.37±25.83g,A	323.33±3.76f,A
	5	103.73±5.58h,D	209.93±0.80f,B	165.6±6.84h,C	313.65±3.18f,A
Locust bean gum	2	497.65±31.88c,B	511.13±11.78b,B	580.75±15.94e,A	476.30±22.69d,B
	5	371.70±32.46e,B	435.53±21.76c,A	375.3±16.58g,B	401.20±15.86e,B
Iota carrageenan	2	480.53±9.42c,B	312.10±13.52ed,C	483.23±17.44f,B	747.675±9.22a,A
	5	712.70±22.71a,B	535.20±22.07b,C	799.1±30.73a,A	777.70±32.28a,A
Gelpro HC30	2	623.98±27.26b,B	535.75±21.38b,D	662±7.43cd,AB	674.90±25.45b,A
	5	611.10±13.16b,AB	586.85±16.76a,B	646.92±27.54d,A	656.90±28.44b,A
Gelpro HC715	2	334.53±4.17f,B	301.63±13.15e,C	481.9±23.90f,A	471.40±6.23d,A
	5	342.65±31.26f,B	341.53±31.99d,B	354.45±18.51g,B	454.08±23.63d,A
Bestek	2	437.78±12.96d,C	465.40±13.32c,B	526.95±29.06f,A	569.73±24.07c,A
	5	414.90±4.67d,B	398.53±24.49cd,B	453.5±16.38f,A	433.83±4.57de,B

Values are given as mean ±SD from five determinations. Different letters in the same column indicate significant differences ($p<0.05$). Different capital letters in the same row indicate significant differences ($p<0.05$).

Table 3 Deformation of mince gels from bigeye snapper added with various hydrocolloids at different levels and subjected to different freeze-thaw cycles

Hydrocolloids	Concentration (%)	Freeze-thaw cycle			
		0	1	3	5
Control		7.22±0.39c,C	7.07±0.19bc,C	8.50±0.48a,B	11.94±0.52a,A
Xanthan	2	4.42±0.30e,A	4.13±0.06fg,A	1.72±010g,B	1.38±0.38g,B
	5	3.44±0.43f,A	3.50±0.37g,A	1.19±0.78g,B	0.99±0.34g,B
Locust bean gum	2	6.39±0.29d,A	6.39±0.82de,A	5.51±0.32c,B	4.72±0.34e,C
	5	4.09±0.12ef,A	4.26±0.31f,A	3.91±0.39ef,A	3.77±0.39F,A
Iota carrageenan	2	7.68±0.72c,A	6.59±0.48cd,B	4.30±0.17de,D	5.08±0.54e,C
	5	7.65±0.37c,A	7.30±0.19bc,A	5.28±0.39c,B	6.50±0.75d,A
Gelpro HC30	2	9.97±0.67a,A	9.45±0.71a,A	6.55±0.42b,B	9.80±0.57c,A
	5	8.82±0.71b,B	7.63±0.69b,C	6.57±0.35b,C	11.13±0.84b,A
Gelpro HC715	2	5.82±0.26d,A	4.78±0.17f,B	3.59±0.22f,C	4.42±0.18ef,B
	5	4.40±0.31e,B	4.35±0.35f,B	3.65±0.40f,B	4.43±0.25ef,A
Bestek	2	6.29±0.11d,B	7.27±0.78bc,A	4.40±0.32de,C	4.64±0.24e,C
	5	6.08±0.71d,A	5.76±0.23e,B	4.67±0.43d,C	4.72±0.30e,C

Values are given as mean ±SD from five determinations. Different letters in the same column indicate significant differences ($p<0.05$). Different capital letters in the same row indicate significant differences ($p<0.05$).

2.2 Expressible moisture content

Table 4 shows the changes in expressible moisture content as affected by the addition of hydrocolloids and multiple freeze-thaw cycles. The marked decreases in expressible moisture content were observed with addition of all hydrocolloids ($p<0.05$). Hydrocolloids at 5% resulted in the lower expressible moisture content than at 2%. However, gel added with xanthan gum at a level of 5% had the higher expressible moisture content than that containing 2% xanthan gum. Those hydrocolloids might hold the water in the interstitial spaces of the gel network to a high extent. Nakayama *et al.* (1988) reported the increased water holding capacity in sardine muscle mince gels added with iota carrageenan. Increased water holding capacity was reported in beef gels (Foegeding and Ramsey, 1987), in surimi gels (Bullens *et al*., 1990) and in giant squid (*Dosidicus gigas*) gels (Go´mez- Guille´n and Montero, 1997) with addition of iota carrageenan. Xiong *et al.* (1999) pointed out that the main advantage of polysaccharide gums seemed to be more to improve water binding in low-fat beef sausage than to improve texture. Gums and myofibrillar proteins, as charged, may compete for water required to form a viscous sol or a viscoelastic gel, thereby interfering with their structure-forming ability and gel matrix formation (Xiong *et al.*, 1999). Additionally, starches are known as a gelling agents with their water-holding and thickening properties (Dickinson, 2003; Singh *et al*., 2003), thereby contributing to the structural and textural properties. After freeze-thawing, the control gels had the slight increase in expressible moisture content ($p<0.05$), particularly after 3 freeze-thaw cycles. No marked changes in expressible moisture content were observed in the gel added with Gelpro HC715 and Bestek at both levels with increasing freeze-thaw cycles ($p>0.05$). On the other hand, the expressible moisture content increased in all gels added with other hydrocolloids as freeze-thaw cycles increased ($p<0.05$).

From the results, the ability of gel in holding water varied, depending upon the types of hydrocolloids used. This might be associated with the resistance to freeze-thawing of different hydrocolloids.

Table 4 Expressible moisture content of mince gels from bigeye snapper added with various hydrocolloids at different levels and subjected to different freeze-thaw cycle

Hydrocolloids	Concentration (%)	Freeze-thaw cycle			
		0	1	3	5
Control		3.84±0.062a,B	3.46±0.050a,C	3.99±0.032a,A	3.80±0.043a,B
Xanthan gum	2	1.74±0.012d,C	1.83±0.059e,C	2.51±0.043b,A	2.28±0.035b,B
	5	2.28±0.073c,C	3.24±0.078b,B	4.72±0.117a,A	1.74±0.013cd,D
Locust bean gum	2	1.61±0.012d,A	1.96±0.004e,A	1.83±0.021d,A	1.82±0.017c,A
	5	0.89±0.018f,A	0.99±0.011hi,A	1.11±0.032e,A	0.79±0.005f,B
Iota carrageenan	2	1.63±0.024d,B	1.53±0.006g,B	1.64±0.051d,AB	1.81±0.005cd,A
	5	0.90±0.008f,B	0.87±0.015i,AB	1.10±0.017e,A	1.16±0.11d,A
Gelpro HC30	2	1.39±0.006e,C	1.88±0.004e,B	2.83±0.024b,A	2.04±0.020c,B
	5	0.75±0.030f,C	1.19±0.025h,B	2.24±0.154c,A	1.41±0.018d,AB
Gelpro HC715	2	2.57±0.004b,B	2.62±0.053c,A	2.72±0.014b,A	1.97±0.014c,C
	5	1.17±0.019ef,A	1.15±0.015h,A	1.21±0.009e,A	1.00±0.010e,B
Bestek	2	2.68±0.060b,A	2.16±0.044d,B	2.20±0.024c,B	2.38±0.054b,B
	5	1.33±0.005e,B	1.55±0.009f,A	1.29±0.013e,B	1.26±0.004d,C

Values are given as mean ±SD from five determinations. Different letters in the same column indicate significant differences ($p<0.05$). Different capital letters in the same row indicate significant differences ($p<0.05$).

2.3 Color and whiteness

*L**, *a** and *b**-values and whiteness of fish mince gels with and without hydrocolloids and subjected to multiple freeze-thaw cycles are presented in Tables 5, 6, 7 and 8. Higher *a**-value was observed in gel added with locust bean gum (Table 5). The increases in both *a** and *b**-values were found as the level of hydrocolloids increased from 2% to 5%. Slight changes in *a** and *b**- values were observed with increasing freeze-thaw cycles (Tables 5 and 6). However, the degree of changes was dependent on the types of hydrocolloids. *L**-value (Table 5) decreased for all gels when hydrocolloids were added, especially when the higher amount was used ($p<0.05$). Nevertheless, no changes in whiteness were found in gel added with Gelpro HC715 and Bestek at a level of 2%. A slight decrease in whiteness was found when Gelpro HC715 and Bestek at a level of 5% was used ($p<0.05$). Decrease in whiteness (Table 8) was observed in the control gel with repeated freeze-thawing. Different change in whiteness were observed among samples added with hydrocolloids when different freeze-thaw cycles were used. The changes in whiteness were in agreement with those found for *L**-value (Table 7). In general, hydrocolloids could retard thus decrease in whiteness as freeze-thaw cycles increased. It was noted that the whiteness markedly decreased in the control gel.

Table 5 L^* value of gels made from bigeye snapper mince added with various hydrocolloids at different levels and subjected to different freeze-thaw cycles

Hydrocolloids	Concentration (%)	Freeze-thaw cycle			
		0	1	3	5
Control		79.56±0.32bc,A	78.71±0.65de,B	75.59±0.21e,C	75.29±0.82f,C
Xanthan	2	80.52±0.25a,C	80.96±0.44a,BC	81.42±0.13a,B	82.26±0.47a,A
	5	79.20±0.42cd,B	80.14±0.14b,A	79.23±0.87b,B	79.84±0.57b,AB
Locust bean gum	2	76.23±0.34h,A	75.28±0.42g,B	74.47±0.42f,C	75.21±0.38f,B
	5	71.71±0.18i,A	71.26±0.12h,B	70.73±0.31g,C	71.48±0.40g,AB
Iota carrageenan	2	78.60±0.36e,C	78.24±0.32e,C	79.38±0.24b,A	79.88±0.28b,B
	5	78.79±0.32e,B	78.92±0.35cd,B	79.04±0.52bc,A	79.81±0.43b,B
Gelpro HC30	2	77.12±0.23g,A	76.69±0.52f,A	77.28±0.50d,A	77.39±0.66d,A
	5	77.56±0.36f,A	76.54±0.55f,B	75.52±0.48e,C	76.07±0.34e,BC
Gelpro HC715	2	79.46±0.15bc,AB	79.76±0.24b,A	78.50±0.66c,C	79.10±0.40c,B
	5	78.86±0.21de,A	78.36±0.23e,B	77.23±0.33d,D	77.80±0.25d,C
Bestek	2	79.77±0.21b,A	79.24±0.23c,B	77.26±0.35d,D	77.75±0.12d,C
	5	78.62±0.28e,A	78.38±0.26e,A	77.73±0.39d,B	78.69±0.37e,A

Values are given as mean ±SD from five determinations. Different letters in the same column indicate significant differences ($p<0.05$). Different capital letters in the same row indicate significant differences ($p<0.05$).

Table 6 *a** value of gels made from bigeye snapper mince added with various hydrocolloids at different levels and subjected to different freeze-thaw cycles.

Hydrocolloids	Concentration (%)	Freeze-thaw cycle			
		0	1	3	5
Control		-1.06±0.09b,A	-1.00±0.13def,A	-1.36±0.12e,B	-1.04±0.07e,A
Xanthan	2	-2.89±4.42c,A	-1.05±0.09ef,A	-1.10±0.03cde,A	-1.04±0.07e,A
	5	-0.51±0.07b,A	-0.70±0.07c,B	-0.72±0.07c,B	-0.58±0.11c,A
Locust bean gum	2	0.28±0.04b,A	0.08±0.09b,C	0.38±0.20b,A	0.23±0.17b,AB
	5	2.03±0.08a,AB	1.89±0.10a,B	2.04±0.12a,AB	2.13±0.17a,A
Iota carrageenan	2	-1.07±0.09b,A	-1.18±0.07fgB,	-1.05±0.03cde,A	-1.03±0.01e,A
	5	-0.73±0.12b,A	-0.89±0.07de,C	-0.88±0.05cd,BC	-0.78±0.06d,AB
Gelpro HC30	2	-1.54±0.09bc,B	-1.62±0.06h,B	-0.93±0.71cd,A	-1.51±0.11g,B
	5	-1.29±0.08bc,A	-1.45±0.09h,A	-1.23±0.61de,A	-1.49±0.09g,A
Gelpro HC715	2	-1.04±0.12b,AB	-1.04±0.08ef,AB	-0.94±0.08cd,A	-1.10±0.08e,B
	5	-0.99±0.05b,AB	-0.83±0.41cd,A	-1.18±0.07de,B	-1.07±0.15e,AB
Bestek	2	-1.02±0.08b,A	-1.11±0.04fg,A	-1.41±0.07e,B	-1.37±0.10fg,B
	5	-1.07±0.05b,A	-1.24±0.03g,B	-1.27±0.06de,B	-1.25±0.16f,B

Values are given as mean ±SD from five determinations. Different letters in the same column indicate significant differences ($p<0.05$). Different capital letters in the same row within indicate significant differences ($p<0.05$).

Table 7 *b** value of gels made from bigeye snapper mince added with various hydrocolloids at different levels and subjected to different freeze-thaw cycles

Hydrocolloids	Concentration (%)	Freeze-thaw cycle			
		0	1	3	5
Control		14.47±0.17f,A	14.54±0.15de,A	14.21±0.14de,B	14.20±0.13f,B
Xanthan	2	14.16±0.20g,A	14.07±0.20gh,A	14.12±0.16e,A	14.12±0.12fg,A
	5	14.63±0.12ef,A	14.36±0.16ef,B	14.59±0.09c,A	14.73±0.19d,A
Locust bean gum	2	14.20±0.12g,A	14.19±0.10fg,A	14.10±0.09e,AB	13.95±0.18g,B
	5	15.29±0.24ab,A	15.12±0.10a,A	15.20±0.21ab,A	15.22±0.19ab,A
Iota carrageenan	2	14.93±0.17cd,A	15.04±0.17a,A	15.16±0.15ab,A	15.08±0.17bc,A
	5	15.40±0.34a,A	14.97±0.23ab,B	14.95±0.21b,B	14.83±0.20d,B
Gelpro HC30	2	14.02±0.15g,B	13.92±0.22h,B	14.41±0.15cd,A	14.47±0.21e,A
	5	14.72±0.12de,B	14.59±0.19cd,B	15.12±0.37b,A	14.75±0.25d,B
Gelpro HC715	2	14.45±0.10f,B	14.77±0.11bc,A	14.57±0.24c,B	14.82±0.07d,A
	5	15.14±0.14bc,B	14.89±0.15ab,C	15.40±0.07a,A	15.34±0.06a,A
Bestek	2	14.51±0.12ef,AB	14.48±0.12de,B	14.62±0.15c,AB	14.69±0.14d,A
	5	14.75±0.14de,B	14.93±0.10ab,AB	15.07±0.08b,A	14.94±0.22cd,AB

Values are given as mean ±SD from five determinations. Different letters in the same column indicate significant differences ($p<0.05$). Different capital letters in the same row indicate significant differences ($p<0.05$).

Table 8 Whiteness of gels made from bigeye snapper mince added with various hydrocolloids at different levels and subjected to different freeze-thaw cycles

Hydrocolloids	Concentration (%)	Freeze-thaw cycle			
		0	1	3	**5**
Control		79.24±0.32bc,A	78.40±0.64de,B	75.33±0.21e,C	75.03±0.81f,C
Xanthan	2	80.23±0.24a,C	80.62±0.44a,BC	81.07±0.13a,B	81.89±0.46a,A
	5	78.87±0.42cd,B	79.80±0.14b,A	78.89±0.85b,B	79.50±0.56b,AB
Locust bean gum	2	75.93±0.34h,A	74.99±0.42g,B	74.19±0.41f,C	74.93±0.37f,B
	5	71.40±0.18i,A	70.97±0.11h,B	70.44±0.31g,C	71.18±0.40g,AB
Iota carrageenan	2	78.28±0.35e,C	77.93±0.32e,C	79.04±0.24b,B	79.54±0.27b,A
	5	78.44±0.32e,B	78.59±0.34cd,B	78.71±0.51bc,B	79.46±0.42b,A
Gelpro HC30	2	76.85±0.22g,A	76.42±0.51f,A	76.98±0.50d,A	77.10±0.65d,A
	5	77.26±0.35f,A	76.26±0.54f,B	75.24±0.47e,C	75.80±0.33e,BC
Gelpro HC715	2	79.13±0.15bc,AB	79.43±0.24b,A	78.18±0.65c,C	78.77±0.39c,B
	5	78.53±0.21de,A	78.03±0.22e,B	76.92±0.33d,D	77.48±0.25d,C
Bestek	2	79.44±0.20b,A	78.92±0.23c,B	76.97±0.34d,D	77.45±0.13d,C
	5	78.30±0.28e,A	78.06±0.25e,A	77.42±0.39d,B	78.37±0.37c,A

Values are given as mean ±SD from five determinations. Different letters in the same column indicate significant differences ($p<0.05$). Different capital letters in the same row indicate significant differences ($p<0.05$).

2.4 Acceptability

Likeness scores of mince gels with and without hydrocolloids and subjected to multiple freeze-thaw cycles are depicted in Figure 18. Addition of hydrocolloids had no impact on overall linking, color, appearance, texture and juiciness, except locust bean gum which caused the lower liking for all attributes mentioned above. Xanthan gum (2%) also lowered juiciness score of resulting gel. Gum as dry powder was not soluble completely, thereby interfering gel matrix formation and resulting in lower scores of likeness. It was noted that iota carrageenan at a level of 2% rendered the gel with the highest texture score. After being subjected to 5 freeze-thaw cycles, the control gel and gel added with locust bean gum had the lowest overall score ($p<0.05$). Lowest appearance and color score were observed in gel added with locust bean gum ($p<0.05$). Addition of 2% iota carrageenan or 2% Gelpro HC30 rendered the gel with the highest overall liking after 5 freeze-thaw cycles, and showed no much changes in mechanical properties after freeze-thawing. Thus, iota carrageenan and Gelpro HC30 at 2% were used as the most appropriate hydrocolloids to reduce the quality losses of mince gel from bigeye snapper induced by freeze-thawing.

Mince gels added with 2 % iota carrageenan and 2% Gelpro HC30 exhibited the highest breaking force and deformation and could lower the expressible moisture content. Both hydrocolloids were found to improve both textural property and water holding capacity of resulting gels. Also, the use of Gelpro HC30 and iota carrageenan rendered the gel with the high acceptability score. Therefore, both hydrocolloids were used for further study.

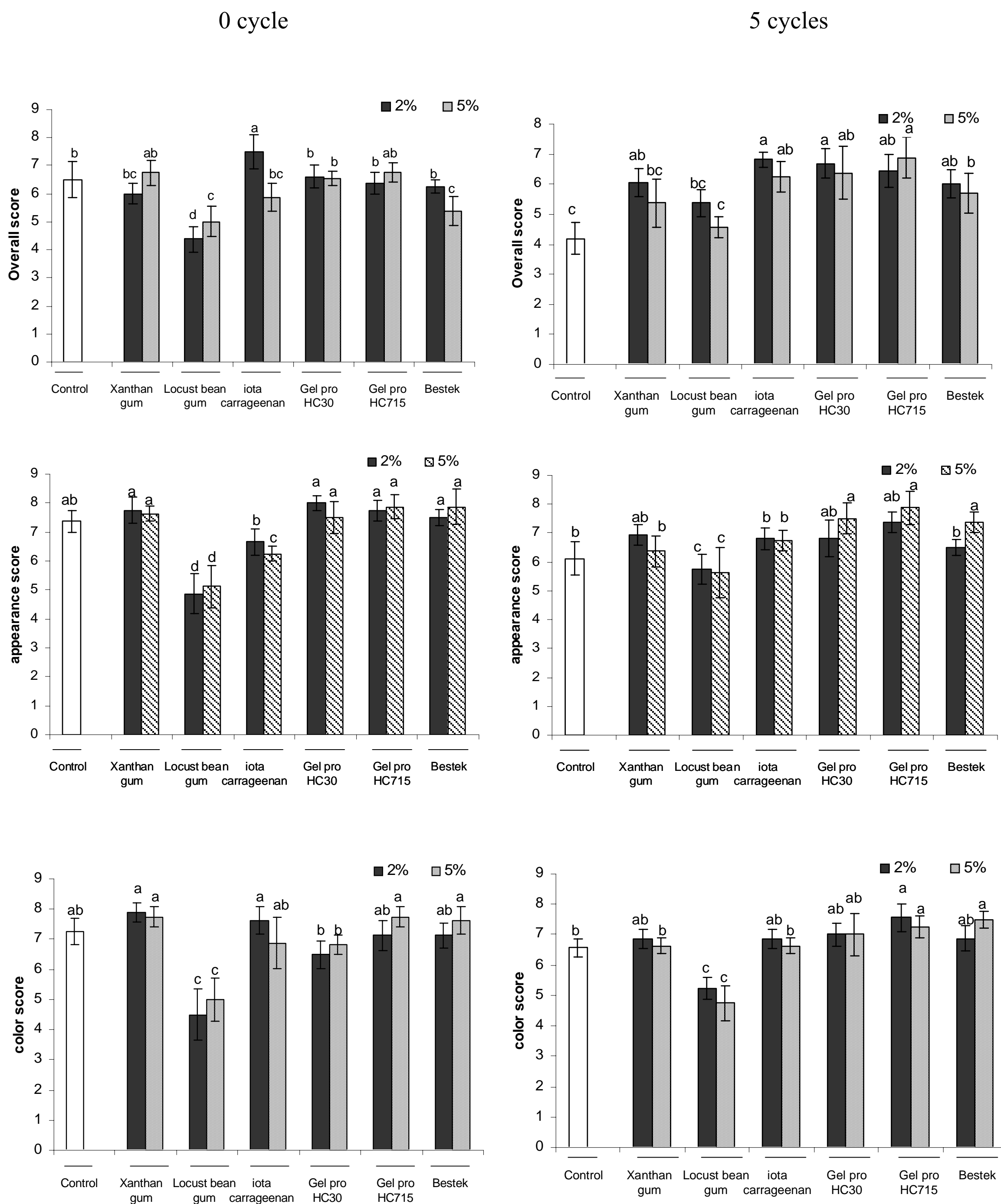


Figure 18. Likeness score of mince gels from bigeye snapper added with various hydrocolloids at different levels and subjected to different freeze-thaw cycles. Bars represent standard deviations from five determinations. Different letters on the bars indicate significant differences ($p<0.05$).

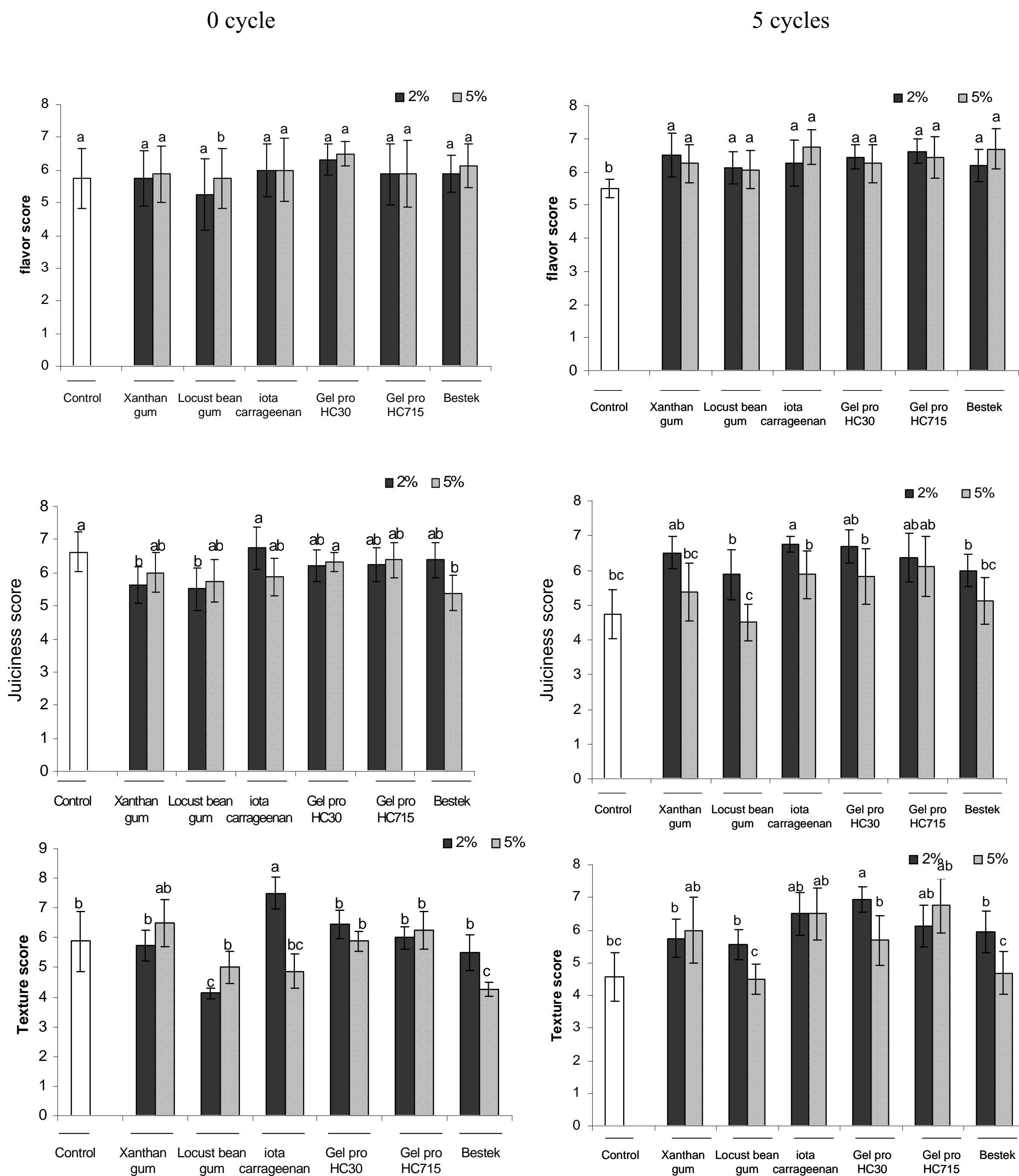


Figure 18. Likeness score of mince gels from bigeye snapper added with various hydrocolloids at different levels and subjected to different freeze-thaw cycles. (Continued). Bars represent standard deviations from five determinations. Different letters on the bars indicate significant differences ($p<0.05$).

3. Effect of iota carrageenan (IC) and modified starch (Gelpro HC30) at different levels on the properties and freeze-thaw stability of bigeye snapper mince gels

3.1 Breaking force and deformation

Breaking force and deformation of gels from bigeye snapper mince added with IC and Gelpro HC30 at various levels (0, 1, 2, 5, 7 and 10%) and subjected to multiple freeze-thaw cycles are shown in Tables 9 and 10. Addition of Gelpro HC30 or IC increased the breaking force of resulting gels, especially as the amount added increased. Addition of Gelpro HC30 at level of 10% decreased the deformation of resulting gel ($p<0.05$). It was noted that the continuous decreases in deformation were found with increasing amounts of IC added ($p<0.05$). Gel-forming capacity of Alaska pollack surimi was found to be improved by the addition of iota- and kappa-carrageenans due to the interaction of carrageenan sulphate groups and fish protein (Llanto *et al*., 1990). Nakayama *et al.* (1988) also reported increased gel strength upon adding iota-carrageenan to sardine muscle mince gels. Polysaccharides have been extensively used in meat product manufacturing to provide the emulsifying, viscous and gelation properties in such products (Chin, *et al*., 1998; Glicksman, 1983). Among them, starch is the most widely used as 'filler' ingredient in surimi products. Added ingredients will be entrapped within the surimi protein gel matrix and thus fill the gel (Lee *et al.,* 1992), exerting their functional effects through influencing the formation of the continuous surimi gel matrix during heat induced gelation or modifying the viscosity, mobility and other properties of the aqueous phase. Lee and Chung (1989) mentioned that several factors will influence the physical properties of filled surimi gel including the integrity and properties of the primary gel matrix, the properties of the dispersed coingredients, and the interactions between the continuous matrix and dispersed coingredients.

With increasing freeze-thaw cycles, the control gel had the marked increases in breaking force and deformation, suggesting that the gels become more rubbery. When Gelpro HC30 was added at levels of 5-10%, breaking force of 940-1071 g was obtained after 5 freeze-thaw cycles. For gel added with IC, those added with IC at 2-3% showed the similar value after 5 freeze-thaw cycles. Therefore, addition of Gelpro HC30 or IC at appropriate levels should retard the formation of rubbery texture of mince of mince gel induced by multiple freeze-thawing.

Table 9 Breaking force of mince gels from bigeye snapper added with IC and Gelpro HC30 at different levels and subjected to different freeze-thaw cycles.

Hydrocolloids	Concentration (%)	Freeze-thaw cycle			
		0	1	3	5
control		840.53±12.76g,D	909.33±21.97e,C	984.61±36.86e,B	1308.95±60.86a,A
Gel pro HC30	1%	956.95±13.51e,B	926.25±16.70de,B	793.10±29.02g,C	1113.28±62.90b,A
	2%	991.40±30.20de,AB	979.28±9.53c,B	1024.95±35.91d,A	982.28±29.21c,B
	3%	1145.81±41.94a,A	960.05±30.85cdC,	978.05±26.98e,C	1060.80±53.98b,B
	5%	967.70±3.23e,A	860.03±24.95f,D	907.38±3.52f,C	940.90±25.03c,B
	7%	1111.55±43.03a,AB	961.45±32.34cd,B	917.58±2.22f,C	1086.38±48.58b,A
	10%	900.90±30.23f,B	801.53±11.05g,C	926.03±16.96f,B	1071.10±18.19b,A
Iota carrageenan (IC)	1%	1012.53±6.11cdC,	858.75±33.17f,D	1111.75±35.93bc,B	1227.15±115.24a,A
	2%	984.15±4.95e,C	958.95±23.72cd,C	1258.18±24.84a,A	1076.93±23.89b,B
	3%	996.58±27.55deB,	948.70±44.85cde,B	1140.33±30.29b,A	1111.98±79.12b,A
	5%	1038.23±4.41c,B	986.33±13.26c,C	1095.78±18.67c,A	1079.35±21.23b,A
	7%	1137.55±43.34a,B	1237.25±26.54a,A	1231.35±21.54a,A	1257.63±86.23a,A
	10%	1081.83±18.78ab,B	1264.88±27.27a,A	1095.43±20.25c,B	1230.65±64.09a,A

Values are given as mean ±SD from five determinations. Different letters in the same column indicate significant differences ($p<0.05$). Different capital letters in the same row indicate significant differences ($p<0.05$).

Table 10 Deformation of mince gels from bigeye snapper added with IC and Gelpro HC30 at different levels and subjected to different freeze-thaw cycles.

Hydrocolloids	Concentration	Freeze-thaw cycle			
	(%)	0	1	3	5
Control		7.93±0.24d,C	9.42±0.21a,B	9.67±0.06a,B	12.47±0.29a,A
Gel pro HC30	1%	9.33±0.24ab,B	8.54±0.78bc,B	8.73±0.46cd,B	12.35±0.31a,A
	2%	8.84±0.82bc,BC	8.01±0.30cd,C	9.51±0.24ab,B	11.42±0.31b,A
	3%	9.65±0.65a,B	8.85±0.42b,C	8.92±0.35bc,BC	10.99±0.43b,A
	5%	7.94±0.36d,B	7.60±0.23de,BC	7.13±0.44fg,C	8.58±0.33de,A
	7%	8.52±0.45cd,B	7.34±0.28e,C	7.18±0.17fg,C	9.59±0.48c,A
	10%	6.40±0.25f,C	6.40±0.17f,C	8.14±0.31de,B	8.69±0.26d,A
iota carrageenan (IC)	1%	8.18±0.19d,C	7.70±0.39deC,	9.12±0.72abc,B	11.09±0.41b,A
	2%	7.29±0.21e,B	7.37±0.46e,B	8.89±0.33bc,A	8.73±0.23d,A
	3%	6.51±0.12f,C	6.59±0.52f,C	7.68±1.09ef,B	8.69±0.27d,A
	5%	5.63±0.14g,D	6.24±0.24f,C	7.21±0.21fg,B	7.75±0.13fg,A
	7%	4.98±0.10h,C	6.69±0.31f,B	6.67±0.21gh,B	7.83±0.35fg,A
	10%	4.86±0.47h,C	6.48±0.36f,B	6.20±0.40h,B	7.64±0.32g,A

Values are given as mean ±SD from five determinations. Different letters in the same column indicate significant differences ($p < 0.05$). Different capital letters in the same row indicate significant differences ($p < 0.05$).

3.2 Expressible moisture content

The effect of Gelpro HC30 and IC on expressible moisture content of mince gel with multiple freeze-thaw cycles is shown in Table 11. The content of water released is inversely associated with the water holding capacity. The continuous decreases in expressible moisture content were observed as both Gelpro HC30 and IC increased up to 10% ($p<0.05$). As freeze-thaw cycles increased, the control gel had the increase in expressible moisture content ($p<0.05$). No changes in expressible moisture content were observed in the gel added with Gelpro HC30 greater than 5% ($p>0.05$) after 5 freeze-thaw cycles. The result indicated that Gelpro HC30 improved the water holding capacity of mince gel. Takagi and Shimidu (1972) reported that hydrocolloids act as network fillers and strengtheners. Their high water holding capacity causes them to swell and augment elasticity by reducing the moisture content of the mesh (Niwa *et al.,* 1988; Iso *et al.,* 1985) and increasing the density of the surrounding protein matrix (Niwa *et al.,* 1989).

For gel added with IC, higher expressible moisture content was observed, compared with that found in gel added with Gelpro HC30 at the same level. This indicated the poorer water holding capacity of IC. These results suggested that during the gelling of fish mince containing Gelpro HC30, Gelpro HC30 might hold water to a greater extent, especially when the amount increased.

The mechanisms whereby starch and carrageenan act were presumed to be different. Starch shows little affinity for myofibrillar protein during heating and thus does not interact with the protein matrix. According to Ziegler and Foegeding (1990), starch acts as a “simple” or “passive filler”. With regard to carrageenan, kappa-carrageenan (Llanto *et al*. 1990) and iota carrageenan (Niwa, 1992) bind the water in the system without any linkage to the myofibrillar protein. Therefore, Gelpro HC30 was found to be effective in holding the water in the gel matrix, even after repeated freeze-thawing.

Table 11 Expressible moisture content of mince gels from bigeye snapper added with Gelpro HC30 and IC at different levels and subjected to different freeze-thaw cycles.

Hydrocolloids	Concentration	Freeze-thaw cycle			
	(%)	0	1	3	5
Control		2.34±0.12ab,B	2.48±0.20a,B	3.01±0.29a,AB	3.54±0.03b,A
Gelpro HC30	1%	1.65±0.15ab,C	1.57±0.20bcdC,	2.00±0.29de,B	2.77±0.13a,A
	2%	1.33±0.06ab,B	1.40±0.21cd,AB	1.59±0.24e,AB	2.10±0.03e,A
	3%	1.14±0.17ab,C	0.80±0.14efg,D	1.52±0.13f,B	1.89±0.01f,A
	5%	0.68±0.08ab,A	0.62±0.10fg,A	0.89±0.02g,B	1.16±0.07g,A
	7%	0.50±0.13ab,A	0.60±019fg,A	0.79±0.10e,A	0.90±0.09h,A
	10%	0.30±0.05b,C	0.56±0.07g,B	0.66±0.13e,AB	0.74±0.07i,A
iota carrageenan (IC)	1%	2.07±0.11ab,C	2.07±0.21ab,C	3.21±0.13a,B	4.31±0.04a,A
	2%	1.68±0.22ab,C	1.80±0.04bc,C	3.16±0.20a,B	3.78±0.04b,A
	3%	1.36±0.26ab,C	1.16±0.21def,C	2.89±0.04ab,B	3.53±0.14c,A
	5%	1.08±0.08ab,C	1.08±0.12defg,C	2.76±0.04b,B	3.44±0.05c,A
	7%	0.81±0.13ab,D	1.64±0.13bcd,C	2.40±0.03c,B	2.89±0.08c,A
	10%	0.59±0.14ab,D	1.33±0.24cde,C	2.22±0.03d,B	2.64±0.05d,A

Values are given as mean ±SD from five determinations. Different letters in the same column indicate significant differences ($p<0.05$). Different capital letters in the same row indicate significant differences ($p<0.05$).

3.3 Color and whiteness

Color and whiteness of all mince gels from bigeye snapper with and without Gelpro HC30 and IC addition as affected by multiple freeze–thaw cycles is shown in Table 12. L*-value of the control gel decreased as the level of both hydrocolloids added increased ($p<0.05$). This was in accordance with the increases in a* and L*- values and the decreases in whiteness. However, after 5 freeze-thaw cycles, gel decreased in L*-value and whiteness, regardless of the amount of hydrocolloids added. Repeated freeze-thawing might induce the changes in color, particularly via browning reaction. Gels added with both hydrocolloids had higher L*-values and whiteness, compared with the control gel after freeze-thawing, except for gel added with 2% Gelpro HC30 ($p<0.05$). From the result, the addition of 10% IC increased the redness of mince gel as indicated by the increase in a*-value.

Table 12 *L*, a*, b**-values and whiteness of mince gels from bigeye snapper added with Gelpro HC30 and iota carrageenan at different levels and subjected to different freeze-thaw cycles.

Cycle	Treatment	Concentration (%)	L*	a*	b*	Whiteness
0	control		79.20±0.20a,A	-0.64±0.07hi,B	14.19±0.09h,B	78.88±0.20a,A
	Gelpro HC30	1%	78.36±0.31b,A	-0.75±0.10i,A	14.50±0.15g,B	78.04±0.30b,A
		2%	78.35±0.14b,B	-0.52±0.03fg,C	14.85±0.10f,B	78.02±0.13b,A
		3%	77.29±0.34c,A	-0.66±0.08h,C	14.74±0.13f,B	76.98±0.33c,A
		5%	76.88±0.30c,A	-0.43±0.07ef,BC	15.51±0.07e,AB	76.55±0.29c,A
		7%	75.60±0.29d,B	-0.31±0.08d,B	15.82±0.06d,B	75.28±0.29d,B
		10%	75.46±0.61d,A	-0.17±0.08c,B	16.70±0.19b,A	75.13±0.61d,A
	iota carrageenan (IC)	1%	78.65±0.06ab,A	-0.58±0.09gh,B	14.39±0.09g,A	78.33±0.05ab,A
		2%	77.40±0.35c,A	-0.51±0.02fg,B	14.84±0.13f,AB	77.08±0.35c,A
		3%	77.57±0.78c,A	-0.38±0.04de,BC	14.92±0.10f,B	77.25±0.77c,A
		5%	76.00±0.21d,A	-0.33±0.06d,C	15.45±0.11e,B	75.69±0.20d,A
		7%	74.76±0.63e,A	-0.07±0.05b,A	16.29±0.11c,A	74.44±0.62e,A
		10%	73.95±1.28f,AB	0.19±0.13a,A	16.98±0.33a,A	73.63±1.27f,AB
1	control		78.54±0.69ab,A	-0.65±0.04i,B	14.53±0.11ef,A	78.21±0.68ab,A
	Gelpro HC30	1%	78.37±0.31b,A	-0.44±0.10fg,B	15.15±0.23d,A	78.03±0.31b,A
		2%	79.08±0.44a,A	-0.41±0.05ef,B	15.06±0.14d,A	78.73±0.43a,A
		3%	76.96±0.35cd,AB	-0.61±0.10hi,BC	15.10±0.14d,A	76.65±0.35cd,AB
		5%	76.69±0.49de,AB	-0.46±0.08fg,C	15.47±0.17c,B	76.37±0.49de,AB
		7%	76.34±0.34ef,A	-0.31±0.06de,B	16.16±0.19b,A	76.01±0.33ef,A
		10%	75.42±0.17g,A	-0.12±0.06c,B	17.01±0.51a,A	75.08±0.17g,A
	iota carrageenan (IC)	1%	78.45±0.49b,A	-0.52±0.06gh,B	14.43±0.24f,A	78.13±0.48b,A
		2%	77.40±0.32c,A	-0.48±0.06fg,B	14.75±0.07e,BC	77.08±0.31c,A
		3%	77.35±0.51c,A	-0.44±0.10fg,C	15.07±0.18d,B	77.03±0.50c,A
		5%	76.06±0.69f,A	-0.23±0.03d,AB	15.45±0.22c,B	75.74±0.68f,A
		7%	73.93±0.11h,A	-0.02±0.07b,A	16.11±0.20b,A	73.63±0.11h,A
		10%	74.44±0.30h,A	0.09±0.12a,A	16.75±0.23a,AB	74.11±0.30h,A
3	control		78.65±0.49a,A	-0.53±0.10e,B	14.37±0.11f,AB	78.33±0.49a,A
	Gelpro HC30	1%	77.63±0.39b,B	-0.48±0.10e,B	15.05±0.15d,A	77.31±0.38b,B
		2%	76.80±0.82cd,C	-0.54±0.07e,C	14.98±0.21de,AB	76.49±0.81cd,B
		3%	76.61±0.35 cdeB,	-0.52±0.07e,B	15.15±0.13d,A	76.30±0.35cde,B
		5%	76.21±0.24 def,B	-0.34±0.06d,B	15.74±0.20c,A	75.89±0.24def,B
		7%	75.83±0.28 ef,B	-0.19±0.10bc,A	16.29±0.16b,A	75.50±0.27ef,B
		10%	73.41±0.60 I,B	-0.06±0.13b,B	16.96±0.34a,A	73.09±0.60i,B

Table 12 *L**, *a**, *b**-values and whiteness of mince gels made from bigeye snapper added with Gelpro HC30 and iota carrageenan at different levels and subjected to different freeze-thaw cycles (Continued)

Cycle	Treatment	Concentration (%)	*L**	*a**	*b**	Whiteness
		1%	77.27±0.31 bc,B	-0.54±0.07e,B	14.61±0.20f,A	76.96±0.30bc,B
		2%	76.27±0.39 def,B	-0.53±0.05e,B	14.68±0.09ef,C	75.97±0.38def,B
	iota carrageenan	3%	76.02±0.21 def,B	-0.34±0.06d,B	15.07±0.13d,B	75.71±0.20def,B
	(IC)	5%	75.46±0.43 fg,AB	-0.31±0.05cd,BC	15.68±0.06c,A	75.15±0.42fg,AB
		7%	74.71±0.94 gh,A	-0.14±0.11b,A	16.07±0.40b,A	74.40±0.94gh,A
		10%	74.32±1.32h,A	0.10±0.22a,A	16.93±0.51a,A	73.99±1.32h,A
5	control		76.86±0.63b,B	-0.29±0.11bcd,A	14.40±0.36f,AB	76.55±0.62b,A
		1%	75.54±0.49c,C	-0.38±0.17cd,B	14.69±0.34ef,B	75.25±0.49c,AA
		2%	77.71±0.27a,B	-0.19±0.08b,A	15.17±0.11cd,A	77.38±0.27a,C
		3%	75.64±0.48c,C	-0.41±0.08c,A	14.81±0.35e,B	75.34±0.47c,C
	Gelpro HC30	5%	75.45±0.47c,C	-0.20±0.08b,A	15.60±0.16b,AB	75.14±0.46c,C
		7%	73.72±0.28d,C	-0.26±0.08bc,AB	15.67±0.24b,B	73.43±0.28d,B
		10%	73.57±0.19d,B	0.08±0.11a,A	16.60±0.28a,A	73.26±0.19d,C
		1%	76.65±0.32b,C	-0.33±0.13bcd,A	14.65±0.27ef,A	76.35±0.32b,B
		2%	76.53±0.14b,B	-0.34±0.04bcd,A	14.94±0.14de,A	76.22±0.14b,B
	iota carrageenan	3%	76.42±0.48b,B	-0.19±0.07b,A	15.35±0.22bc,A	76.10±0.48b,B
	(IC)	5%	75.17±0.17c,B	-0.20±0.09b,A	15.39±0.19bc,B	74.87±0.17c,B
		7%	72.76±0.49e,B	-0.03±0.13a,A	16.33±0.23a,A	72.46±0.49e,B
		10%	72.92±0.54e,B	0.11±0.13a,A	16.33±0.44a,B	72.62±0.53e,B

Values are given as mean ±SD from five determinations. Different letters in the same column within the same freeze-thaw cycle indicate significant differences ($p<0.05$). Different capital letters in the same column within the same level of hydrocolloids indicate significant differences ($p<0.05$).

3.4 Microstructure

Microstructures of mince gels from bigeye snapper (without Gelpro HC30 and IC) (A and B), gel with 2% Gelpro HC30 (C and D) and 2% IC (E and F) before and after 5 freeze-thaw cycles are illustrated in Figure 19. The mince gel without hydrocolloids had a larger void, compared with those containing 2% Gelpro HC30 and IC. These observations suggested that 2% IC resulted in the formation of an ordered structure with finer strands. However, mince gel added with 2% Gelpro HC30 exhibited a finer and more compact structure with smaller pores in the matrix than that containing 2% IC.

Structure with the larger voids was observed after 5 freeze-thaw cycles. Larger fibrous aggregate was also found. However, the addition of 2% Gelpro HC30 yielded the gel after freeze-thawing similar to that found in control gel before freeze-thawing. Therefore, Gelpro HC30 would be a means to maintain the quality of mince gel during frozen storage, particularly with the fluctuated temperature. Slow-freezing appears to cause some structural damage to gel proteins, which lose their water binding ability. Rate of freezing and storage temperature are both factors that influence the formation of ice crystals (Shenouda, 1980; Sikorsky *et al.,* 1976).

Hydrocolloids added might reduce the migration of water to form the ice crystals via the water-hydrocolloids interaction. As a consequence, the lower ice crystals were formed and the disruption of gel matrix was impeded.

Mince gel added with 2% Gelpro HC30 could maintain the textural property and decreased the expressible moisture of mince gel subjected to multiple freeze-thawing most effectively. As starch granules absorb water from the surrounding during heating, the expanded starch granules exert pressure to the gel matrix. Gelpro HC30 was effective in holding the water in the gel matrix even after repeated freeze-thawing. As a consequence, the lower ice crystals were formed and the disruption of gel matrix was impeded. The addition of Gelpro HC30 yielded the gel network with fine structure with smaller voids after freeze-thawing. Thus Gelpro HC30 was selected for further study.

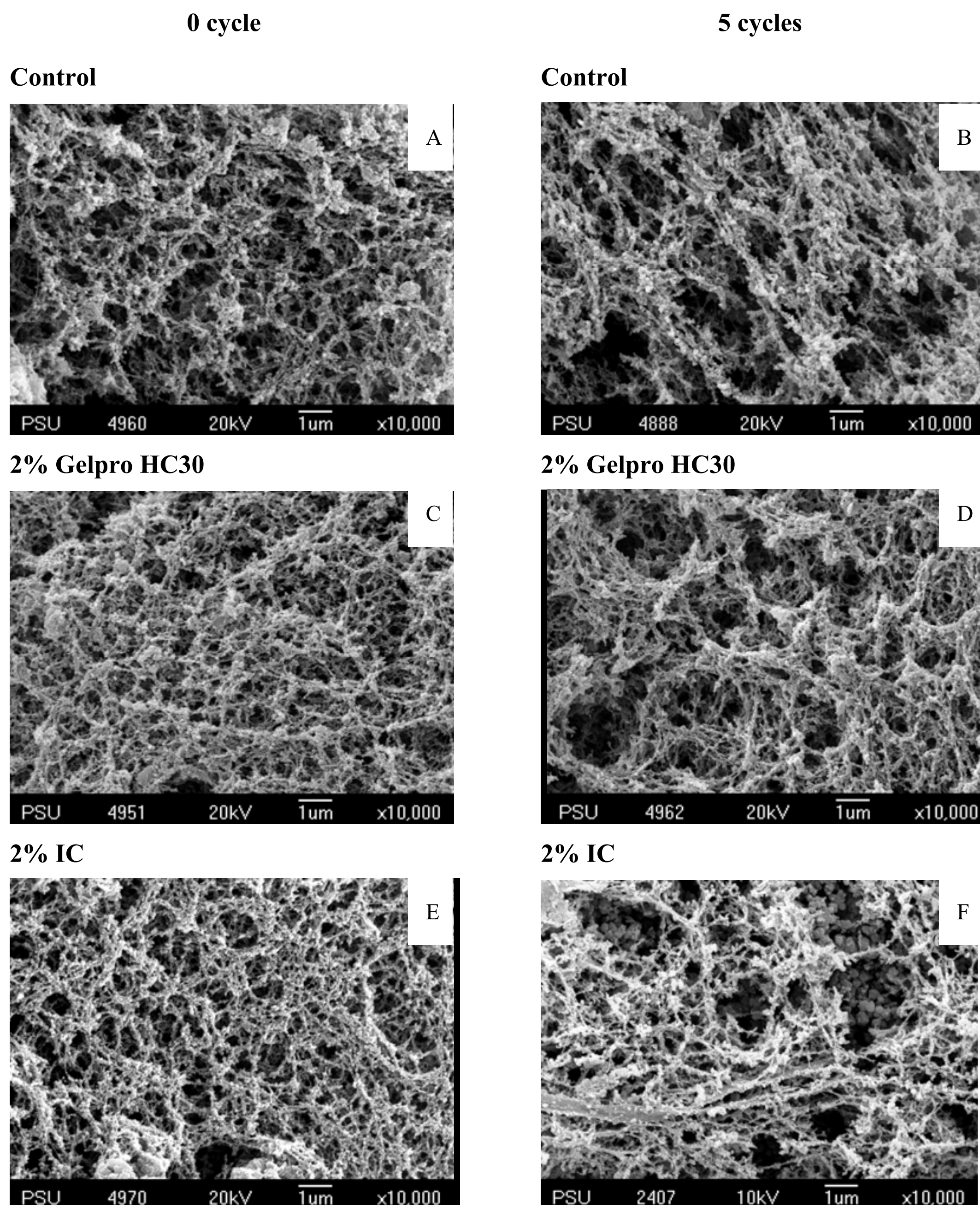


Figure 19. Microstructure of bigeye snapper mince gels added without and with 2% Gelpro HC30 or 2% IC and subjected to different freeze-thaw cycles. (Magnification: x10,000)

4. Effect of Gelpro HC30 on the stability of mince and surimi gels subjected to multiple freeze-thawing

4.1 Effect of Gelpro HC30 at different levels on textural properties of mince and surimi gel subjected to different freeze-thaw cycles

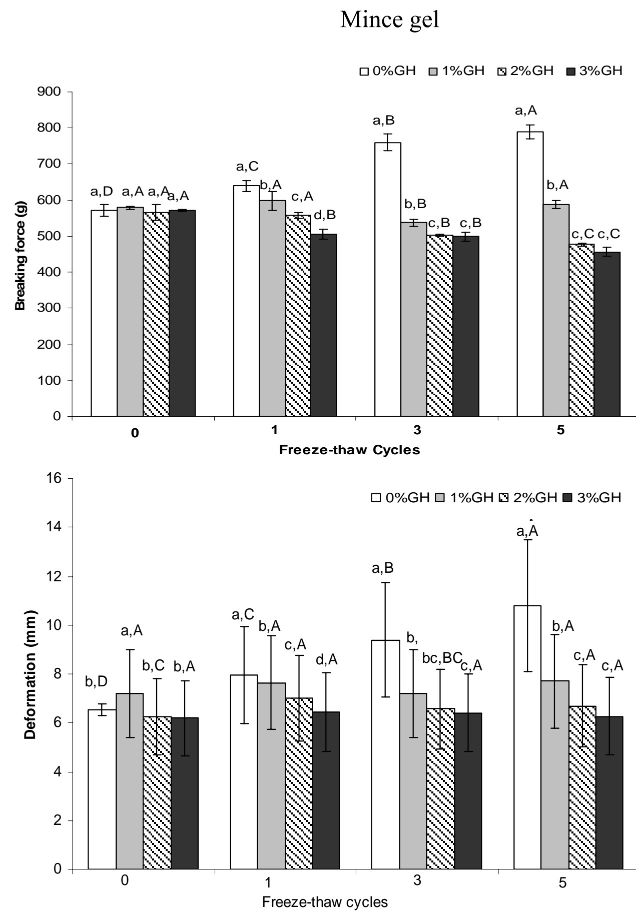
Breaking force and deformation of gels from bigeye snapper mince and surimi added with different levels of Gelpro HC30 and subjected to multiple freeze-thaw cycles are presented in Figure 20. In the absence of Gelpro HC30, mince gel showed the lower breaking force and deformation than surimi gel. During washing, sarcoplasmic proteins as well as lipids, blood, enzyme were removed, leading to the concentrated myofibrillar proteins, which play a role in gel formation. The addition of Gelpro HC30 in the range of 1-3% had no impact on breaking force and deformation of resulting gels ($p > 0.05$). Increases in breaking force and deformation were observed in the control mince and surimi gels (without Gelpro HC30) when the freeze-thaw cycles increased ($p < 0.05$). After 5 freeze-thaw cycles, the breaking force and deformation of the control mince increased by 37% and 64%, whereas the control surimi gel had the increases by 25% and 33%, respectively. It indicated that the control gel became more rubbery in texture. This suggested that mechanical damage was more pronounced in the control gel, particularly with repeated freeze-thawing.

From the result, mince gels added with Gelpro HC30 had the decreases in breaking force as freeze-thaw cycles increased. Nevertheless, no changes in breaking force were found in gel added with 1% Gelpro HC30 with all freeze-thaw cycles ($p>0.05$), except for the gel subjected to 3 freeze-thaw cycles, which had slightly decreased breaking force. At the same freeze-thawing cycle, breaking force was lower in mince gel added with higher amount of Gelpro HC30. However, no differences in deformation were observed among gels added with Gelpro HC30 ranging from 1 to 3% ($p>0.05$), regardless of freeze-thaw cycles. Gels added with Gelpro HC30 at higher amount turned to be mushy after freeze-thawing, leading to the weakened gel (Lee *et al*., 1992). During frozen storage, amylose in starch undergoes severe retrogradation, resulting in gel with higher expressible moisture and increased brittleness (Park, 2000).

For surimi gels, the increase in breaking force was found when Gelpro HC30 at levels of 1 and 3% were added ($p<0.05$). Lower breaking force was found when gel was subjected

to 1 cycle of freeze-thawing, compared with the control gel. It was noted that the addition of 1% Gelpro HC30 could prevent the increases in breaking force of surimi gel subjected to multiple freeze-thawing. The similar trend was found for deformation (Figure 20d).

The rubberiness of a heated surimi-based product can be somewhat reduced by increasing the level of starches or more effectively by adding hydroxypropyrated and cross-linked waxy-maize starch (Wu, 1985c). Hydroxypropyl starch is modified by the addition of hydroxyl propyl groups to some of the hydroxyl groups, usually by treatment with propylene oxide under highly alkaline conditions with sodium sulfate to prevent excessive granular swelling (Thomas and Atwell, 1999). Cross-linking of hydroxypropyl starch imparts viscosity stability and a desired short texture properties to the paste. However, for each application there is an optimum level and balance between hydroxypropyl substitution and cross-linking (Wootton and Kensington, 1983). Hydroxypropyl starch plays a role in textural maintainance and freeze-thaw stability (Goff, 2000). Hydroxypropyl groups inhibit the alignment of polymers that causes a change in the structure of the food product through ionic repulsion as well as steric hindrance (Tuschhoff, 1986). This modification prevents gelling and syneresis and maintains textural appearance. Inhibiting retrogradation imparts texture and freeze-thaw stability, thus prolonging the shelf-life of the food product (Goff, 2000). From the result, the addition of 1% Gelpro HC30 in bigeye snapper mince and surimi gels could maintain the mechanical properties of resulting gel effectively, irrespective of freeze-thaw cycles. Effects of starch on the texture of surimi-starch gels depend on its concentration, modification, and the ratio of amylose and amylopectin. The amylopectin component (present in modified starch) made the granule swell and greatly increased gel strength and the larger size starch granules also produced the stronger gel (Yang and Pang, 1998). The effect of modified cassava starch in the surimi gels may be explained by the following theory: the starch granules embedded in protein gel absorb water from the matrix and push the matrix as they swell during cooking. At the same time, the protein matrix loses moisture and becomes firmer (Kim and Lee, 1987).



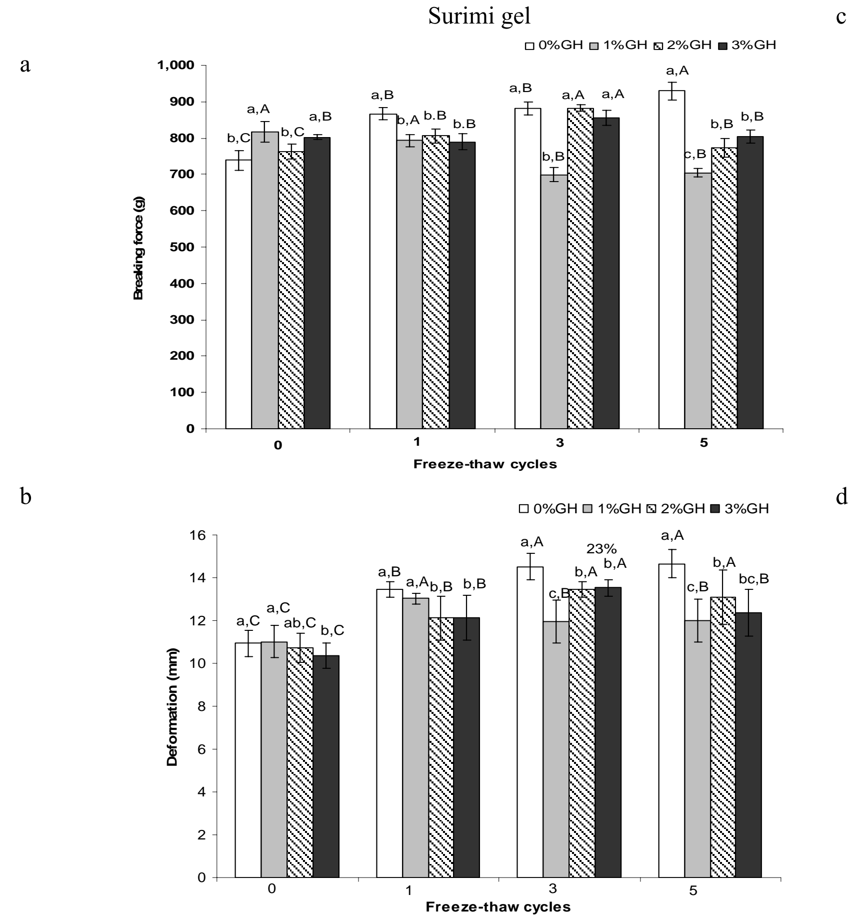


Figure 20. Breaking force and deformation of gels from bigeye snapper mince (a,b)and surimi (c,d) added with different levels of Gelpro HC30 and subjected to different freeze-thaw cycles. GH: Gelpro HC30. Bars represent standard deviations from five determinations. The different letters within the same freeze-thaw cycle indicate significant differences ($p<0.05$). The different capital letters within the same level of Gelpro HC30 added indicate significant differences ($p<0.05$)

4.2 Effect of Gelpro HC30 on expressible moisture content of mince and surimi gels subjected to different freeze-thaw cycles

Expressible moisture contents of bigeye snapper mince and surimi gel added with different levels of Gelpro HC30 and subjected to multiple freeze-thaw cycles are shown in Figure 21. Both mince and surimi gels added with 3% Gelpro HC30 had the lowest expressible moisture content, compared to other samples ($p<0.05$). After freeze-thawing, particularly with increasing cycles, the control gels had the marked increases in expressible moisture content ($p<0.05$). The increases in expressible moisture content by 42 and 15% were found in the control mince and surimi gel, respectively, after 5 freeze-thaw cycles. The sample gels added with 1 % and 2% Gelpro HC30 had the decreases in expressible moisture content when the freeze-thawing cycles increased ($p<0.05$). For gel added with 3% Gelpro HC30, no changes in expressible moisture content were observed with increasing freeze-thaw cycles ($p>0.05$). The results suggested that water holding capacity of mince gel was increased with the addition of Gelpro HC30. Thus, freeze-thawing had no marked impact on expressible moisture content of mince gel added with Gelpro HC30. For surimi gel, expressible moisture content increased with increasing freeze-thaw cycles, regardless of amount of Gelpro HC30 added. However, the lower expressible moisture content was found in gel added with higher Gelpro HC30 content. The higher amount of Gelpro HC30 might imbibe more water in the gel matrix.

Starch granules bound in the surimi proteins had a "packing effect" on the surimi protein due to the internal pressure (Kong, 1999). The modified starch has reduced syneresis, and increased stability to freezing and freeze-thawing (Wong, 1989). Retrogradation is caused by close alignment of the starch chains to form a tree-dimensional network, resulting in a more fluid (syneresis) (Tuschhoff, 1986). Chemical substitution of these chain, however, prevents close alignment that is resistant to retrogradation (Tuschhoff, 1986). Chemical modification of the starch can minimize the aggregation of the starch molecules, allowing the modified starch to rehydrate well on thawing (Mallett, 1993).

From the results, the addition of 1% Gelpro HC30 could reduce the expressible moisture content as effectively as 2% Gelpro HC30. Furthermore, Gelpro HC30 at 3% might cause the dryness of gel since water could be imbibed much more in starch granules. The unique property of modified starch is evidenced in improved freeze-thaw or cold storage stability.

Hydrophilic nature of the hydroxypropyl group keeps the water in the starch paste from separating or syneresis when subjected to freeze-thawing (Tuschhoff, 1986). White *et al*. (1989) reported that hydroxypropyl distarch phosphate has no recrystallization after ten freeze-thaw cycles, and no free water is released (Smith, 1982). Hydroxypropyrated and cross-linked waxy maize starch (1.0-2.0%) are popularly used to overcome severe expressible moisture problems on thawing after frozen storage (Park, 2000). Water molecules intimately adsorbed to macromolecules are called “binding water” and reflect the ability of a molecular surface to form weak non covalent bindings with water (Rickard *et al*., 1991). In the cold water-starch system, starch granules can reversibly absorb water, swell slightly and become partially hydrated with excessive comminution (Waniska and Gomez, 1992). Thermal changes of the starch granule in the surimi-starch system are different from those in the starch-water system. Gelatinization of starch occurs concomitantly with thermal gelation of fish proteins. However, it is delayed by the presence of myofibrillar proteins, salt, sucrose, and sorbitol in the surimi-starch system. Myofibrillar proteins are thermally denatured before the starch is completely gelatinized (Wu, *et al.,* 1985 a,b). Water is entrapped in the protein-gel network, limiting the availability of water for starch gelatinization and resulting in competition for water between starch and protein (Kim and Lee, 1987). Starch granules absorb water and expand themselves until they are limited by the gel matrix. Even though the starch granules expand in surimi seafood, they cannot expand as much as in the starch-water system because fish proteins take part of the water (Okada and Migita, 1956).

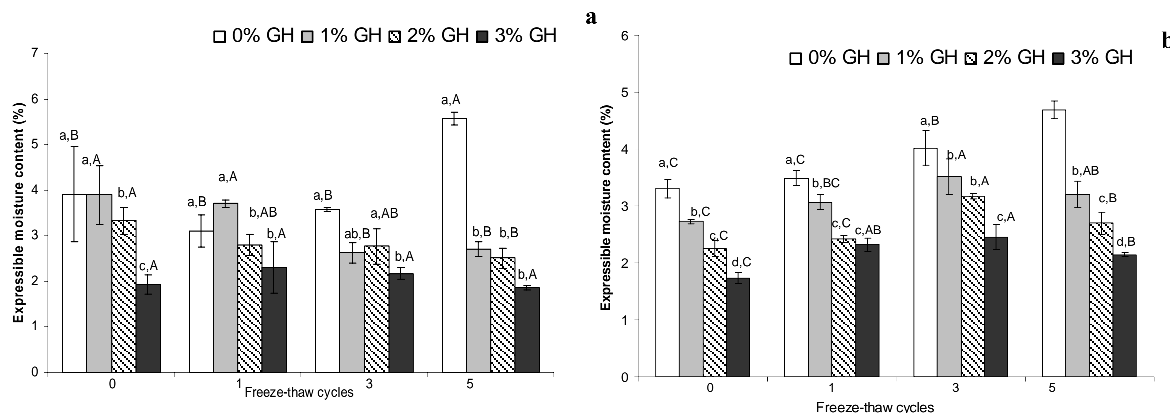


Figure 21. Expressible moisture content of gels from bigeye snapper mince (a) and surimi (b) added with different levels of Gelpro HC30 and subjected to different freeze-thaw cycles. GH: Gelpro HC30. Bars represent standard deviations from five determinations. The different letters within the same freeze-thaw cycle indicate significant differences ($p<0.05$). The different capital letters within the same level of Gelpro HC30 added indicate significant differences ($p<0.05$)

4.3 Effect of Gelpro HC30 on color and whiteness of mince and surimi gel subjected to different freeze-thaw cycles

Color and whiteness of mince and surimi gels from bigeye snapper with and without Gelpro HC30 addition as affected by multiple freeze–thaw cycles are shown in Table 13. The addition of Gelpro HC30 had no effect on $L*$-value and whiteness of resulting mince and surimi gels ($p>0.05$). Nevertheless, $a*$-value decreased in mince gels but $a*$-value increased in surimi gels added with Gelpro HC30 ($p<0.05$). The $b*$-value of mince gels increased as the amount of Gelpro HC30 added increased ($p<0.05$), but no change in $b*$-value were observed in surimi gels ($p>0.05$). After 1 cycle of freeze-thawing, the control mince gel (without Gelpro HC30) had the marked decreases in $L*$-value. However, freeze-thaw cycles had no influence on the change in $L*$-value of gel added with Gelpro HC30 (1-3%). The decreases in $a*$-value with the increases in $b*$-value were found in all samples as freeze-thawing cycles increased ($p<0.05$). Thus, the decreases in redness with a slight increases in yellowness of mince gel were obtained as the freeze-thawing cycles increased. For surimi gels, freeze-thawing cycles had no effect on whiteness, regardless of level of Gelpro HC30 used.

Table 13 *L**, *a**, *b**-values and whiteness of gels from bigeye snapper mince and surimi added with different levels of Gelpro HC30 and subjected to different freeze-thaw cycles

	Cycle	Concen tration (%)	*L**	*a**	*b**	Whiteness
Mince	0	0	76.91 ± 0.75a,A	0.93 ± 0.06a,A	15.84 ± 0.16b,B	71.98 ± 0.56a,A
		1	74.99 ±1.09b,C	0.91 ± 0.05a,A	15.77 ± 0.13b,C	70.42 ± 0.89b,B
		2	77.10 ± 0.22a,A	0.82 ± 0.09a,A	16.43 ± 0.16a,C	71.80 ± 0.11a,A
		3	77.24 ± 0.11a,A	0.65 ± 0.09b,A	16.41 ± 0.10a,C	71.93 ± 0.14a,A
	1	0	72.99 ± 1.02b,C	0.95 ± 0.09a,A	15.43 ± 0.23c,C	68.88 ±0.81c,B
		1	77.05 ± 0.29a,A	0.76 ± 0.09c,A	16.53 ± 0.16ab,B	71.70 ± 0.16a,A
		2	76.87 ± 0.29a,A	0.68 ±0.08bc,AB	16.47 ± 0.15b,C	71.60 ±0.29ab,A
		3	76.43 ± 0.22a,B	0.82 ± 0.10ab,A	16.75 ±0.15a,B	71.07 ± 0.14b,B
	3	0	73.32 ± 0.87b,C	0.79 ± 0.37a,A	15.88 ± 0.22c,B	68.94 ± 0.76b,B
		1	76.26 ± 0.29a,AB	0.32 ± 0.25b,B	16.93 ± 0.33b,A	70.84 ± 0.31a,B
		2	76.41 ± 1.00a,AB	0.36 ± 0.34b,B	17.21 ± 0.18ab,A	70.79 ± 0.86a,B
		3	76.02 ± 0.43a,C	0.21 ± 0.23b,B	17.51 ± 0.15a,A	70.31 ± 0.43a,C
	5	0	74.67 ± 0.30b,B	0.21 ± 0.15b,B	16.68 ± 0.18b,A	69.68 ± 0.33b,B
		1	75.55 ± 0.64c,B	0.35 ± 0.21ab,B	16.89 ± 0.14b,A	70.28 ± 0.55a,B
		2	75.81 ± 0.89a,B	0.58 ± 0.41a,AB	16.90 ± 0.29b,B	70.48 ± 0.62a,B
		3	76.03 ± 0.11a,C	0.16 ± 0.06b,B	17.43 ± 0.03a,A	70.36 ± 0.11a,C
Surimi	0	0	78.49±0.43a,A	0.79±0.11a,A	10.21±0.12ab,C	76.18±0.38b,A
		1	78.95±0.79a,B	0.79±0.03a,B	10.14±0.13ab,C	76.62±0.72ab,C
		2	79.18±0.51a,B	0.81±0.11a,B	10.04±0.22b,B	76.87±0.40a,C
		3	77.65±0.14b,B	0.90±0.08a,A	10.29±0.14a,B	75.38±0.12c,B
	1	0	79.74±3.28a,A	0.80±0.26a,A	12.08±0.41b,AB	76.37±0.03a,A
		1	82.07±0.51a,A	1.10±0.03a,A	11.87±0.18b,B	78.46±0.33a,A
		2	81.74±0.29a,A	1.09±0.11a,A	12.02±0.25b,A	78.10±0.28a,A
		3	80.95±0.42a,A	1.11±0.07a,A	12.51±0.38a,A	77.18±0.55a,A
	3	0	80.19±0.65a,A	0.76±0.25a,A	12.66±0.21a,A	76.48±0.59b,A
		1	81.31±0.65a,A	0.87±0.05a,AB	12.10±0.23c,A	77.71±0.54a,B
		2	80.75±0.45a,A	1.07±0.09a,A	12.35±0.09bc,A	77.11±0.38ab,BC
		3	80.93±0.54a,A	1.00±0.39a,A	12.43±0.25ab,A	77.21±0.56a,A
	5	0	80.60±0.25a,A	0.93±0.20a,A	12.39±0.18a,AB	76.96±0.23c,A
		1	81.68±0.33a,A	1.05±0.17a,A	12.06±0.10b,A	78.04±0.30a,AB
		2	81.19±0.65a,A	0.94±0.09a,AB	12.24±0.24ab,A	77.54±0.56b,B
		3	80.65±0.38a,A	1.17±0.09a,A	12.38±0.20a,A	77.00±0.32c,A

Values are given as mean ±SD from five determinations. Different letters in the same column within the same freeze-thaw cycle indicate significant differences ($p<0.05$). Different capital letters in the same column within the same level of Gelpro HC30 under the same sample indicate significant differences ($p<0.05$).

4.4 Effect of Gelpro HC30 on protein solubility of mince and surimi gel subjected to different freeze-thaw cycles

Protein solubility in 2% SDS of gels from bigeye snapper mince and surimi added with different levels of Gelpro HC30 and subjected to multiple freeze-thaw cycles is depicted in Figure 22. Protein solubility of all samples decreased when the freeze-thaw cycles increased ($p<0.05$). The decreases were more pronounced in the control gel. Gelpro HC30 at the levels of 1 and 2% could retard the decrease in protein solubility at all freeze-thaw cycles tested, particularly after 5 freeze-thawing cycles ($p<0.05$). The result indicated that higher hydrogen bonding or ionic interaction were formed as the freeze-thawing cycles increased. The addition of Gelpro HC30 at 1 or 2 % might hold the water thereby, preventing the migration of water to form the ice. As a consequence, the water could be imbibed in the gel matrix to some extent. This might retard the cross-linking of proteins, which caused the loss in solubility. When the freeze-thaw cycles increased, a decrease in protein solubility was the result of a shift from a balance of protein intermolecular interaction and protein–water interaction, resulting in a situation where protein intermolecular interaction was formed, while protein water interaction was weakened (Vojdani, 1996). Nevertheless, the lower solubility of gel added with 3% Gelpro HC30 was found. This might be caused by the competitive absorption of water between starch and protein molecules. As a result, less water might be held by proteins, leading to the ease of intermolecular cross-linking of protein molecules. For surimi gel, Gelpro HC30 at level of 1% showed the greater preventive effect on the loss of solubility, compare with 2 and 3%.

Solubility of mince and surimi gels added with different levels of Gelpro HC30 was determined using a mixture of 1% SDS+8M urea + 2% βME in 20 mM tris-HCl, pH 8.0 (Figure 23), The higher solubility was noticeable in all gels when compared with that observed with 2% SDS (Figure 22). Samples added with Gelpro HC30 at all levels showed the similar solubility than the control gel at all freeze-thawing cycles used ($p<0.05$). The difference in solubility between using 2% SDS and the mixture of 1% SDS+8M urea + 2% βME in 20 mM tris-HCl, pH 8.0 was more intense as the freeze-thaw cycles increased, indicating the major role of disulfide bond in sample subjected to multiple freeze-thawing. From the result, the addition of Gelpro HC30 of 1-2% could retard the loss of solubility of protein induced by repeated freeze-thawing process. Starch might hold water in the gel matrix (Park *et al*. 1997). Thus, the migration

of water to form the ice could be retarded with the lower disruption of gel matrix and lower cross-linking of protein molecules. Though most bondings were formed during the thermal gelation process, some free reaction groups were still available and could undergo bonding as the freeze-thawing took place.

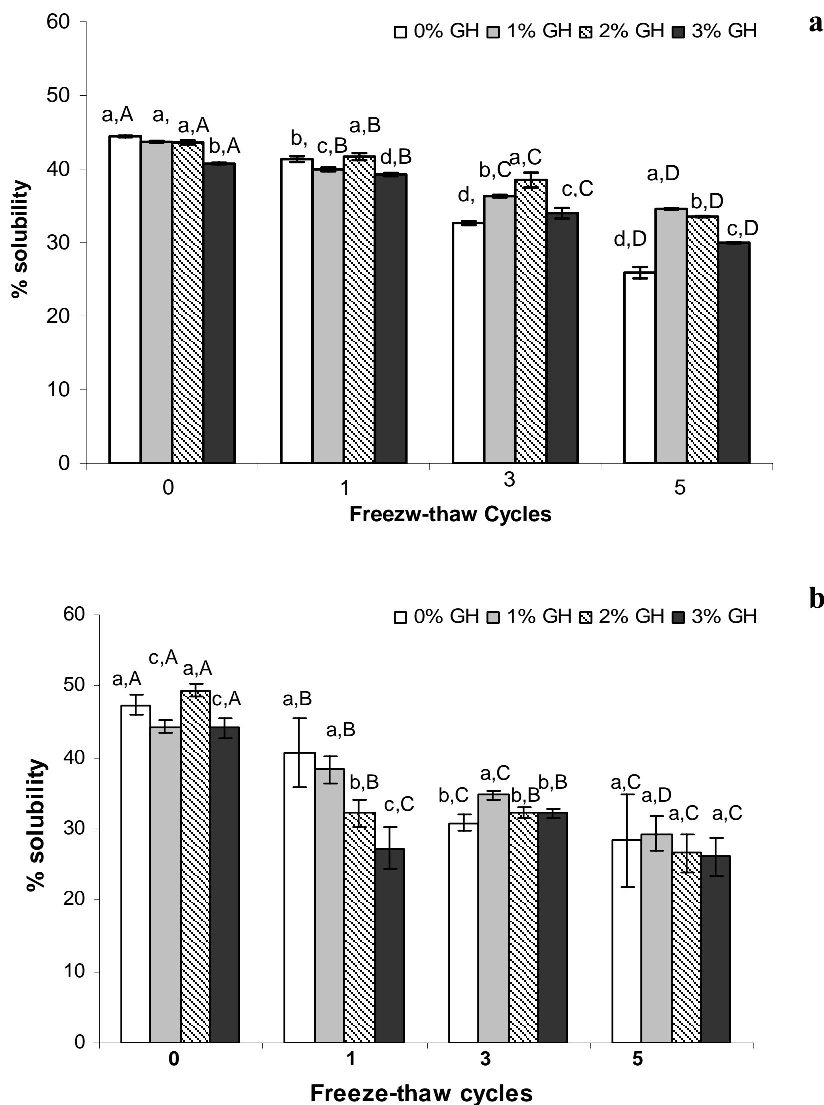


Figure 22. Changes in protein solubility of gels from bigeye snapper mince (a) and surimi (b) added with different levels of Gelpro HC30 and subjected to different freeze-thaw cycles in 2% SDS. GH: Gelpro HC30. Bars represent standard deviations from five determinations. The different letters within the same freeze-thaw cycle indicate significant differences ($p<0.05$). The different capital letters within the same level of Gelpro HC30 added indicate significant differences ($p<0.05$)

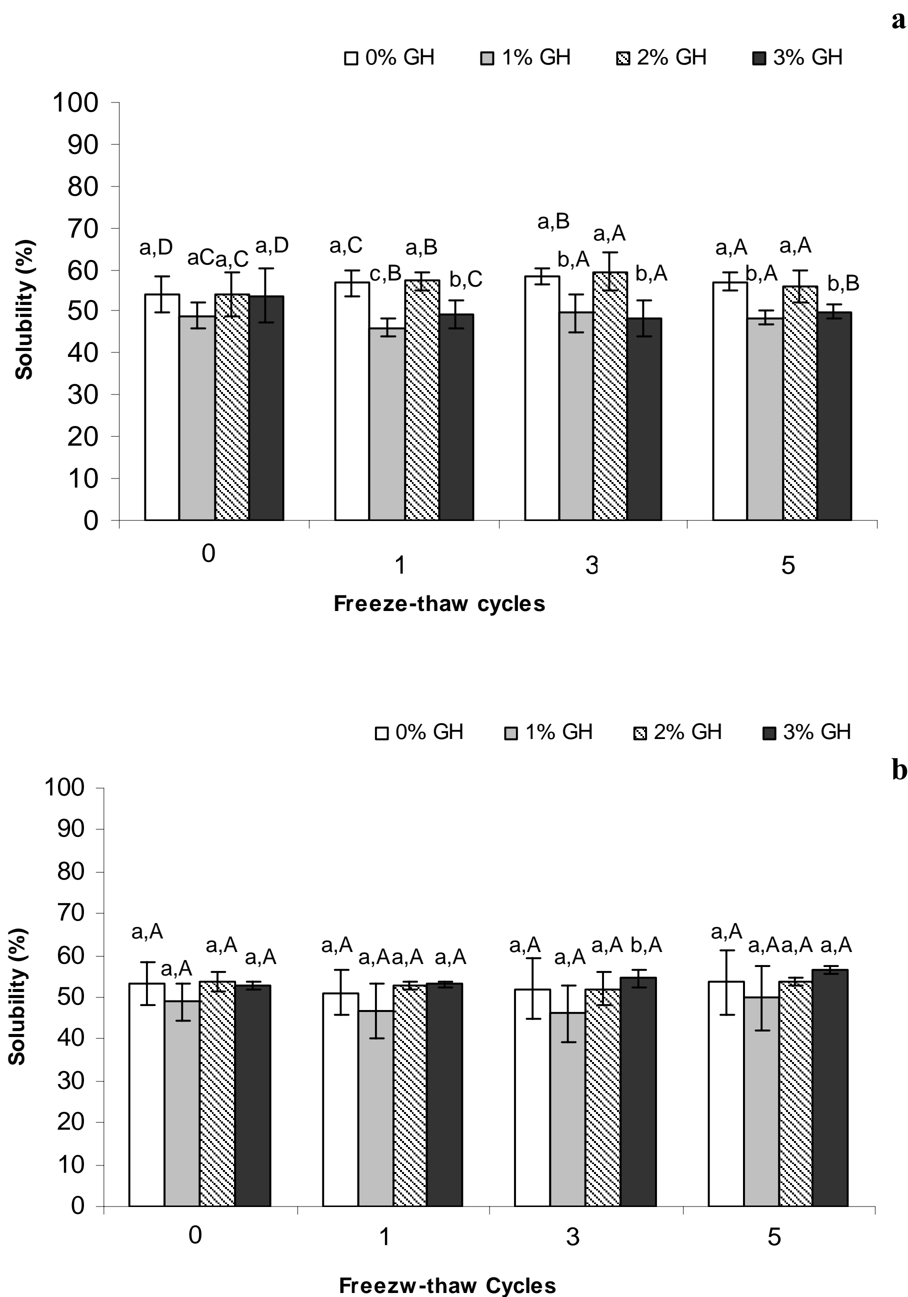


Figure 23. Changes in protein solubility of gels from bigeye snapper mince (a) and surimi (b) added with different levels of Gelpro HC30 and subjected to different freeze-thaw cycles in a solution containing 1% SDS, 8 M urea and 2% β-mercaptoethanol. GH: Gelpro HC30. Bars represent standard deviations from five determinations. The different letters within the same freeze-thaw cycle indicate significant differences ($p<0.05$). The different capital letters within the same level of Gelpro HC30 added indicate significant differences ($p<0.05$)

4.5 Effect of Gelpro HC30 on likeness of mince and surimi gel subjected to different freeze-thaw cycles

The likeness scores of mince and surimi gel samples with and without Gelpro HC30 at different levels and subjected to multiple freeze-thaw cycles are presented in Figure 24. No differences in color and flavor likeness were found in all samples tested, regardless of amount of Gelpro HC30 and freeze-thaw cycles. The juiciness of the control mince gel continuously decreased as the freeze-thaw cycles increased ($p<0.05$). Nevertheless, no differences in juiciness likeness were found in samples added with Gelpro HC30 at 1-3 %. For the control mince gel, all attributes except color and flavor, markedly decreased to a greater extent when freeze-thawing cycles increased ($p<0.05$). For gel added with Gelpro HC30, the decrease in likeness of all attributes was lowered. From the result, the addition of 1% Gelpro HC30 rendered the mince gel with the highest overall, appearance and texture likeness at all freeze-thawing cycles used. Thus, it can be concluded that Gelpro HC30 at 1% was the most appropriate to reduce the quality losses of freeze-thawed mince gel from bigeye snapper. For surimi gel (Figure 25), similar results were observed, compared to those found in mince gels. However, gels from surimi showed the higher likeness for all attributes tested, particularly for texture, color and flavor. During washing, blood and some compounds causing off-odor or fishy odor could be removed. This led to the improved odor, flavor as well as color. Since myofibrillar proteins became concentrated, the gel forming ability should be enhanced as evidenced by the higher score of texture for surimi gel.

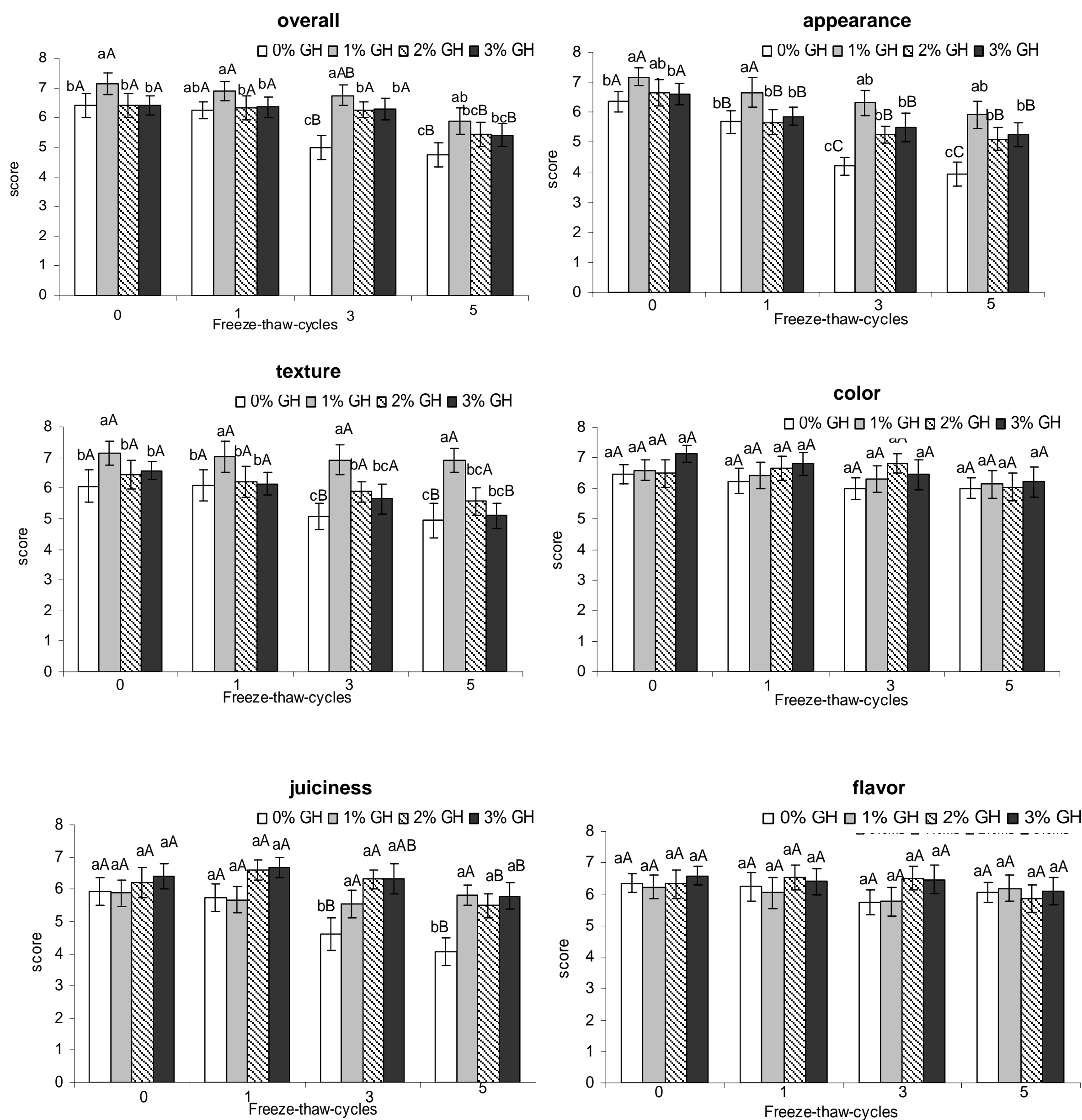


Figure 24. Changes in sensory property of gels from bigeye snapper mince added with different levels of Gelpro HC30 and subjected to different freeze-thaw cycles. GH: Gelpro HC30. Bars represent standard deviations from five determinations. The different letters within the same freeze-thaw cycle indicate significant differences (p<0.05). The different capital letters within the same level of Gelpro HC30 added indicate significant differences (p<0.05)

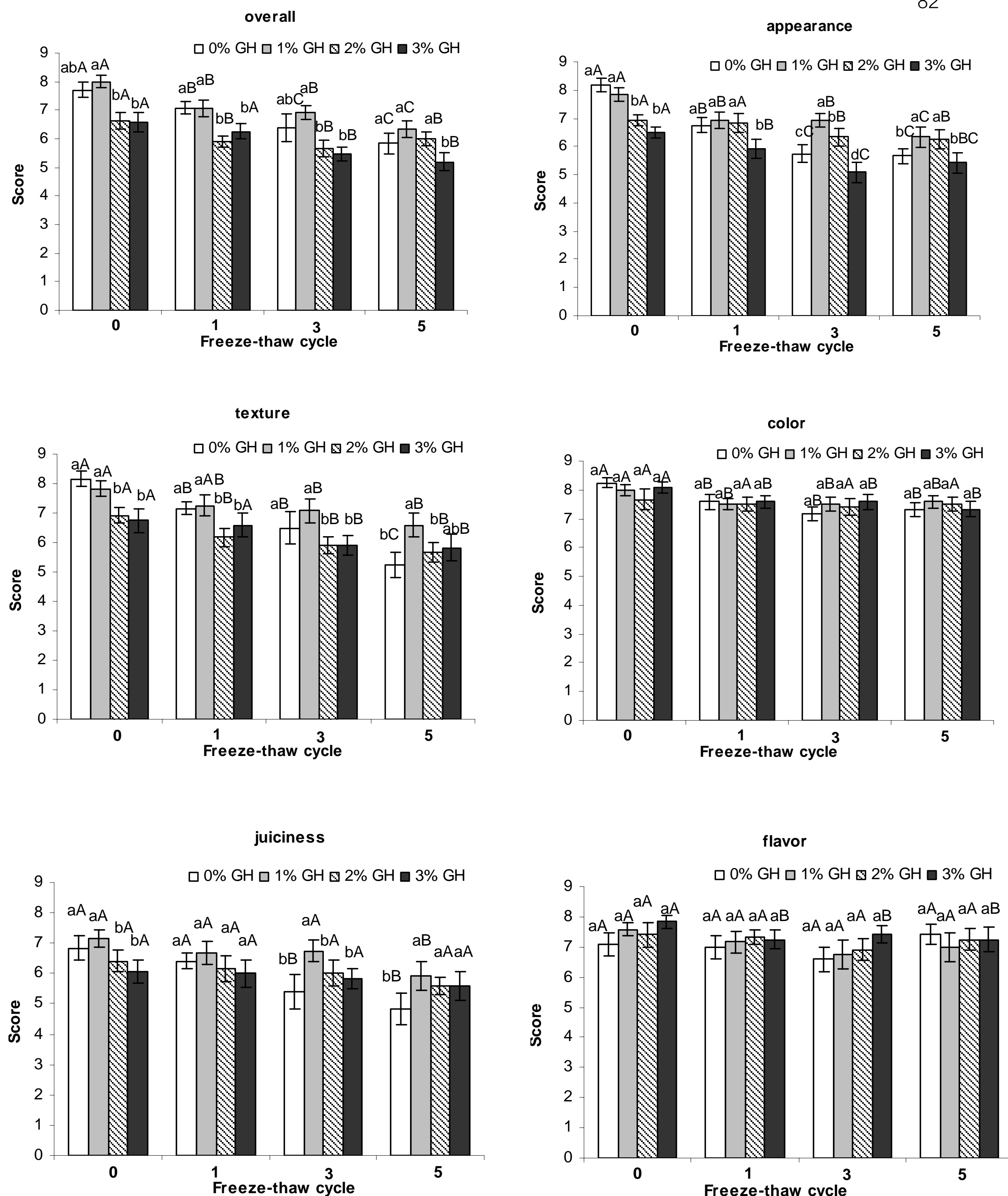


Figure 24. Changes in sensory property of gels from bigeye snapper surimi added with different levels of Gelpro HC30 and subjected to different freeze-thaw cycles. GH: Gelpro HC30. Bars represent standard deviations from five determinations. The different letters within the same freeze-thaw cycle indicate significant differences ($p<0.05$). The different capital letters within the same level of Gelpro HC30 added indicate significant differences ($p< 0.05$) (Continued)

4.6 Effect of Gelpro HC30 on microstructure of mince and surimi gels subjected to different freeze-thaw cycles

Figure 25 shows microstructures of mince and surimi gels from bigeye snapper without and with 1 % Gelpro HC30 addition at different freeze-thaw cycles. The results revealed that the control gels had the larger strands with the irregular structure as the freeze-thaw cycles increased. However, gel added with 1% Gelpro HC30 still had the fine structure without the large aggregates or strands even with 5 freeze-thaw cycles. Starch might prevent the cross-linking of proteins subjected to the repeated freeze-thawing by holding the water or being the barrier between of protein molecules in forming the large aggregates. Thus, the addition of 1% Gelpro HC30 would be a means to maintain the quality of mince gel during frozen storage, particularly with the fluctuated temperature. When comparing the changes in microstructure between gels from mince and surimi, it was found that mince gel was more susceptible to alteration, compared with surimi gel. Lipid and blood remaining in the mince might facilitate lipid oxidation, in which the oxidation products could enhance the protein aggregation via the remaining reactive groups of protein gel.

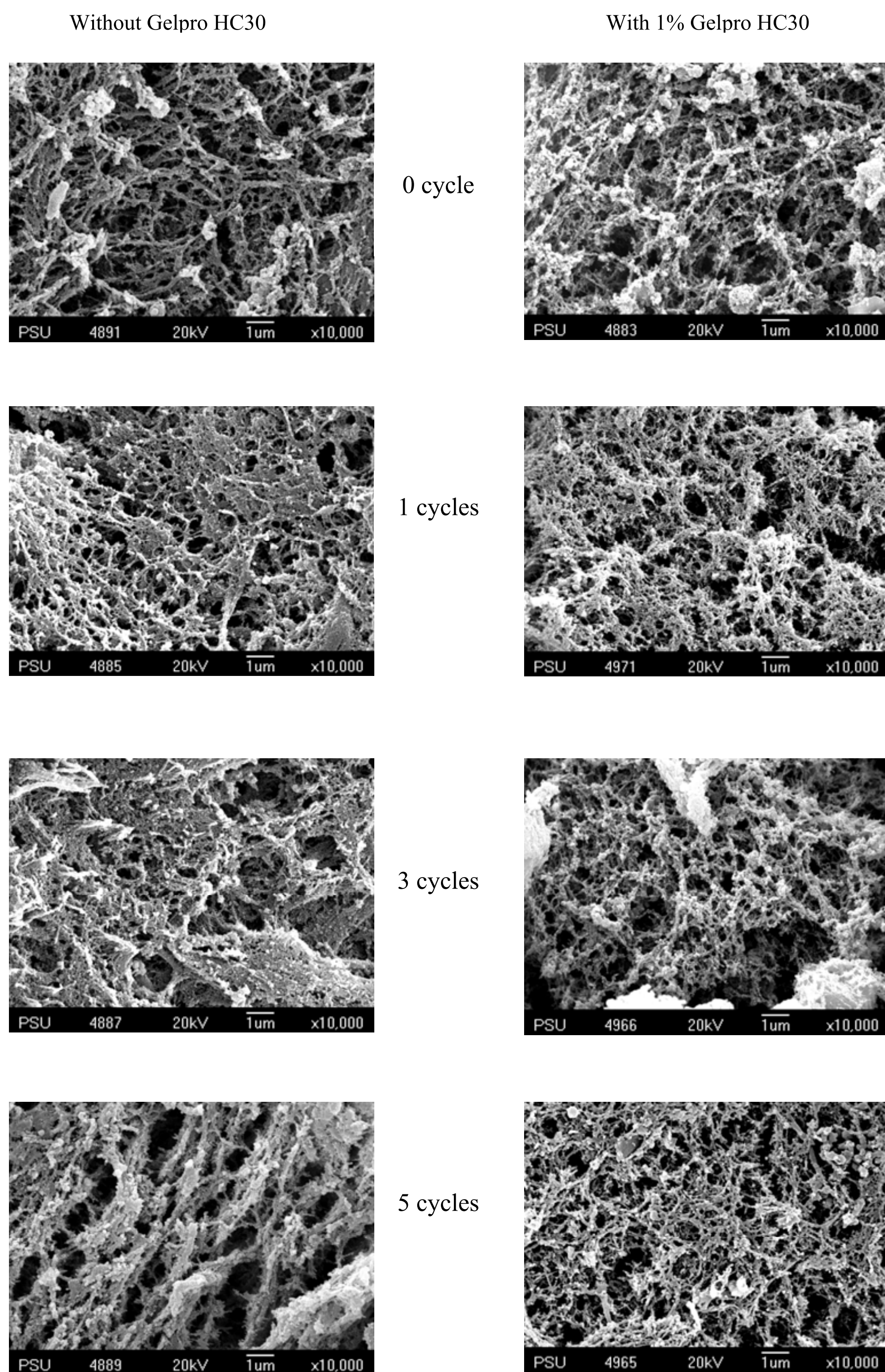


Figure 25. Microstructure of bigeye snapper mince gels without and with 1% Gelpro HC30 added and subjected to different freeze-thaw cycles (Magnification: x10,000)

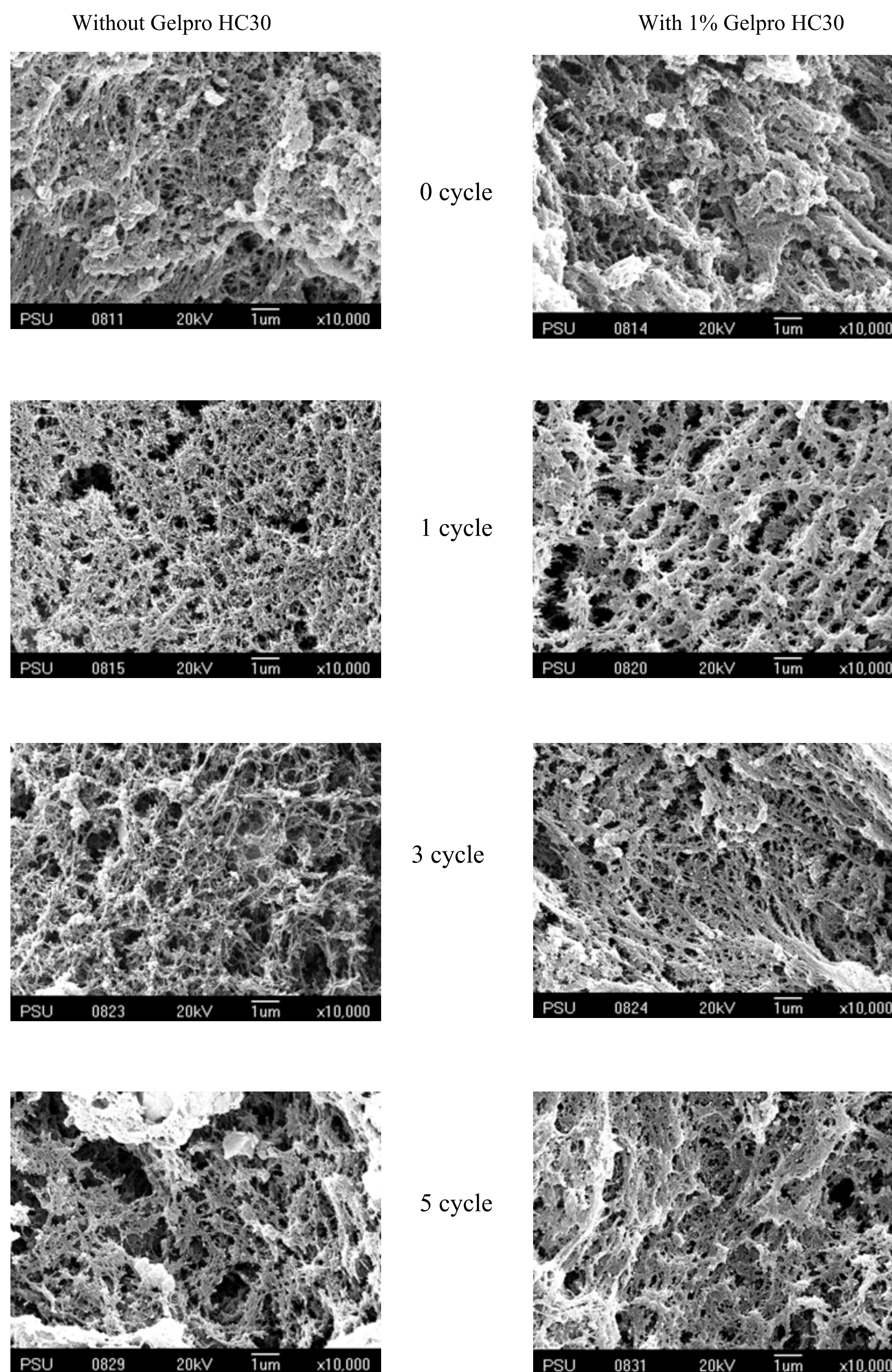


Figure 25. Microstructure of bigeye snapper surimi gels without and with 1% Gelpro HC30 added and subjected to different freeze-thaw cycles. (Magnification: x10,000) (Continued)

5. Changes of chemical and physical properties of gels from mince and surimi containing Gelpro HC30 during frozen storage

5.1 Breaking force and deformation

Breaking force of mince and surimi gels with and without 1% Gelpro HC30 during storage at -18°C up to 10 weeks are shown in Figure 26. Without the addition of Gelpro HC30, gel of mince had the marked increases in breaking force than did gel containing Gelpro HC 30 throughout the storage time ($p<0.05$). Deformation of mince gel containing Gelpro HC30 was greater than the control gel within the first 4 weeks of storage. Thereafter, the control gel showed the higher value ($p<0.05$). For surimi gel, both gels with and without Gelpro HC30 had the increases in breaking force up to 10 weeks of storage and that containing 1% Gelpro HC30 tended to possess a slightly higher breaking force. Nevertheless, the addition of 1% Gelpro HC30 increased deformation of gel within the first week of frozen storage and maintain the deformation of surimi gel up to 10 weeks of storage. Conversely, the continuous increase in deformation was observed for the control surimi gel (without 1% Gelpro HC30) throughout the storage. The rate of moisture migration is between different regions in a complex food, and hence 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 have been the main objectionable textural changes found in frozen surimi-based products (Lee and Chung, 1989). From the result, Gelpro HC30 could imbibe water and reduced the migration of water to form the ice crystal.

After 10 weeks, deformation of surimi gels added with modified starch was 11.35 mm, which was similar to that of 0 week. At week 8 and 10, no differences in deformation were found between mince and surimi containing Gelpro HC30 ($p< 0.05$). When modified starch was added, the properties of mince and surimi gels were maintained to some extent.

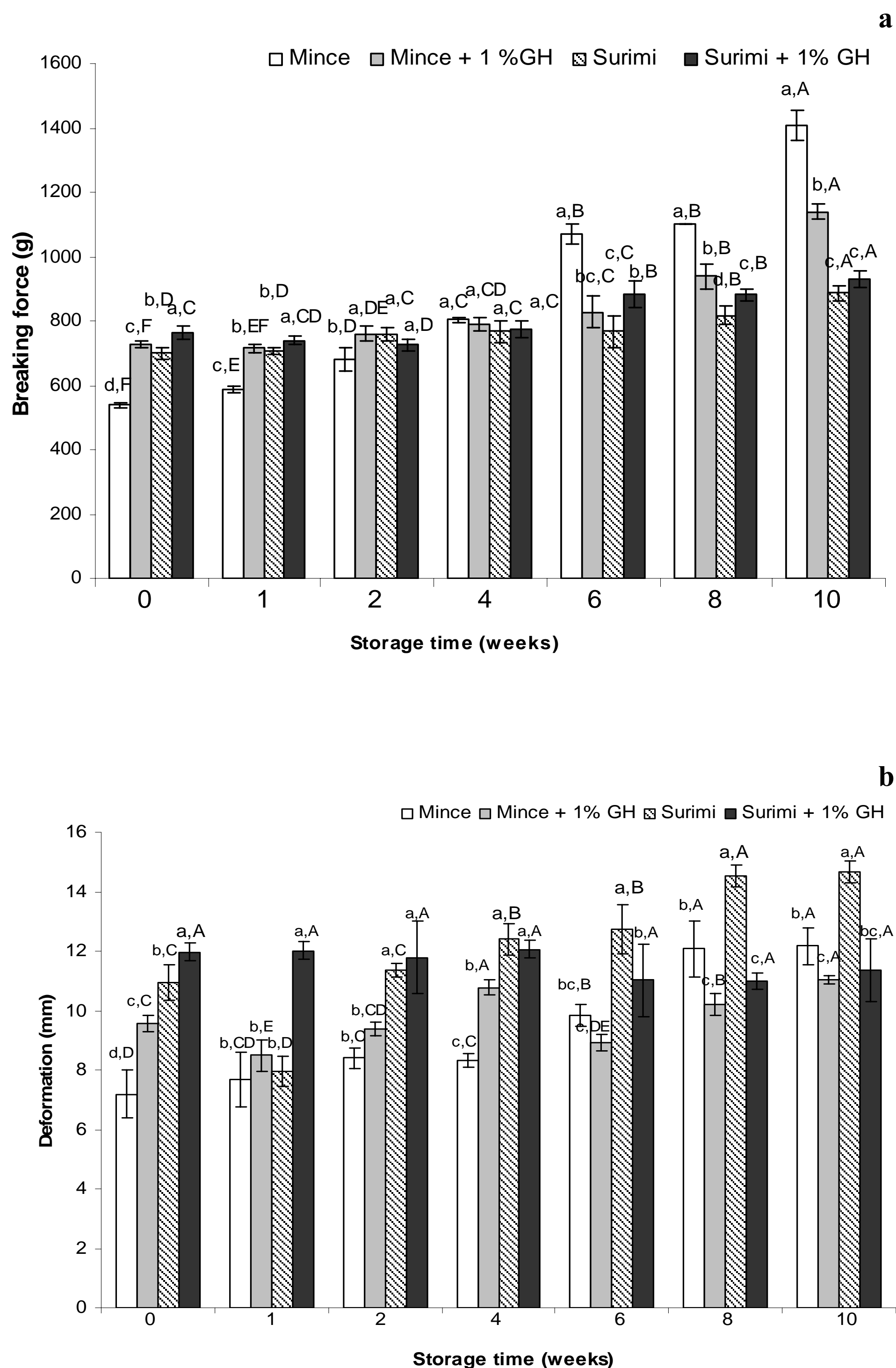


Figure 26. Breaking force (a) and deformation (b) of gels from bigeye snapper mince and surimi with and without Gelpro HC30 during frozen storage GH: Gelpro HC30. Bars represent standard deviations from five determinations. The different letters within the same storage time indicate significant differences ($p<0.05$). The different capital letters within the same treatment indicate significant differences ($p<0.05$)

5.2 Expressible moisture content

Expressible moisture content in mince and surimi gel with and without 1% Gelpro HC30 during frozen storage is shown in Figure 27. Expressible moisture content increased as the storage time increased up to 10 weeks ($p<0.05$). It indicated that less water was imbibed in the gel matrix. No marked differences in expressible moisture content were found among all gels, either with or without Gelpro HC30 addition ($p>0.05$) within the first 6 weeks of storage. Nevertheless, much lower expressible moisture content was observed in gels containing Gelpro HC30 during week 8 and 10 of storage ($p<0.05$). After 8 weeks of storage time, the expressible moisture content was increased to approximately 4.64% and 4.63% in the mince and surimi gels without Gelpro HC30, respectively, while gels from mince and surimi added with 1% Gelpro HC30 had the expressible moisture content of 4.09% and 3.57%, respectively. Additionally, the gel matrix formed possessed the lower water holding capacity. From the result, it was suggested that the expressible moisture content increased because the texture deteriorated with the increment of the free water during frozen storage (Samson and Regenstein, 1986). Expressible moisture was an effective measure to determine the textural change of surimi in frozen storage (Jahncke *et al*., 1992). Ice crystal formed resulted in the tissue damage and the leakage of various organelles (Mishara and Srikar, 1989). The expressible drip refers to as an amount of water exuded when the external force is applied (Muyonga and Regenstein, 1997).

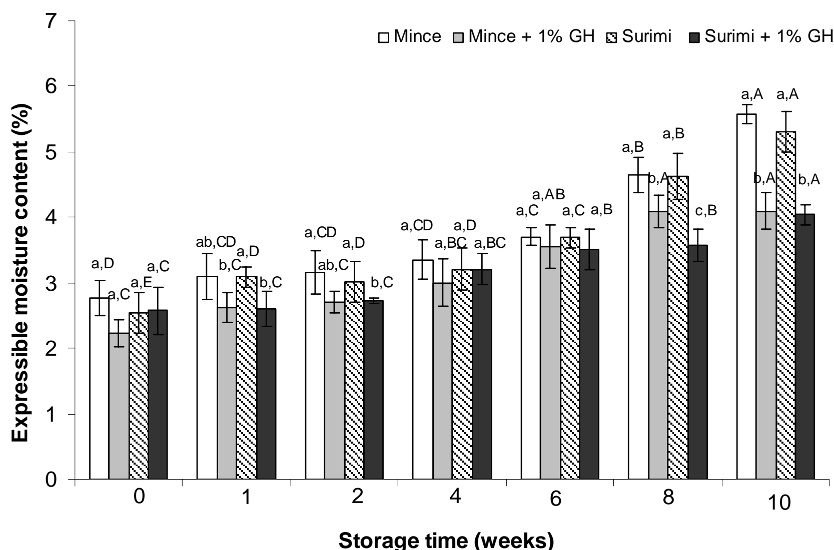


Figure 27. Expressible moisture content of gels from bigeye snapper mince and surimi with and without Gelpro HC30 during frozen storage GH: Gelpro HC30. Bars represent standard deviations from five determinations. The different letters within the same storage time indicate significant differences (p<0.05). The different capital letters within the same treatment indicate significant differences (p<0.05)

5.3. *L**, *a**, *b** and whiteness

L, a*, b** values and whiteness of mince and surimi gels with and without 1% Gelpro HC30 during frozen storage are shown in Table 14. The addition of Gelpro HC 30 had no pronounced effect on *L**-value and whiteness of mince and surimi gels. (p>0.05). In general, *L**-value of mince gel decreased with increasing storage time (p<0.05). This was coincidental with the increase in *b**-value throughout the storage time. When comparing *L**-value of both gels, mince and surimi gels, surimi gel had the higher *L**-value than mince counterpart. Washing process was able to remove the blood and some lipids. During storage, those components might undergo the changes, particularly oxidation, leading to the formation of brown or yellows finished compounds. However, storage time had no influence on the change in whiteness of mince and surimi gel, irrespective of Gelpro HC30 addition.

Table 14 *L*, a*, b**-values and whiteness of gels from mince and surimi added with and without Gelpro HC 30 during frozen storage

	Storage time (weeks)		*L**	*a**	*b**	Whiteness
Mince	0	0%	80.04±2.48b,A	-1.10±0.20a,E	13.14±1.06a,E	76.07±2.63b,A
		1%	80.27±0.36b,A	-1.20±0.15a,D	13.58±0.24a,C	76.01±0.23b,A
	1	0%	75.12±0.68d,B	-0.48±0.08a,D	15.11±0.40a,CD	70.88±0.55c,B
		1%	76.58±0.36c,B	-0.90±0.16b,C	15.56±0.46a,B	71.87±0.50c,B
	2	0%	73.32±0.87d,BC	0.79±0.37a,A	15.88±0.22b,B	68.93±0.76d,CD
		1%	76.26±0.29c,B	0.32±0.25b,A	16.93±0.33a,A	70.83±0.31c,BC
	4	0%	74.67±0.30c,B	0.21±0.15b,B	16.68±0.18a,A	69.67±0.33b,BC
		1%	75.55±0.64b,B	0.35±0.21b,A	16.89±0.14a,A	70.27±0.55b,C
	6	0%	74.26±0.96b,B	-0.36±0.12b,CD	14.95±0.13b,D	70.23±0.85b,BC
		1%	75.55±2.05b,B	-0.84±0.33c,C	15.49±0.69a,B	71.04±2.07b,BC
	8	0%	73.93±1.68b,B	-0.37±0.13a,CD	15.04±0.31a,CD	69.89±1.53b,BC
		1%	72.26±1.51b,C	-0.37±0.05a,B	15.08±0.32a,B	68.42±1.24b,D
	10	0%	75.34±1.50a,B	-0.13±0.27a,C	15.68±0.33a,BC	67.52±1.28c,D
		1%	71.57±1.45b,C	-0.52±0.12b,B	15.62±0.48a,B	70.79±1.28b,BC
Surimi	0	0%	82.13±1.00a,A	-1.35±0.14a,D	12.17±0.82b,AB	78.33±1.27a,A
		1%	83.76±0.41a,A	-1.21±0.28a,BC	11.33±0.20b,B	80.16±0.33a,A
	1	0%	78.88±1.60b,BC	-1.24±0.13c,CD	11.75±0.36b,BC	75.79±1.56b,B
		1%	80.13±0.43a,B	-1.13±0.13c,BC	11.52±0.30b,B	77.00±0.35a,BC
	2	0%	80.19±0.65b,B	0.76±0.25a,B	12.66±0.21c,A	76.48±0.59b,B
		1%	81.30±0.64a,B	0.86±0.04a,A	12.10±0.22d,A	77.71±0.54a,B
	4	0%	80.75±0.44a,AB	1.07±0.08a,A	12.35±0.09b,AB	77.11±0.38a,AB
		1%	80.93±0.54a,B	0.99±0.38a,A	12.43±0.24b,A	77.21±0.56a,B
	6	0%	80.60±0.24a,AB	0.93±0.20a,AB	12.38±0.17c,AB	76.96±0.23a,AB
		1%	81.67±0.32a,B	1.04±0.17a,A	12.05±0.10c,A	78.04±0.30a,B
	8	0%	78.27±0.58a,CD	-1.24±0.18b,CD	11.30±0.38b,C	75.47±0.56a,B
		1%	78.28±2.13a,C	-1.31±0.31b,BC	11.43±0.52b,B	75.42±2.08a,C
	10	0%	76.65±2.80a,D	-1.00±0.27c,C	11.99±0.63b,B	73.72±2.70a,C
		1%	75.66±2.66a,D	-0.88±0.38bc,B	12.07±0.37b,A	72.80±2.53b,D

Values are given as mean ±SD from five determinations. Different letters in the same column within the same storage time indicate significant differences ($p<0.05$). Different capital letters in the same column within the same level of Gelpro HC30 under the same sample indicate significant differences ($p<0.05$).

5.4. Protein solubility

Solubility in 2% SDS of mince and surimi gels with and without 1% Gelpro HC30 during frozen storage is shown in Figure 28. During frozen storage of 10 weeks, protein solubility in 2% SDS of the control mince and surimi gels decreased sharply, while that of mince and surimi gel containing 1% Gelpro HC30 decreased to a lower extent (Figure 28(a)). After 10 weeks of storage, protein solubility of mince and surimi gel with 1% Gelpro were 8.1 and 20.8%, respectively. From the result, the surimi gel had the higher solubility than mince gel, regardless of Gelpro HC30 addition. The result suggestion that mince gel underwent aggregation to a greater extent than surimi counterpart, particularly as the storage time increased. From the result, slightly lower solubility in 2% SDS was observed when Gelpro HC30 was added, when compared with the control gel. Hydrocolloids dispersed in the gel matrix might bring about the closer alignment of protein molecules in which the greater cross-linking could be formed.

Solubility of mince and surimi gels with and without Gelpro HC30 is the solution containing 1% SDS, 8 M urea and 2% βME was not changed during prolonged storage (Figure 28(b)). As the storage time increased, the greater formation of cross-links mediated by various bonds, including hydrogen bond, hydrophobic interaction as well as disulfide bonds was presumed. Formation of disulfide, hydrogen and hydrophobic bonds has been known to cause the aggregate during frozen storage (Jiang *et al*., 1988). The increase in number of hydrophobic interactions suggested that slow freezing could cause proteins to unfold, which resulted in the exposure of hydrophobic groups. The change during extended storage might occur and the new bonding could be formed, mostly weaker bonds and disulfide bonds. Those alteration might be associated with the formation of ice crystals, in which lower water was retained in the gel matrix. This might favor the aggregation of protein molecules via the remaining reactive groups. Differences in solubility of mince and surimi gels when 2 % SDS and the mixture containing 1% SDS, 8M urea and 2% βME were used indicated the formation of disulfide, hydrogen, and hydrophobic bonds in gels during extended frozen storage. Furthermore, the loss of fluid water may favor protein aggregations by increasing the concentration of reactive solutes (Buttkus, 1970). From the result, the decreased solubility in 2% SDS without any change in solubility in solution containing SDS, urea and βME of gels indicated the aggregation of proteins caused by freezing and frozen storage.

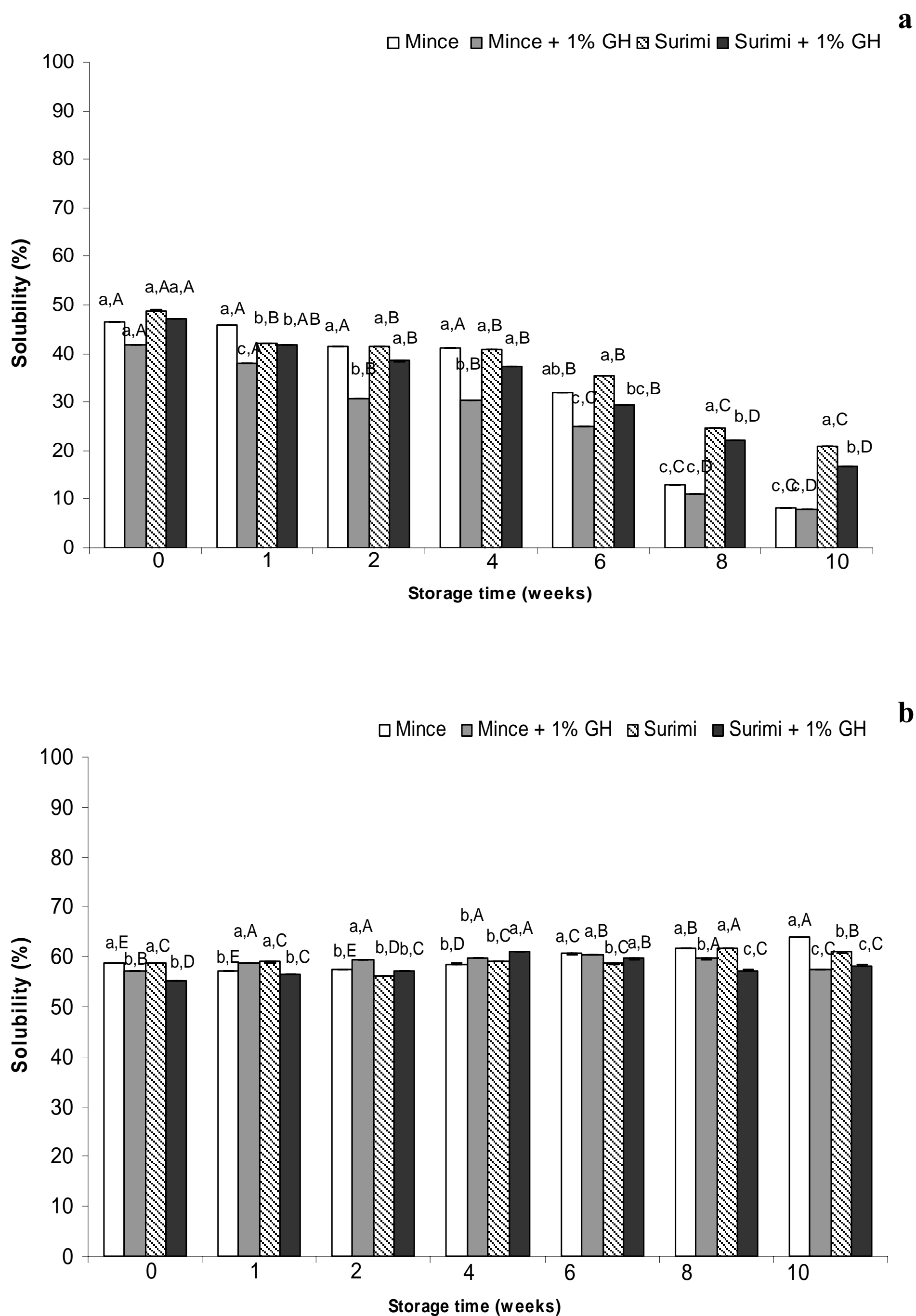


Figure 28. Changes in protein solubility in 2% SDS (a) and solution containing 1% SDS, 8 M urea and 2% β-mercaptoethanol (b) of gels from mince and surimi with and without 1% Gelpro HC30 during frozen storage. GH: Gelpro HC30. Bars represent standard deviations from five determinations. The different letters within the same storage time indicate significant differences ($p<0.05$). The different capital letters within the same treatment indicate significant differences ($p<0.05$)

5.5. Thiobarbituric acid reactive substances (TBARS)

It is known that during frozen storage of fish, products of lipid hydrolysis and oxidation can accumulate and cause the deterioration of the product (Mackie, 1993). TBARS is a widely used indicator for the assessment of degree of lipid oxidation. In general, the higher TBARS values were obtained in mince gel, compared with surimi gel, regardless of Gelpro HC30 addition. It was presumed that hemoglobin and other prooxidants were greater in mince. Additionally, surimi contained less lipid than mince, caused by the removal during washing process. TBARS in mince and surimi gels with and without Gelpro HC30 increased slightly during the first 6 weeks of storage (Figure 29). After 6 weeks of storage, the sharp increases in TBARS of mince and surimi gels were noticeable. After 10 weeks of frozen storage, no differences in TBARS values were found between gels with and without Gelpro HC30. Therefore, Gelpro HC30 had no impact on the lipid oxidation of both mince and surimi gels. Polyunstaurated fatty acids are more prone to oxidation, compared to saturated fatty acids. Perez-Villarreal *et al.* (1991) found the increase in TBARS and peroxide value in European hake muscle during frozen storage. 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; Xiong, 1997a). Carbonyl groups of oxidised lipids may participate in covalent bonding, leading to the formation of stable protein– lipid aggregates (Saeed and Howell, 2002). Also reaction of proteins with lipid oxidation products results in the formation of protein-centred radicals (Saeed *et al*., 1999). Many lipid degradation products are also capable of cross-linking polypeptides and thus are responsible for the generation of insoluble protein aggregate (Buttkus, 1970). This possibly resulted in the loss in solubility of protein in both mince and surimi gels, especially when the storage time increased.

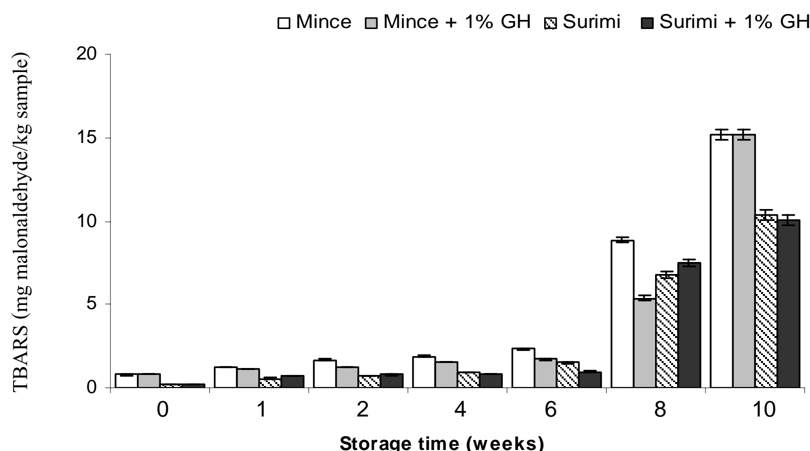


Figure 29. Changes in thiobarbituric acid reactive substances of gels from mince and surimi with and without 1% Gelpro HC30 during frozen storage. GH: Gelpro HC30. Bars represent standard deviations from five determinations.

5.6. Microstructure

Microstructures of mince and surimi gels with and without 1% Gelpro HC30 during frozen storage are shown in Figure 30. Mince and surimi gels without Gelpro HC30 had the similar microstructure to those added with Gelpro HC30 at week 0. However, after 10 weeks, gel with a larger void was found in the control mince and surimi gels (without Gelpro HC30), whereas more regular network was found in gels added with Gelpro HC30. Uniformity of gel microstructure resulted in improved water-binding characteristics (Woodward and Cotterill, 1986). During frozen storage, Gelpro HC30 could hold water as the bound form, which could not migrate freely to form the ice crystals. The lower amount of ice crystals could be in accordance with the lower disruption of network as evidenced by the smaller voids. These holes were attributed to formation of ice crystals through migration of water molecules from myofibrillar proteins. Loss of water holding ability and a tendency of proteins to aggregate chemically and become more physically compact led to the changes of gel structure (Montero *et al*., 1997). Montero *et al*. (1997) reported that the storage life of slow-frozen gels was strongly affected by storage temperature; at -12°C the growth of crystal size was greater and at the same time the gels became harder. This did not occur in quick-frozen gels.

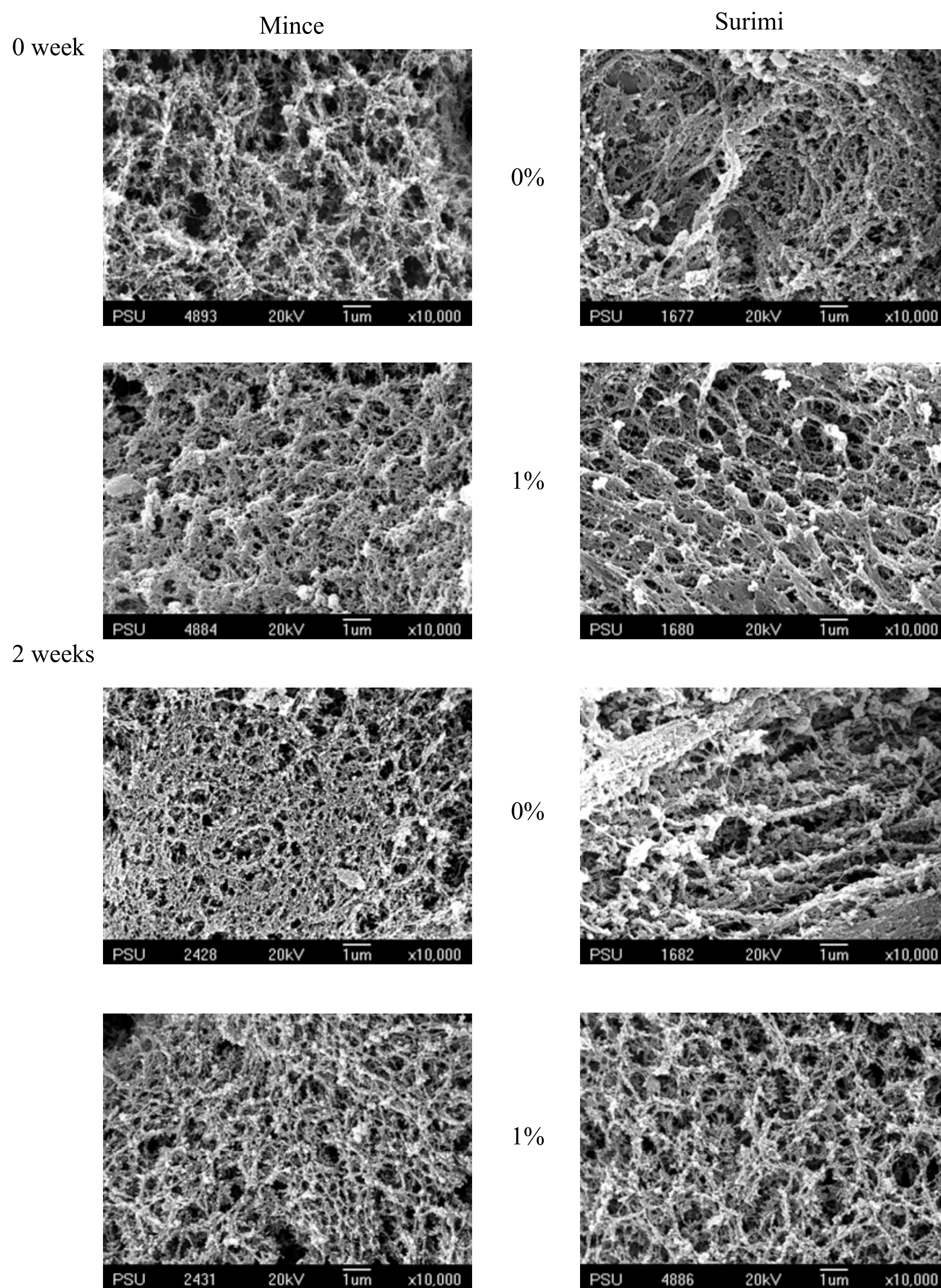


Figure 30. Microstructure of mince and surimi gels added without and with 1% Gelpro HC30 during frozen storage time (Magnification: x10,000)

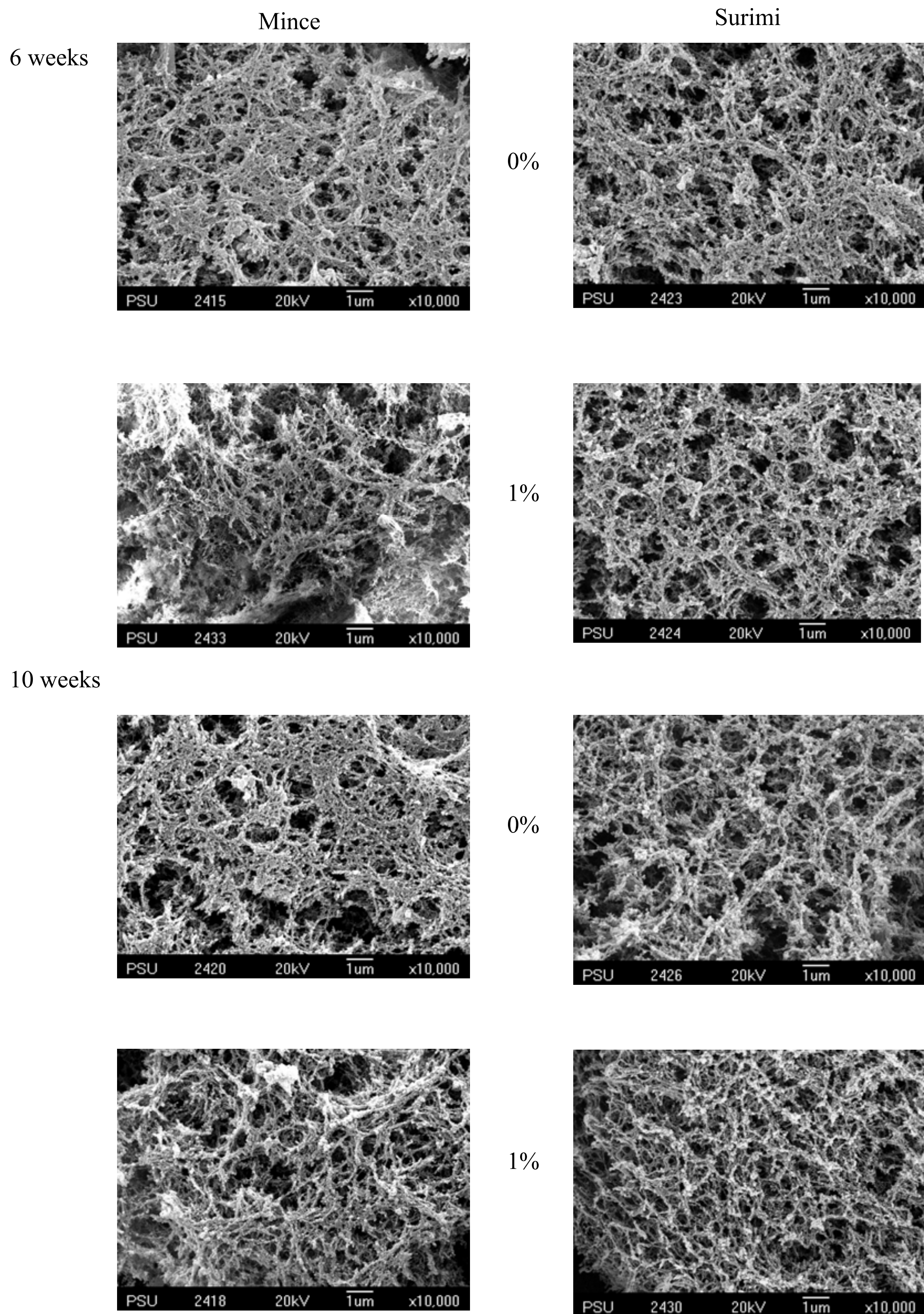


Figure 30. Microstructure of mince and surimi gels added without and with 1% Gelpro HC30 during frozen storage time (Magnification: x10,000) (Continued)

5.7. Likeness

Likeness score of mince and surimi gels with and without 1% Gelpro HC30 during frozen storage up to 10 weeks at -18°C is shown in Figure 31. Surimi gel showed the higher score for all attributes tested, compared with mince gel ($p<0.05$). Likeness for all attributes decreased gradually as the frozen storage time increased. The rate of decrease in likeness was greater in mince gel in comparison with surimi gel. This might be associated with the greater alteration of mince gel including lipid oxidation (Figure 29). In general, the gels had the much lower likeness for all attributes tested after 6 weeks of storage. Thus, storage time was another factor determining the shelf-life of mince and surimi gels. During 8-10 weeks of storage, it was found that the gels added with 1% Gelpro HC30 yielded the higher likeness in comparison with the control for both mince and surimi gels. This was in agreement with the lower mechanical damage of gel in the presence of 1% Gelpro HC30.

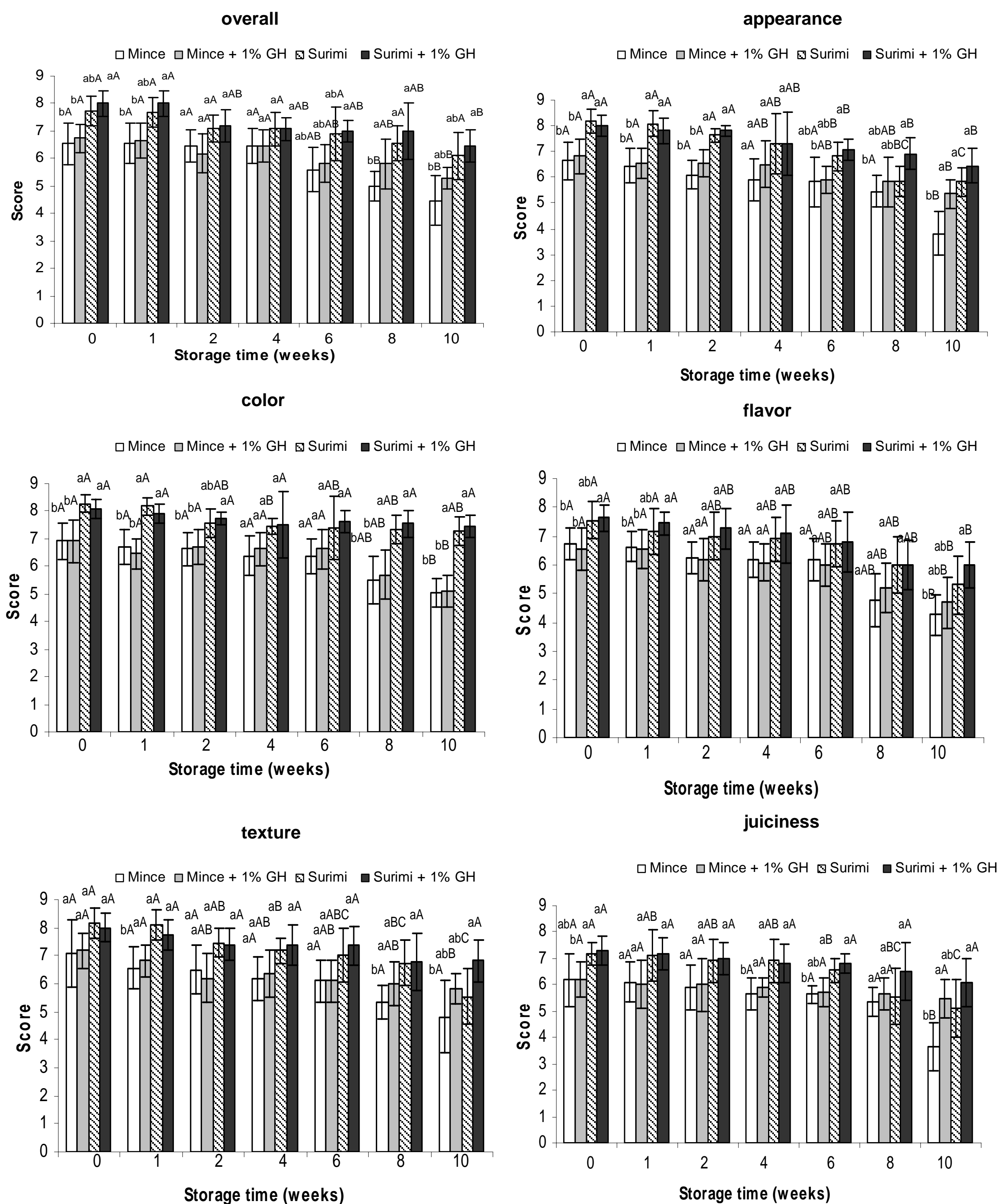


Figure 31. Changes in likeness score of gels from mince and surimi with and without 1% Gelpro HC30 during frozen storage. GH: Gelpro HC30. Bars represent standard deviations from five determinations. The different letters within the same storage time indicate significant differences ($p<0.05$). The different capital letters within the same treatment indicate significant differences ($p<0.05$)

5.8. Protein pattern

Figure 32 shows the protein pattern of mince and surimi gels with and without 1% Gelpro HC30 during frozen storage up to 10 weeks at -18°C. For surimi gel, protein bands were more retained under reducing condition, compared to that found under non-reducing condition. It was suggested that disulfide bonds were formed during extended frozen storage. Under reducing condition, βME in sample buffer effectively dissociated disulfide. However, no marked differences in protein pattern were found in mince gels analyzed under both conditions. It was noted that cross-linked proteins were more pronounced with increasing storage time as indicated by the greater band intensity in the stacking gel. Those cross-links became lower under reducing condition. This confirmed the role of disulfide bond in cross-linking of protein in gel during frozen storage. The formation of cross-links was in agreement with the loss in solubility in 2%SDS (Figure 28a). From the result, no differences in protein pattern were observed between mince and surimi gels with and without Gelpro HC30 addition. Cross-linking of MHC through disulfide and non disulfide covalent bonds contributed to the formation of high-molecular-weight polymers and aggregates (Ragnarsson and Regenstein, 1989).

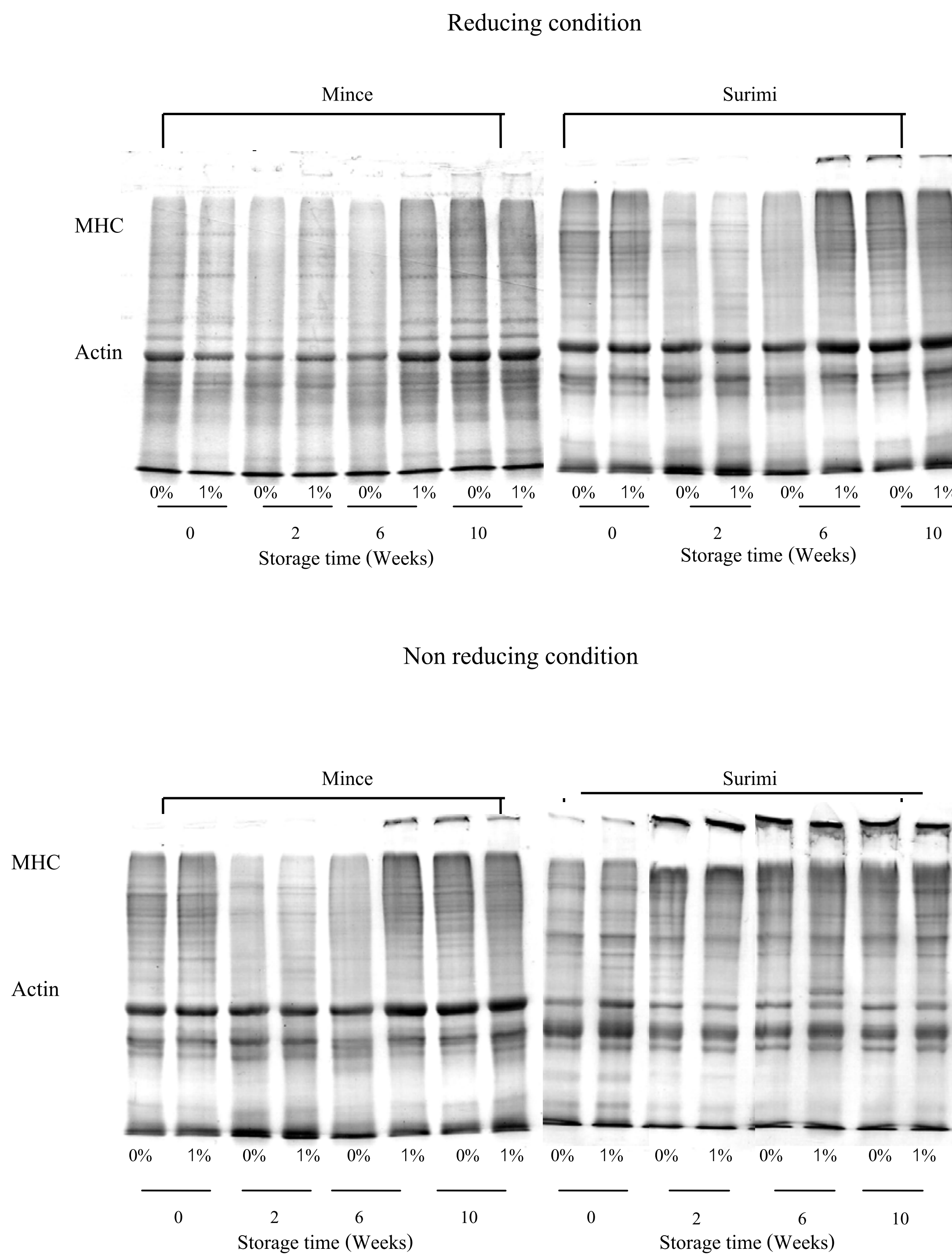


Figure 32. SDS-PAGE pattern of mince and surimi gels added without and with 1% Gelpro HC30 during frozen storage under reducing and non reducing condition. MHC: myosin heavy chain

6. Effect of modified atmosphere packaging (MAP) on quality of mince gel during storage at 4°C

6.1 Microbiological changes of mince gel

Changes in mesophilic bacterial counts of mince gel with and without 1% Gelpro HC30 during storage at 4°C are shown in Table 15. Initial mesophilic plate counts of mince gel added with and without Gelpro HC30 were 2.67 log CFU/g and 2.74 log CFU/g, respectively. Mesophilic bacterial counts increased most rapidly in both samples stored in air and the lowest counts were found in sample stored under MAP3 and MAP4. For sample stored under MAP1 and MAP2, lower count was found when Gelpro HC30 at a level of 1% was added. However no differences were found in sample added with and without Gelpro HC30 when MAP3 and MAP4 were applied. The shelf-life of samples stored in air and under all MAP was estimated to be 9 and 15 days, respectively. Total bacterial count in fish products indicating the rejection of the fish products frozen, chilled or stored with ice is < 6 log CFU/g. (ICMSF, 1978). The result indicated that MAP was effective in retarding the growth of bacteria. CO_2 used for MAP played a role in inhibiting the growth of microorganisms (Sivertsvik *et al*., 2002). O_2 generally stimulates the growth of aerobic bacteria and may inhibit the growth of exclusively anaerobic bacteria (Farber, 1991). Thus, the use of O_2 in modified atmospheres is generally avoided with this kind of fish, in order to minimize such effects. MAP associated to high CO_2 levels improves the stability of fresh fish, increasing its shelf-life (Baker *et al.* 1986). Carbon dioxide atmosphere, with variable proportions in oxygen, can be considered effectively inhibitory on the total aerobic flora (Stammen *et al*., 1990). MAP can affect the quality of the product, mainly owing to CO_2 dissolved in the muscle tissue, which is associated with an increase of carbonic acid (Sivertsvik *et al*., 2004). Ozogul *et al.* (2002) reported that the amount of mesophilic bacteria count in herring flesh at the time of rejection in MAP (60% CO_2/ 40% N_2) (10 days) and in vacuum (8 days) is < 6 log CFU/g.

Psychrophilic bacteria counts of mince gel with and without 1% Gelpro HC30 during storage at 4°C are shown in Table 16. Initial psychrophilic bacteria counts of mince gel added with and without 1% Gelpro HC30 were 3.13 log CFU/g and 2.41 log CFU/g, respectively. Psychrophic plate counts increased rapidly in both samples stored in air and the lowest counts were found in sample stored under MAP3 and MAP4. In general, a minimum of 20% CO_2 is

needed for significant effects on microbiological growth, but increasing CO_2 levels increase the efficiency of the gas. CO_2 is easily absorbed in meat, and the absorption depends on some factors such as temperature, pH, partial CO_2 pressure, headspace to meat volume ratio, meat surface area, total meat volume, water and fat content (Jakobsen and Bertelsen, 2002). When meat is exposed to CO_2, carbonic acid (H_2CO_3) is formed which further dissociates to bicarbonate and hydrogen ions (Sorheim, 2004). Psychotropic bacteria count of gutted cod under MAP (25% CO_2/ 75% N_2) was lower than that of air packed samples during the storage at $0^{\circ}C$ (Villemure *et al.*, 1986). From the result, higher content of CO_2 in MAP showed the inhibitory effect on psychrophilic bacterial, leading to the lower counts obtained.

Changes in yeast of mince gels without and with 1% Gelpro HC30 during the storage under MAP are shown in Table 17. Yeast of mince gels kept in air was observed after 6 days of storage. Yeasts were found in mince gels stored under MAP after 9 days of storage. Thus, MAP resulted in the retardation of yeast growth in mince gel. Arkoudelos *et al*. (2007) reported that farmed eel (*Anguilla anguilla*), stored under MAP contained no yeast within the first 24 days and finally reached the log 3.1 at day 37. The result suggested that Gelpro HC30 was not the source of yeast which was contaminated in the gel samples.

Changes in LAB of mince gels with and without at 1% Gelpro HC30 kept in air or under MAP during storage at 4°C are shown in Table 18. Generally, LAB was found at day 9 of storage at at 4°C. Addition of 1% Gelpro HC30 resulted in a slightly lower LAB count. At days 12 and 15 of storage, higher LAB was found in sample stored under MAP with greater CO_2 ratio. Under MAP conditions, LAB growth was observed in hake steaks (Ordonez *et al*., 2000) and refrigerated sea bass (Masniyom *et al*., 2002). LAB inhibit growth of other bacteria because of the formation of lactic acid and bacteriocins, and this may contribute to their selective growth during spoilage of seafood products. LAB has been known to produce bacteriocin, which showed inhibitory activity towards some microorganisms (Adams and Marteau, 1995). However, bacteriocins not only inhibited the growth of spoilage bacteria, but also LAB (Gill and Penney, 1986). CO_2 can favor the growth of some Gram-positive bacteria, i.e. Lactobacillus that would compete with spoilage bacteria (Stenstrom, 1985). CO_2, the inhibitory gas, has a strong antimicrobial action, which varies between different microorganisms (Gould, 2000). It is

responsible for extending the lag phase of bacterial growth and for decreasing the growth rate during the log phase (Farber, 1991).

Table 15 Changes in mesophilic bacterial counts (log CFU/g) of mince gel stored in air and under MAP during storage at 4°C

Treatment		Storage time (days)					
		0	3	6	9	12	15
Air	0 %	2.74±0.04	3.30±0.49	4.33±0.70	5.44±0.30	6.80±0.49	
	1 %	2.67±0.13	3.06±0.52	3.51±0.27	5.15±0.54	6.80±0.79	
60-35-5	0 %	2.74±0.04	3.16±0.48	3.98±0.19	5.15±0.50	5.58±0.40	5.71±0.77
(MAP1)	1 %	2.67±0.13	3.25±0.24	3.65±0.24	4.93±0.38	5.23±0.43	5.06±0.79
60-30-10	0 %	2.74±0.04	3.43±0.41	3.67±0.63	3.98±0.30	5.17±0.40	5.94±0.67
(MAP2)	1 %	2.67±0.13	3.37±0.64	3.54±0.09	4.37±0.36	5.00±0.43	5.37±0.49
80-15-5	0 %	2.74±0.04	3.94±0.56	4.16±0.00	4.19±0.11	5.04±0.04	5.52±0.63
(MAP3)	1 %	2.67±0.13	2.66±0.51	3.25±0.50	3.93±0.64	5.10±0.26	5.59±0.26
80-10-10	0 %	2.74±0.04	2.62±0.23	3.54±0.48	4.55±0.50	5.54±0.45	5.61±0.24
(MAP4)	1 %	2.67±0.13	1.98±0.52	3.38±0.64	4.20±0.00	5.20±0.32	5.62±0.39

Values are given as mean ± SD from triplicate determinations.

Table 16 Changes in psychrophic bacterial counts (log CFU/g) of mince gel stored in air and under MAP during storage at 4°C

Treatment		Storage time (days)					
		0	3	6	9	12	15
Air	0 %	0	2.41±0.57	4.54±0.51	5.51±0.59	6.48±0.50	
	1 %	0	3.13±0.45	4.39±0.50	5.48±0.50	6.49±0.40	
60-35-5	0 %	0	1.70±0.21	2.95±0.06	4.32±0.43	4.38±0.32	4.61±0.49
(MAP1)	1 %	0	1.60±0.33	2.71±0.19	3.48±0.23	4.14±0.85	4.60±0.45
60-30-10	0 %	0	1.30±0.32	3.11±0.22	3.30±0.22	4.23±0.32	4.67±0.39
(MAP2)	1 %	0	1.78±0.22	3.48±0.31	3.85±0.33	4.39±0.40	4.76±0.09
80-15-5	0 %	0	2.81±0.27	2.86±0.06	3.20±0.31	3.39±0.42	3.80±0.12
(MAP3)	1 %	0	2.11±0.20	2.48±0.07	3.13±0.40	3.71±0.27	3.76±0.33
80-10-10	0 %	0	2.47±0.01	2.61±0.10	3.26±0.43	3.63±0.31	3.79±0.42
(MAP4)	1 %	0	1.95±0.35	3.30±0.09	3.45±0.30	3.69±0.49	3.90±0.37

Values are given as mean ± SD from triplicate determinations.

Table 17 Changes in yeast (log CFU/g) of mince gel stored in air and under MAP during storage at 4°C

Treatment		Storage time (days)					
		0	3	6	9	12	15
Air	0 %	0	0	3.60±0.23	3.78±0.36	5.13±0.49	
	1 %	0	0	3.00±0.60	3.43±0.30	3.63±0.25	
60-35-5	0 %	0	0	0	3.64±0.45	4.00±0.23	5.48±0.50
(MAP1)	1 %	0	0	0	3.50±0.40	3.63±0.35	4.93±0.51
60-30-10	0 %	0	0	0	2.43±0.55	3.64±0.40	4.34±0.29
(MAP2)	1 %	0	0	0	3.20±0.46	3.63±0.45	4.68±0.37
80-15-5	0 %	0	0	0	3.32±0.60	3.62±0.70	3.64±0.50
(MAP3)	1 %	0	0	0	3.47±0.50	3.63±0.63	4.28±0.47
80-10-10	0 %	0	0	0	3.20±0.45	3.64±0.50	4.00±0.52
(MAP4)	1 %	0	0	0	3.00±0.40	3.36±0.50	3.63±0.56

Values are given as mean ± SD from triplicate determinations.

Table 18 Changes in lactic acid bacteria (log CFU/g) of mince gel stored in air and under MAP during storage at 4°C

Treatment		Storage time (days)					
		0	3	6	9	12	15
Air	0 %	0	0	0	3.0±0.20	3.0±0.27	
	1 %	0	0	0	2.6±0.19	3.0±0.09	
60-35-5	0 %	0	0	0	3.0±0.75	3.42±0.35	4.59±0.35
(MAP1)	1 %	0	0	0	2.0±0.3	2.60±0.42	3.95±0.6
60-30-10	0 %	0	0	0	3.00±0.2	3.11±0.57	3.22±0.45
(MAP2)	1 %	0	0	0	2.95±0.2	3.00±0.75	3.12±0.65
80-15-5	0 %	0	0	0	3.00±0.4	4.78±0.14	4.88±0.21
(MAP3)	1 %	0	0	0	2.60±0.1	4.55±0.13	4.60±0.47
80-10-10	0 %	0	0	0	3.00±0.7	3.50±0.74	4.49±0.14
(MAP4)	1 %	0	0	0	2.60±0.7	3.49±0.23	3.80±0.02

Values are given as mean ± SD from triplicate determinations.

6.2 TVB content

Changes in TVB contents of mince gels with and without 1% Gelpro HC30 stored under MAP and in air during storage at 4 °C are presented in Table 19. Generally, TVB content increased in all samples as storage time increased. However, the rate of increases was lowered when MAP was applied, particularly as the higher CO_2 ratio (MAP3 and MAP4) was used. TVB of sample kept in air increased rapidly and reached 40 mg/100 g after 9 days of storage. In contrast, samples stored under CO_2-enriched atmosphere, particularly with 80%, had TVB less than 28 mg/100 g within 15 days. For sample kept under 60% CO_2 atmosphere, TVB reached 51 mg/100 g after 15 days of storage. TVB-N limit of acceptability (35 mg per 100 g) for consumption of hake steaks was reported by Ordonez (2000) as 7 days of storage in air and 11 days packaged and stored in 20% CO_2 and 14 days stored in 40% CO_2. TVB-N and TMA are products of bacterial spoilage and their contents are often used as an index to assess the keeping quality and shelf life of seafood products. Higher TVB-N values in the range 25–35 mg per 100 g indicate fish that are slightly decomposed/edible and decomposed/inedible (El Marrakchi *et al*., 1990; Ababouch *et al*., 1996). Fraser and Sumar (1998) indicated that bacterial catabolism of amino acids in fish muscle results in the accumulation of ammonia and other volatile bases. The lowered TVB content in sample stored under MAP was coincidental with the retarded growth of bacteria as evidenced by the lower mesophilic and psychrophic count, in comparison with the sample stored in air.

Table 19 Changes in total volatile base content (mgN/100g sample) of mince gel stored in air and under MAP during storage at 4 ºC.

Treatment		Storage time (days)					
		0	3	6	9	12	15
Air	0%	4.20±0.40b,D	17.92±0.00c,C	34.44±1.98a,B	39.76±0.79a,A		
	1%	4.76±0.40a,D	17.36±1.58c,C	33.32±0.40a,B	40.32±0.00a,A		
60-35-5	0%	4.20±0.40b,E	17.64±0.40c,D	30.24±1.58b,C	35.28±0.79b,B	36.12±4.36b,B	51.24±0.40b,A
(MAP1)	1%	4.76±0.40a,C	19.88±1.19b,D	23.24±1.19c,C	24.64±2.38c,C	39.20±0.79a,B	56.84±0.40a,A
60-30-10	0%	4.20±0.40b,E	24.92±3.56a,D	33.88±0.40a,C	35.56±0.40b,C	39.48±1.19a,B	46.76±1.19c,A
(MAP2)	1%	4.76±0.40a,E	17.36±0.79c,D	22.96±1.58c,C	28.28±0.40c,B	28.56±0.40d,B	34.44±0.40e,A
80-15-5	0%	4.20±0.40b,E	17.92±0.79c,D	22.68±0.40c,C	26.04±2.77c,B	33.32±0.40b,B	33.32±0.40e,A
(MAP3)	1%	4.76±0.40a,E	19.88±2.77b,D	24.36±1.19c,C	28.56±0.79c,B	28.28±0.40e,A	39.48±0.40d,A
80-10-10	0%	4.20±0.40b,F	17.64±0.44c,E	17.92±0.79d,D	21.28±3.96d,C	28.56±1.58cd,B	28.28±0.40f,A
(MAP4)	1%	4.76±0.40a,E	17.36±.079c,D	22.68±0.40c,D	27.72±0.40c,C	29.12±0.00c,A	33.88±0.40e,A

Values are given as mean ±SD from five determinations. Different letters in the same column indicate significant differences ($p<0.05$). Different capital letters in the same row indicate significant differences ($p<0.05$).

6.3 Changes in TBARS

Changes in TBARS values of mince gels with and without 1% Gelpro HC30 stored in air or under MAP were monitored during storage at 4°C (Table 20). Those stored under MAP showed the lower TBARS than those kept in air. However, the sample stored under MAP with higher CO_2 content (MAP3 and MAP4) had the higher TBARS than others ($p<0.05$). This was probably because carbonic acid formed may induce the release of free haem iron, a potential pro-oxidant in the muscle system. At higher concentrations of CO_2 particularly 80 and 100%, carbonic acid formed in muscle probably caused the inactivation of antioxidative enzymes, e.g. gluthathione peroxidase, resulting in the higher oxidation in the muscle (Renerre *et al*., 1996). Therefore, CO_2-enriched packaging effectively inhibited the spoilage caused by microorganisms, but it somehow accelerated lipid oxidation. Furthermore, the lower TBARS values of samples kept in air might result from the direct microbial utilization of malonaldehyde and other TBARS (Wang and Brown, 1983). The presence of oxygen may cause oxidative rancidity problems in mince gels, promoting the formation of low molecular weight aldehydes, ketones, alcohols and carboxylic acids. An increase in TBARS was observed in all samples when the storage time increased ($p<0.05$), indicating that lipid oxidation still occurred. It was suggested that a maximum level of TBA value indicating the good quality of the fish frozen, chilled or stored with ice is 5 mg malonaldehyde/kg (Nunes *et al*., 1992). Lipid in fish muscle typically has a high percentage of polyunsaturated fatty acids and is consequently prone to oxidative reaction (Stammen *et al*., 1990). From the result, the addition of 1% Gelpro HC30 had no impact on lipid oxidation in mince gel under all packaging atmospheres used.

Table 20 Changes in TBARS (mg malonaldehyde/kg sample) of mince gel stored in air and under MAP during storage and air atmosphere at 4 ºC.

Treatment		Storage time (days)					
		0	3	6	9	12	15
Air	0%	6.20±0.05a,D	11.26±2.34a,C	15.59±0.35a,B	18.13±0.27a,A		
	1%	5.10±0.04b,D	9.49±0.60bc,C	13.42±1.42b,B	14.75±0.09b,A		
60-35-5	0%	6.20±0.05a,E	10.01±0.09b,D	10.02±0.34de,D	11.28±0.37e,C	13.02±0.27b,B	22.08±0.94b,A
(MAP1)	1%	5.10±0.04b,E	9.28±0.25bc,D	10.90±0.02d,C	12.01±0.18d,B	12.36±0.19c,B	20.97±0.18b,A
60-30-10	0%	6.20±0.05a,D	10.05±1.55b,C	11.19±0.38d,C	11.16±0.02f,C	13.75±0.41a,B	21.00±0.73b,A
(MAP2)	1%	5.10±0.04b,D	9.52±0.26bc,C	9.79±0.15e,C	13.26±0.18c,B	13.36±0.86a,B	19.23±1.07c,A
80-15-5	0%	6.20±0.05a,D	10.41±0.01b,C	10.55±0.73d,C	12.70±0.12d,B	13.67±0.29a,B	22.51±0.75ab,A
(MAP3)	1%	5.10±0.04b,F	9.28±0.15c,E	10.26±0.14de,D	11.58±0.24e,C	14.53±0.74a,B	26.39±0.86a,A
80-10-10	0%	6.20±0.05a,E	9.14±0.20c,D	11.63±0.07c,C	12.470.25±d,C	14.81±0.42a,B	25.24±0.45a,A
(MAP4)	1%	5.10±0.04b,E	8.39±0.13d,D	11.80±0.17c,C	12.39±0.28d,C	15.13±0.44a,B	24.00±1.20a,A

Values are given as mean ±SD from five determinations. Different letters in the same column indicate significant differences (p<0.05). Different capital letters in the same row indicate significant differences (p<0.05).

6.4 Changes in expressible moisture content

Expressible moisture content of mince gels during storage in air or under MAP in the presence and absence of 1% Gelpro HC30 is shown in Table 21. Slight increases in expressible moisture content were observed in sample kept in air, regardless of Gelpro HC30 addition throughout the storage ($p<0.05$). Mince gels stored under MAP with a high content of CO_2 (MAP3 and MAP4) had the increase in expressible moisture content as the storage time increased ($p<0.05$). This may be due to greater loss of water holding capacity of gel at lower pH values. Carbon dioxide dissolution into the tissue leads to an acidification of the medium and the subsequent formation of carbonic acid. This might affect the water holding capacity and thus the texture properties of the product. Conversely, slight decrease in expressible moisture content was found in sample stored under MAP1 and MAP2 when the storage time increased. The addition of 1% Gelpro HC30 resulted in the decreased expressible moisture content, possibly via holding the water in the molecule of modified starch. From the result, it was noted that the expressible moisture content of mince gel was not increased as much as fish meat under MAP, particularly as the storage time increased. Therefore, gel matrix formed might resist to CO_2 penetration. Also, aggregated proteins were much less prone to acid denaturation, compared with native protein in fish muscle.

6.5 Breaking force and deformation

Breaking force and deformation of mince gels with and without 1% Gelpro HC30 kept under different conditions are shown in Table 22 and 23. For mince gels stored in air, breaking and deformation rapidly increased up to 9 days of storage ($p<0.05$), except for day 6 when the low breaking force was found. Gel added with 1% Gelpro HC30 had no changes in breaking force between day 0 and 9 of storage in air ($p>0.05$). Overall, inconsistent changes were observed in all gels, during storage up to 9 and 15 days of storage for the sample kept in air and under MAP, respectively. However, gels tended to have the increase in breaking force but decreased deformation when stored under MAP with high CO_2 content, (MAP3 and MAP4).

Table 21 Changes in expressible moisture content (%) of mince gel stored in air and under MAP during storage at 4ºC

Treatment		Storage time (days)					
		0	3	6	9	12	15
Air	0%	2.13±0.0038a,B	2.18±0.0212a,B	2.22±0.0167a,B	2.35±0.0235a,A		
	1%	2.12±0.0012a,B	2.12±0.0183a,B	2.20±0.0210a,A	2.29±0.0129a,A		
60-35-5	0%	2.13±0.0038a,B	2.18±0.0218a,A	2.15±0.0149a,A	2.11±0.0211b,A	2.12±0.0212a,A	2.11±0.0184b,A
(MAP1)	1%	2.12±0.0012a,A	2.01±0.0201b,B	1.81±0.0181a,C	1.75±0.0175e,C	1.99±0.0199b,B	1.99±0.0160c,B
60-30-10	0%	2.13±0.0038a,A	2.14±0.0221a,A	2.17±0.0193a,A	1.91±0.0154d,B	1.89±0.0189c,B	1.96±0.0196c,B
(MAP2)	1%	2.12±0.0012a,A	2.11±0.0244a,A	1.93±0.0217e,B	1.54±0.0191f,E	1.66±0.0166e,D	1.80±0.0180d,C
80-15-5	0%	2.13±0.0038a,B	2.12±0.0212a,B	2.21±0.0191a,A	2.02±0.0202c,C	2.00±0.0200b,C	2.25±0.0215a,A
(MAP3)	1%	2.12±0.0012a,B	1.80±0.0180c,C	2.09±0.0185d,B	1.80±0.0180e,C	1.82±0.0182d,C	2.22±0.0182a,A
80-10-10	0%	2.13±0.0038a,B	2.05±0.0205a,C	2.26±0.0256a,A	2.19±0.0229b,A	1.96±0.0196b,D	2.21±0.0211a,A
(MAP4)	1%	2.12±0.0012a,B	1.86±0.0186c,D	2.17±0.0172c,A	1.83±0.0183e,D	2.19±0.0219a,A	2.00±0.0190c,C

Values are given as mean ±SD from five determinations. Different letters in the same column indicate significant differences ($p<0.05$). Different capital letters in the same row indicate significant differences ($p<0.05$).

Table 22 Changes in breaking force (g) and deformation of mince gel stored in air and under MAP during storage at 4ºC

Treatment		Storage time (days)					
		0	3	6	9	12	15
Air	0%	548.23±34.36a,B	707.68±30.40ab,A	557.2±16.86c,B	735.60±26.23b,A		
	1%	665.25±29.70a,B	740.98±40.12a,A	538.93±15.82c,B	668.05±30.00c,B		
60-35-5	0%	548.23±34.36a,C	649.88±36.62c,B	673.65±18.04b,AB	751.68±37.98b,A	748.18±14.49b,A	640.80±43.54c,B
MAP1	1%	665.25±29.70a,C	666.33±21.46c,C	650.95±36.22b,C	739.85±11.99b,A	792.73±41.49b,A	641.23±25.97c,C
60-30-10	0%	548.23±34.36a,B	732.13±23.00a,A	734.93±35.05a,A	742.18±27.80b,A	751.23±13.40b,A	753.48±26.90a,A
MAP2	1%	665.25±29.70a,C	743.85±6.75a,B	756.65±22.77a,B	843.38±24.10a,A	842.88±34.78a,A	765.23±15.96a,B
80-15-5	0%	548.23±34.36a,C	653.88±20.92c,B	538.08±28.77c,C	748.80±44.67b,A	743.33±5.21b,A	576.35±29.04d,C
MAP3	1%	665.25±29.70a,B	743.33±31.34a,A	653.9±15.79b,B	758.38±19.22b,A	732.48±24.41b,A	642.93±20.88d,B
80-10-10	0%	548.23±34.36a,C	653.00±13.49c,B	534.83±35.75c,C	718.25±26.71b,AB	751.13±22.48b,A	683.65±12.39b,B
MAP4	1%	665.25±29.70a,C	731.68±23.66a,A	671.15±15.62b,BC	711.98±32.53b,AB	679.15±34.34c,BC	724.28±12.66b,A

Values are given as mean ±SD from five determinations. Different letters in the same column indicate significant differences ($p<0.05$). Different capital letters in the same row indicate significant differences ($p<0.05$).

Table 23 Changes in deformation (mm) and deformation of mince gel stored in air and under MAP during storage at 4ºC

Treatment		Storage time (days)					
		0	3	6	9	12	15
Air	0%	8.48±0.33a,C	9.27±0.36cd,B	9.28±0.22a,B	10.09±0.29ab,A		
	1%	9.90±0.34a,B	9.53±0.10a,A	8.54±0.32b,D	9.45±0.33de,C		
60-35-5	0%	8.48±0.33a,B	8.86±0.39e,B	7.19±0.10d,C	9.70±0.22de,A	8.76±0.07b,B	8.57±0.32c,B
MAP1	1%	9.90±0.34a,AB	9.18±0.36de,C	8.31±0.20b,D	10.30±0.35bcd,A	8.61±0.18b,D	9.63±0.23c,B
60-30-10	0%	8.48±0.33a,B	9.43±0.23cd,A	7.50±0.17cd,C	9.41±0.28de,A	8.26±0.15c,B	8.65±0.56b,B
MAP2	1%	9.90±0.34a,AB	9.76±0.40bc,BC	8.65±0.16b,D	10.33±0.30a,A	8.54±0.35bc,D	9.33±0.06a,C
80-15-5	0%	8.48±0.33a,B	9.38±0.18cd,A	7.70±0.21c,C	9.55±0.26cde,A	9.17±0.28a,A	7.58±0.17b,C
MAP3	1%	9.90±0.34a,A	10.05±0.17ab,A	7.34±0.38cd,C	9.92±0.27abc,A	8.60±0.13b,B	7.46±0.45a,C
80-10-10	0%	8.48±0.33a,B	9.14±0.25de,A	7.42±0.24cd,C	9.30±0.29e,A	7.60±0.12d,C	7.67±0.28c,C
MAP4	1%	9.90±0.34a,A	10.06±0.17ab,A	7.37±0.38b,E	9.31±0.34e,B	8.56±0.16bc,C	7.89±0.09a,D

Values are given as mean ±SD from five determinations. Different letters in the same column indicate significant differences (p<0.05).Different capital letters in the same row indicate significant differences (p<0.05).

6.6 Color and whiteness

L, a*, b**- value and whiteness of gel samples during storage in air and under MAP are shown in Table 24. No marked changes in all parameters were noticeable during the storage up to 15 days. Since the proteins in gel network were denatured and cross-linked during gelation process, no much change in color occurred during MAP storage. Unlike fish fillet or meat, the meat turns to be opaque under MAP, especially with the high CO_2 content. Thus, atmosphere packing condition and storage time did not show marked effect on color and whiteness of mince gel. Gelpro HC30 at a level of 1% also exhibited the negligible effect on color and whiteness of resulting gels at all storage time studied.

Table 24 Changes in *L**, *a**, *b** and whiteness of mince gel stored in air and under MAP during storage at 4ºC

			Storage time (days)					
	Condition		**0**	**3**	**6**	**9**	**12**	**15**
L*	Air	0 %	76.74±0.40a,A	76.92±0.36abc,A	76.76±0.12ab,A	76.36±0.60b,A		
		1%	75.65±0.70a,A	76.15±0.71bc,A	76.14±0.24b,A	75.30±0.25c,A		
	60-35-5	0%	76.74±0.40a,A	74.94±0.23c,C	75.90±0.31b,B	76.44±0.25b,A	76.50±0.19a,A	76.29±0.53a,AB
	MAP1	1%	75.65±0.70a,C	77.33± 0.33a,A	76.01±0.29b,BC	76.43±0.24b,B	76.34±0.42a,BC	76.17±0.69a,BC
	60-30-10	0%	76.74±0.40a,AB	76.26±0.37bc,BC	76.74±0.48a,AB	77.16±0.53a,A	75.66±0.63a,C	76.89±0.58a,AB
	MAP2	1%	75.65±0.70a,A	76.04±0.59b,A	75.63±0.18bc,A	75.90±0.38b,A	76.05±0.34a,A	74.43±0.23b,B
	80-15-5	0%	76.74±0.40a,A	77.03±0.61ab,A	77.22±1.15a,A	77.15±0.33a,A	75.62±1.35a,B	76.56±0.51a,AB
	MAP3	1%	75.65±0.70a,A	76.22±0.29bc,A	76.02±0.21b,A	76.11±0.64b,A	75.78±0.59a,A	74.55±1.19b,B
	80-10-10	0%	76.74±0.40a,A	76.47±1.42bc,A	77.08±0.07a,A	77.09±0.47a,A	76.14±0.72a,A	77.15±0.73a,A
	MAP4	1%	75.65±0.70a,BC	75.00±0.18c,C	75.21±0.45cB,C	77.04±0.53a,A	76.33±0.19a,AB	76.39±1.22a,AB
a*	Air	0%	-1.20±0.16a,C	-1.00±0.12ab,B	-1.48±0.03bc,D	-0.79±0.14a,A		
		1%	-1.09±0.14a,A	-0.80±0.29a,A	-1.55±0.21bc,B	-1.09±0.11b,A		
	60-35-5	0%	-1.20±0.16a,B	-1.26±0.23bc,B	-1.32±0.18a,B	-0.85±0.12ab,A	-0.71±0.19abc,A	-0.78±0.17ab,A
	MAP1	1%	-1.09±0.14a,AB	-1.12±0.20abc,AB	-1.18±0.18a,B	-1.09±0.27b,AB	-0.86±0.19bcA	-0.91±0.20b,AB
	60-30-10	0%	-1.20±0.16a,C	-1.39±0.10c,D	-1.43±0.06ab,D	-1.01±0.29ab,BC	-0.69±0.17abc,A	-0.81±0.11ab,AB
	MAP2	1%	-1.09±0.14a,B	-1.14±0.16abc,B	-1.74±0.05c,C	-1.36±0.16c,B	-0.74±0.12bc,A	-1.19±0.29c,B
	80-15-5	0%	-1.20±0.16a,B	-1.04±0.21ab,B	-1.52±0.10bc,C	-0.97±0.15ab,B	-0.45±0.23a,A	-0.57±0.06a,A
	MAP3	1%	-1.09±0.14a,B	-1.04±0.28ab,B	-1.73±0.13c,C	-1.05±0.27ab,B	-0.60±0.14ab,A	-0.72±.180ab,A
	80-10-10	0%	-1.20±0.16a,B	-1.15±0.25abc,B	-1.58±0.09bc,C	-0.95±0.14ab,AB	-0.73±0.30bc,A	-0.83±0.11b,A
	MAP4	1%	-1.09±0.14a,BC	-1.29±0.18bc,C	-1.57±0.29bc,D	-1.07±0.15ab,BC	-0.89±0.16c,AB	-0.80±0.10ab,A
b*	Air	0%	14.74±0.18a,B	15.04±0.12a,B	15.11±0.24a,B	15.77±0.44a,A		
		1%	14.57±0.37a,B	14.95±0.54ab,AB	14.86±0.39abc,B	15.63±0.52a,A		
	60-35-5	0%	14.74±0.18a,A	14.81±0.39ab,A	14.96±0.11ab,A	13.83±0.32c,B	14.19±0.18cd,B	15.13±0.38ab,,A
	MAP1	1%	14.57±0.37a,AB	14.43±0.14bc,AB	14.73±0.05bcd,A	14.22±0.37bc,B	14.40±0.06bc,AB	14.79±0.25abA
	60-30-10	0%	14.74±0.18a,B	14.65±0.21abc,BC	14.51±0.04cd,BCD	14.13±0.29bc,CD	14.01±0.33de,D	15.38±0.66a,A
	MAP2	1%	14.57±0.37a,AB	14.22±0.09c,BCD	14.42±0.14d,ABC	14.07±024bc,CD	13.98±0.17e,D	14.65±0.43b,A
	80-15-5	0%	14.74±0.18a,A	14.70±0.46abc,A	14.59±0.30bcd,AB	14.20±0.43bc,B	13.73±0.16e,C	14.80±0.16ab,A
	MAP3	1%	14.57±0.37a,AB	14.59±0.42abc,A	14.62±0.04bcd,AB	14.54±0.25b,AB	13.80±0.37de,C	14.97±0.41ab,A
	80-10-10	0%	14.74±0.18a,A	14.43±0.53bc,A	14.51±0.14cd,A	13.76±0.72c,B	14.56±0.18b,A	14.80±0.50ab,A
	MAP4	1%	14.57±0.37a,A	14.56±0.28abc,A	14.42±0.25d,B	14.03±0.38bc,B	14.97±0.31a,A	14.68±.034b,A
whiteness	Air	0%	72.44±0.33a,A	72.43±0.36bc,A	72.24±0.23bc,A	71.57±0.58d,B		
		1%	71.59±0.54b,A	71.83±0.42c,A	71.85±0.02cd,A	70.75±0.47e,B		
	60-35-5	0%	72.44±0.33a,AB	70.86±0.29d,D	71.60±0.24de,C	72.66±0.38ab,A	72.46±0.29a,AB	71.93±0.72a,BC
	MAP1	1%	71.59±0.54b,C	73.10±0.32a,A	71.82±0.41d,BC	72.44±0.53bc,B	72.36±0.32a,B	71.86±0.59a,BC
	60-30-10	0%	72.44±0.33a,AB	72.07±0.29bc,B	72.55±0.22ab,AB	73.12±0.26a,A	72.45±0.41a,AB	72.22±0.34a,B
	MAP2	1%	71.59±0.54b,AB	72.11±0.50bc,A	71.63±0.03de,AB	72.06±0.26cd,AB	71.46±0.59bc,B	70.51±0.50b,C
	80-15-5	0%	72.44±0.33a,ABC	72.71±0.18ab,ABC	72.91±0.17a,AB	73.07±0.60a,A	72.14±0.83ab,C	72.27±0.94a,BC
	MAP3	1%	71.59±0.54b,A	72.08±1.00bc,A	71.86±0.19cd,A	72.02±0.40cd,A	71.48±0.57bc,A	70.45±0.88b,B
	80-10-10	0%	72.44±0.33a,A	72.35±0.25bc,A	72.82±0.02a,A	73.25±0.49a,A	72.59±0.25a,A	72.77±0.99a,A
	MAP4	1%	71.59±0.54b,BC	71.04±0.29d,C	71.27±0.27e,C	73.07±0.66a,A	71.33±0.62c,C	72.18±0.99a,B

Values are given as mean ±SD from five determinations. Different letters in the same column with in the same parameter indicate significant differences ($p<0.05$). Different capital letters in the same row indicate significant differences ($p<0.05$).

CHAPTER 4

CONCLUSION

1. Mince gels added with iota carrageenan and Gelpro HC30 exhibited the highest breaking force and deformation and could lower the expressible moisture content. The addition of modified starch (Gelpro HC30) could maintain the textural property and decreased the expressible moisture of mince gel subjected to multiple freeze-thawing most effectively. The addition of 1% Gelpro HC30 yielded the gel network with fine structure with smaller voids which was likely associated with the maintained textural properties and acceptability of bigeye snapper gels after freeze-thawing.

2. Extended frozen storage of mince and surimi gels resulted in the formation of rubbery texture and lower water holding capacity. Gels from mince underwent the textural changes to a higher degree than those from surimi. Addition of 1% Gelpro HC30 could lower the loss of gel properties during extended frozen storage to some degree. Gelpro HC30 most likely retarded protein aggregations induced by migration of water in mince and surimi gels during frozen storage.

3. Mince gels underwent physicochemical and biochemical changes during storage at 4°C, depending on modified atmosphere packaging conditions. MAP was found to be an effective method to prolong the shelf-life of mince gel, mainly due to a substantial reduction of the microbial growth. This led to the delayed spoilage and protein degradation. MAP with CO_2 contents of 60% or 80% could retard the microbial growth. However, the addition of 1% Gelpro HC30 had no pronounced effect on the gel properties during storage at 4°C for up to 15 days.

FUTURE RESEARCHES

1. Novel hydrocolloids, particularly modified counterparts should be used to increase the freeze-thaw stability of mince gel.
2. Uses of some natural antimicrobials in mince gel prior to MAP should be intensively studied to extend the shelf-life of mince gel products.

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APPENDIX

ANALYTICAL METHODS

1. pH determination (Benjakul *et al*., 1997)

Method

1. Weigh 5 g of sample. Add 5 volumes of distilled (w/v).
2. Homogenize for 2 min.
3. Measure pH using pH meter.

2. 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 desiccator to cool. Weigh the empty the dish and lid.
2. Weigh about 3 g of sample to the dish. Spread the sample with spatura.
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

$$\text{Moisture content (\%)} = \frac{(W_1-W_2) \times 100}{W_1}$$

where: W_1 = weight (g) of sample before drying

W_2 = weight (g) of sample after drying

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 burn off. Cool the crucible in the desiccator (30 min).
2. Weigh the crucible and lid to 3 decimal places.
3. 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.

4. Heat at 550°C overnight. During heating, do not cover the lid. Place the lid on after complete heating to prevent loss of fluffy ash. Cool down in the desiccator.
5. Weigh the ash with crucible and lid until turning to gray. If not, return the crucible and lid to the furnace for further ashing.

Calculation

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

4. Determination of protein (AOAC, 1999)

Reagents

1. Kjedahl catalyst: Mix 9 part of potassium sulphate (K_2SO_4) anhydrous, nitrogen free with 1 part of copper sulphate ($CuSO_4$) - Sulfuric acid (H_2SO_4)
2. 40% NaOH solution (w/v)
3. 0.02N HCl solution
4. 4% H_3BO_3 solution (w/v)
5. 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 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 until frothing ceases. Boil briskly until solution clears.
4. Cool and add 60 ml distilled water cautiously.
5. Immediately connect flask to digestion bulb on condenser, and with tip of condenser immersed in standard acid and 5-7 indicator in receiver. Rotate flask to mix content thoroughly; then heat until all NH3 has distilled.
6. Remove receiver, wash tip of condenser, and titrate excess standard acid in 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 0.02N HCl used sample titration

B = volume (ml) of 0.02N HCl used in blank titration

N = Normality of HCl

W = weight (g) of sample

14.007 = atomic weight of nitrogen

6.25 = the protein-nitrogen conversion factor for fish and its byproducts

5. Determination of fat content (AOAC, 1999)

Reagents

- Petroleum ether

Method

1. Place the bottle and lid in the incubator at 105°C overnight to ensure that weight of bottle is stable.
2. Weigh about 3-5 g of sample to paper filter and wrap.
3. Take the sample into extraction thimble and transfer into soxhlet
4. Fill petroleum ether about 250 ml into the bottle and take it on the heating mantle.
5. Connect the soxhlet apparatus and turn on the water to cool them and then switch on the heating mantle.
6. Heat the sample about 14 h (heat rate of 150 drop/min).
7. Evaporate the solvent by using the vacuum condenser.
8. Incubate the bottle at 80-90 °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

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

6. Determination of solubility (Benjakul and Bauer 2000)

Reagent

- Tris-HCl buffer (pH 8.0) containing 1 % (w/v) SDS, 8 M urea and 2 % (v/v) β-mercaptoethanol
- 0.5 M NaOH
- 10 % and 50 % trichloroacetic acid (TCA)

Method

- Cut the sample into small pieces, weigh 1 g of sample, and transfer into a 100 ml conical flask.
- Add 20 ml of solvent, homogenize for 1 min.
- Heat the homogenate in boiling water bath for 2 min and stir at room temperature for 4 h.
- Centrifuge at 10,000xg for 30 min.
- Add 2 ml of 50% TCA (w/v) into10 ml of the supernatant (soluble fraction).
- Kept the mixture at 4°C for 18 h, centrifuged at 10,000xg for 20 min.
- Wash the precipitate with 10 % TCA and solubilize in 0.5 M NaOH.
- Determine protein content by biuret method.

Note: Samples was also solubilized in 0.5 NaOH. Protein content in 0.5 M NaOH extract was used as reference value, i.e., 100 %

7. Determination of protein content using the Biuret method (Robinson and Hodgen, 1940)

Reagents

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

Method

1. To 0.5 ml of sample, 2.0 ml of the biuret reagent were added and mixed well.

2. The mixture was incubated at room temperature for 30 min, then the absorbance at 540 nm was read.

Table: Experimental set up for the biuret's assay.

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

8. Thiobarbituric acid-reactive substance (TBARS) (Buege and Aust, 1978)

Reagents

TBARS solution: 0.375 g of thiobituric acid, 15 g of thichloroacetic acid, and 0.875 ml of hydrochloric were mixed thoroughly in 100 ml of distilled water.

Method

- Disperse ground sample (0.5g) in 10 ml of TBA solution.
- Heat the mixture in boiling water for 10 min, followed by cooling in running tap water.
- Centrifuge the mixture at 3600xg for 20 min and measure the absorbance at 532 nm.
- Prepare the malonaldehyde bis (dimethyl acetal) (MDA) at concentration ranging from 0 to 10 ppm using.
- Calculate and express TBARS as mg malondialdehyde/kg.

9. Determination of trimethylamine (TMA-N) and total volatile basic nitrogen (TVB-N) by Conway's method (Conway, 1950)

Reagents

1. Inner ring solution (1 % boric acid solution containing indicator).
2. Mixed indicator solution: Dissolve bromocresol green (BCG) 0.01 g and methyl red (MR) 0.02 g in 10 ml of ethanol.
3. 0.02 N HCl
4. Satureted K_2CO_3 solution: Take 60 g of potassium carbonate, and add 50 ml of distilled water. Boil gently for 10 min. After cooling down, obtain filtrate through filter paper.
5. 4 % trichloroacetic acid (CCl_3COOH), TCA, solution: Dissolve 40 g of TCA in 960 ml of distilled water.
6. Sealing agent: Take 3 g of Trangacanth gum, add 30 ml of distilled water, 15 ml of glycerine and 15 ml of 50 % saturated K_2CO_3 solution and mix well.
7. Neutralized 10 % formaldehyde solution: Add 10 g of $MgCO_3$ to 100 ml of formaline (35 % formaldehyde solution) and shake in order to neutralize the acidity of formaline. Filter and dilute filtrate 3 volume with distilled water.

Method

Sample extraction:

1. Take 2 g of fish meat in a mortar and grind well.
2. Add 8 ml of 4 % TCA solution and grind well.
3. Stand for 30 min at ambient temperature with occasional grinding.
4. Filter through filter paper (Whatman No. 41) or centrifuge at 3,000 rpm, for 10 min.
5. Keep the filtrate in -20°C freezing if necessary.

9.1 Determination of TVB-N

1. Apply sealing agent to Conway's unit.
2. Pipette 1 ml of inner ring solution into inner ring.
3. Pipette 1 ml of sample extract into outer ring.
4. Slant the Conway's unit with cover.

5. Pipette 1 ml of saturated K_2CO_3 solution into outer ring.
6. Close the unit.
7. Mix gently.
8. Stand for 60 min at 37°C in incubator.
9. Titrate inner ring solution with 0.02 N HCl using a micro-burette until green color turns pink.
10. Prepare the blank using 1 ml of 4 % TCA instead of sample extract.

9.2 Determination of TMA-N

1. Apply sealing agent to Conway's unit.
2. Pipette 1 ml of inner ring solution into inner ring.
3. Pipette 1 ml of sample extract into outer ring.
4. Pipette 1 ml of neutralized 10 % formaldehyde unto outer ring.
5. Slant the Conway's unit with cover.
6. Pipette 1 ml of saturated K_2CO_3 solution into outer ring.
7. Close the unit.
8. Mix gently.
9. Stand for 60 min at 37°C in incubator.
10. Titrate inner ring solution with 0.02 N HCl using a micro-burette until green color turns pink.
11. Prepare the blank using 1 ml of 4 % TCA instead of sample extract.

Calculation

$$\text{TMA-N or TVB-N (mg N/100g)} = \frac{(V_S - V_B) x (N_{HCl} x A_N) x V_E x 100}{W_S}$$

where:

V_S = Titration volume of 0.02 N HCl for sample extract (ml)

V_B = Titration volume

N_{HCl} = Normality of 0.02 N HCl for the blank (ml)

A_N = Atomic weight of nitrogen

W_S = Weight of sample (g)

V_E = Volume of 4 % TCA used in extraction

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

Reagents

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

Method

Pouring the separating gel:

1. Assemble the minigel apparatus according to the manufacturer's detailed instructions. Make sure that the glass and other components are rigorously clean and dry before assembly.
2. Mix the separating gel solution by adding, as defined in following Table.
3. Transfer the separating gel solution using a pasture pipette to the center of sandwich is 2 cm from the top of the shorter glass plate.
4. Cover the top of the gel with a layer of isobutyl alcohol by gently squirting the isobutyl alcohol by gently squirting the isobutyl alcohol against the edge of one of the spacers. Allow the resolving gel to polymerize fully (usually 45 min).

Pouring the stacking gel:

1. Pour off completely the layer of isobutyl alcohol.

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

Reagents	10 % running gel	4 % stacking gel
30 % Acrylamide-bis	3.333 ml	0.665 ml
1.5 M Tris-HCl buffer, pH 8.8	2.5 ml	-
0.5 M Tris-HCl buffer, pH 6.8	-	1.25 ml
10 % SDS	100 µl	50 µl
Distilled water	4.012 ml	3 ml
2 % Ammonium persulfate	50 µl	25 µl
TEMED	5 µl	3 µl

Sample preparation:

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

Loading the gel:

1. Dilute the protein to be 1:1 (v/v) with sample buffer in microcentrifuge tube and boil for 1 h.
2. Remove the comb without tearing the edge of the polyacrylamide wells.
3. Fill the wells with electrode buffer.
4. Place the upper chamber over sandwich and lock the upper buffer chamber to the sandwich. Pour electrode buffer into the lower buffer chamber. Place the sandwich attached to the upper buffer chamber into the lower chamber.

5. Fill the upper buffer chamber with electrode buffer so that the sample wells of the stacking gel are filled with buffer.
6. Use a 10-25 μl syringe with a flate-tipped needle, load the protein sample into the wells by carefully applying the sample as a thin layer at the bottom of the well.
7. Fill the remainder of the upper buffer chamber with additional electrode buffer.

Running the gel:

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

Disassembling the gel:

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

Staining and destaining the gel:

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

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Educational Attainment

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Bachelor of Science (Agriculture)	Prince of Songkla University	2002

Scholarship Awards during Enrolment

The scholarship for development of agro-Industry to the excellence

List of Publication and Proceeding

Tuankriangkrai, S and Benjakul, S. 2007. Effect of modified starch on the stability of fish mince gels subjected to multiple freeze-thawing. J. Muscle Foods (Submitted).

Tuankriangkrai, S. Benjakul, S and Kijroongrojana, K. 2007. Effect of some hydrocolloids on the gelling properties and freeze-thaw stability of bigeye snapper mince gels. The 10th Asean Food Conference (2007). The Premier Food Science and Technology Conference in Asean, Kuala Lumpur, Malaysia Aug 21 -23.

Tuankriangkrai, S. Benjakul, S and Kijroongrojana, K. 2007. Effect of modified starch on the stability of fish mince gels subjected to multiple freeze-thawing.7th National Graduate Research Conference. Prince of Songkla University, Suratthani Campus, 4 to 5 April 2007.