AN ABSTRACT OF THE THESIS OF

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Characteristics of films obtained from fish myofibrillar proteins extracted from various solubilization methods were investigated. In this study, we utilized three agents to solubilize fish myofibrillar proteins from surimi, which are sodium chloride, sodium tripolyphosphate, and alkaline condition. The first part of this study focused on combination of sodium chloride and alkaline solubilization, while the second part discussed combination of sodium chloride and sodium tripolyphosphate solubilization. Surimi concentration (15 and 20%), sodium chloride concentration (0, 2, and 5%), pH (7, 9, and 11), and STP concentration (1.0, 1.5, and 2.0%) were varied.

Our findings suggested that film with high flexibility, indicated by high puncture distance and elongation at break, were obtained from surimi slurries with 20% surimi-0% salt-pH 11, 15 and 20% surimi-0% salt-1% STP, and also from 20% surimi-2% salt-pH 9

and 11. Films with high puncture strength were produced from treatments with 20% surimi-0% salt-pH 11, 15% surimi-2% salt-pH 9, and 15 and 20% surimi-0% salt-1% STP. Increased tensile strength was found mainly on films produced from slurries with 5% salt. We predicted that high tensile strength was associated with salt crystallinity. Therefore, we disregarded this property in selection of fat blocking solution. Considering puncture strength, puncture distance, and elongation at break data together, we considered surimi slurries with 20% surimi-0% salt-pH 11, 15% surimi-0% salt-1% STP, and 20% surimi-0% salt-1% STP should be appropriate for our further study.

Film-forming Ability of Fish Proteins

by

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Zatil Afrah Athaillah, Author

CONTRIBUTION OF AUTHORS

Dr. Jae W. Park was involved in design, experiment, and also writing. Angee Hunt reviewed chapter 2, 3, and 4.

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CHAPTER 1

GENERAL INTRODUCTION

Deep frying is a common way to prepare foods in industry, restaurants, and households. The superiority of frying is that it can create products with crispy crusts, enticing aroma, and visual appeal (Mallikarjunan and others 2010). However, adverse health conditions related to consuming high amounts of fat are worrisome. High fat, especially saturated fat, uptake is associated with obesity, cardiovascular disease, hyperglycemia, and disruption in dietary calcium absorption (Sidery and others 1991, Ikemoto and others 1996, Wohl and others 1997). Nonetheless, fried food consumption is still high. It is assumed that consumer awareness to health is not the only factor influencing consumer preference. Flavor and texture still dictate consumer choices. Therefore, scientists work to develop methods to reduce fat in fried foods while still maintaining the organoleptic characteristics of those products.

Application of coating solutions prior to frying is one of the strategies to reduce fat in the final products. Commercial coating solutions are mostly carbohydrate-based (Mallikarjunan and others 2010), but proteins have also been studied for that purpose. Rayner and others (2000) showed that soy protein isolate solutions (10% SPI) added with 0.05% gellan gum as plasticizer can reduce fat in doughnuts by 55.12%. Mittal and

others (2002) also studied the effectiveness of some protein and carbohydrate solutions to reduce fat uptake in fried pastry mix and found that whey protein isolate generated the maximum fat uptake reduction (86.0%), followed by soy protein isolate with 80.1%. The percentage of fat reduction by soy protein isolate generated by Mittal is different from that studied by Rayner because the frying conditions and the products were different. These findings suggest that protein solutions might be promising coatings as well.

Hunt and Park (2012) found that fried surimi seafoods have significantly lower fat content (2.3%) than their counterparts. For comparison, fat content in fried fish, chicken nuggets, and French fries are 9.5%, 15.7%, and 18.4%, respectively (Hunt and Park 2012). We assumed that fish myofibrillar proteins contained in surimi seafoods, especially on the surface, form layers that later minimize moisture loss from foods and also oil migration into foods. Therefore, fish myofibrillar protein solution might be a potential coating that can reduce fat uptake in fried foods.

Other studies have been conducted to understand the film-forming ability of fish proteins (Shiku and others 2004, Prodpran and Benjakul 2005, Weng and others 2007). Nevertheless, salt and sodium tripolyphosphate solubilization effect on the characteristics of fish protein-based films as well as the combination of alkaline treatments and heating in preparing the film-forming solutions have not been discussed.

In this study, salt was combined with either alkaline or sodium tripolyphosphate extraction for preparing fish protein film solutions. Alaska pollock (*Theragra*

chalcogramma) surimi was used as the fish protein source. According to Guenneugues and Morrissey (2005), this fish is the most widely used fish for surimi production. It has a white flesh and tends to have uniform size. It also produces high quality surimi (Guenneugues and Morrissey 2005).

In order to obtain fish myofibrillar protein solution with optimum film-forming ability, extraction methods of fish myofibrillar proteins that can produce films with optimum mechanical (breaking force and tensile) properties were investigated. In addition, viscosity (after homogenization and after heating), surface hydrophobicity, and surface reactive sulfhydryl contents of the film-forming solutions were measured.

CHAPTER 2

LITERATURE REVIEW

2.1 SURIMI

According to Park and Lin (2005), surimi is stabilized myofibrillar proteins obtained from mechanically deboned fish flesh that is washed with water and blended with cryoprotectants. Surimi originated from Japan. The word ‘surimi’ came from two Japanese words: ‘suru’, that means ‘to process’, and ‘mash/mi’ meaning ‘meat’ (Vidal-Giraud and other 2007). In Japan, surimi is used to produce various surimi seafoods; for example kamaboko, hanpen, chikuwa, and satsuma age (Kibun Foods 2012).

Before the 1960s, surimi production was limited because it did not have a long shelf life (Park and Lin 2005). Freezing was not an option due to protein denaturation that occurred during freezing, which lowered surimi quality. Subsequently, in 1960-1970, cryoprotectants were introduced into surimi manufacturing. Since the discovery of cryoprotectants, surimi production and sales have increased (Park and Lin 2005).

Surimi crabstick, which is called kanikama in Japan, propelled surimi seafood expansion from Japan to other countries (Lin and Park 2005). This product was developed in 1974-1975 while the United States started to produce surimi and surimi seafood crabstick in the 1980s. Currently, surimi and surimi seafood are produced in

many countries, including USA, South Korea, Thailand, India, China, France, Spain, Lithuania, Ukraine, and Russia (Lin and Park 2005, Vidal-Giraud and other 2007).

Surimi manufacturing involves various steps: sorting fish; de-heading, gutting, de-boning, skinning, and or filleting the fish to obtain fish flesh; mincing fish flesh; washing fish mince; refining to remove impurities; dewatering using a screw press; mixing fish mince with cryoprotectants; and freezing (Lin and Park 2005, Vidal-Giraud and other 2007). During surimi production, washing is an essential step. Washing is used to eliminate myoglobin that can decrease the whiteness index and protein functionality, which lowers surimi quality (Chaijan and others 2008). Washing is also intended to remove sarcoplasmic proteins, which were to could impede gelation of myofibrillar protein. However, some recent research showed that addition of sarcoplasmic proteins did not disturb myofibrillar protein gelation and actually improved gel formation (Jafarpour and others 2009; 2012). Apparently, sarcoplasmic protein effects on gelation depend on the type of protein and method of surimi-based gel production. It was believed that among the sarcoplasmic proteins, endogenous transglutaminase induced a positive effect on myofibrillar protein gelation (Jafarpour and others 2012).

Currently, surimi production utilizes 2-3 million metric tonnes of fish worldwide (Vidal-Giraud and other 2007). Surimi is mainly produced from Alaska pollock (*Theragra chalcogramma*) due to its high quality. However, Guenneugues and Morrissey (2005) showed that since its production declined, producers started to use other fish, like

Pacific whiting (*Merluccius productus*) that is found in the Pacific Northwest and threadfin bream (*Nemipterus* spp.) found in the Indo-west Pacific region.

2.2 FISH MYOFIBRILLAR PROTEIN EXTRACTION

Myofibrillar proteins play an important role in gelation. They comprise about 70-79% of total proteins in fish (Lanier and Carvajal 2005). This group of protein is characterized by its solubility in high ionic strength solution (Yongsawatdigul and Park 2004). According to Lanier and Carvajal (2005), myofibrillar protein consists of myosin, actin, and other proteins associated with either actin or myosin, for instance tropomyosin, troponin complexes, actinins, M-proteins, and C-proteins. Among these proteins, myosin and actin contribute the most to gel formation (Xiong and Blanchard 1994).

Upon heating, myosin alone can form a strong gel, while actin is not able to establish gelation on its own (Sun and Holley 2010). Myosin has unique characteristics for forming a gel because it has a double-headed globular region and a helical tail. Samejima and others (1981) suggested that myosin forms a heat-induced gel by head-to-head binding followed by self-associating through cross-linking of the tail portions. In contrast, a suspension containing F-actin, instead of forming a proper gel, produced a curdy sol after heating (Sun and Holley 2010). However, in many processed foods, actomyosin, a complex protein composed of myosin binding tightly with actin, is present and acts as a gelling agent.

A study using a rheometry showed that heat-induced gelation of fish actomyosin, which is indicated by the elastic modulus (G') , generally occurs through three steps: 1) transformation from a viscous solution to an elastic gel network commencing at approximately 5 to 35 ºC, which is indicated by an increasing elastic modulus, 2) break down of the gel in the range of 35-51 ºC, shown by a decreasing elastic modulus, and 3) continuous protein aggregation as indicated by another increase in the elastic modulus (Liu and others 2007). This early G' formation was suggested to be due to the cross-linking of light meromyosin (LMM) (Reed and Park 2011; Fukushima and others 2005; Egelandsdal and others 1986). As the heating proceeded further to around 40 °C, this semi gel-like structure was disrupted and released fluid, leading to decreased G'.

In order to achieve excellent gelation, myofibrillar proteins should be solubilized. Solubility of myofibrillar proteins, and also other types of proteins, achieves minimum values at their isoelectric point (Choi and Park 2002, Yongsawatdigul and Park 2004). The isoelectric point of fish myofibrillar protein is around pH 5.5. At this condition, proteins precipitate and interact through hydrophobic bonds. Shifting pH of the system to be lower or higher than the isoelectric point increases the number of positively or negatively charged residues, respectively. Therefore, repulsion force between proteins becomes more dominant, improving protein dispersion and hydration (Damodaran 2008). Some studies showed that maximum solubility is attained at very alkali conditions (pH 11-12), followed by very acidic environment (pH 1-2) (Choi and Park 2002, Yongsawatdigul and Park 2004).

Myofibrillar proteins can be extracted by chopping with salt, especially sodium chloride (Lanier and others 2005, Yongsawatdigul and Park 2004). According to Puolanne and others (2001), chloride ions, that have a higher affinity compared to sodium ions, bind with proteins and cause myofibrillar filaments to swell due to difference in solute concentrations. Accordingly, the swelling exposes ionic residues. It also causes increase in the net negative charge of proteins. Furthermore, it induces myofibril filament disintegration at NaCl concentration of 0.8 M (in absence of phosphate) or 0.4 M (in presence of phosphate) for cooked sausage (Puolanne and others 2001). Another study on chicken myofibrils showed that swelling started at an ionic strength of 0.5 M and myosin dissociated at 0.6 M (Xiong and others 2000). Phase contrast microscopy data showed that myosin dissociation occurred along the entire length of the A-band (Xiong and others 2000).

Phosphate has been studied for its ability to extract and solubilize muscle proteins. Prusa and others (1984) found that phosphate salt, in the presence of sodium chloride, could increase supernatant protein extractability of turkey muscle. Phosphate increased the ability of myosin to bind meat pieces by dissociating actomyosin (Siegel and others 1979, Xiong and others 2000), which consequently, enhanced gelation. In addition, the solubilization effect of phosphate is mediated by its capacity to increase pH (Shults and Wierbicki 1973).

Some studies suggested that the effect of phosphate on myofibrillar protein functionality highly depends on phosphate type (Julavittayanukul and others 2006),

especially at low ionic strength (Liu and Xiong 1997). Phase contrast macroscopy results showed that pyrophosphate and tripolyphosphate dissociate myosin from both ends of the A-band, whereas orthophosphate and hexametaphosphate extract myosin from the center of the A-band (Liu and Xiong 1997).

Some studies revealed phosphate's synergistic effect with NaCl (Siegel and Schmidt 1979). In the presence of NaCl (6%) and STP (2%), myosin forms a 3-dimensional network of overlapping fibers, while in the absence of these, the structure is sponge-like (Siegel and Schmidt 1979). However, another study reported otherwise. When the concentration of NaCl was higher than 0.5 M, adding phosphate contributed to decreased gel strength (Robe and other 1993).

2.3 PROTEIN-BASED FILMS

Edible film is a consumable thin layer material used to block moisture, oxygen, flavor, aroma, and/or oil, thus improving quality and shelf life of foods (Krochta 2002, Bourtoom 2008). Edible films can also be utilized to carry antimicrobial, antioxidant, flavor, color, and nutrients (Krochta 2002). There are several main sources of edible films: proteins, polysaccharides, lipids, and resins. Protein films have attracted many interests due to their nutritional values.

Protein extraction is a requirement for film formation. Many studies used the pH shift method to achieve protein extraction. Based on studies on soy protein isolate films (Brandenburg and others 1993) and peanut protein films (Jangchud and others 1999), alkali solubilization produced films with higher elongation at break compared to films from the same source but solubilized at neutral pH. In addition, alkali extraction also improved film appearance. The films were clearer, more uniform, and had less air bubbles (Brandenburg and others 1993). However, a study on fish myofibrillar protein-based films showed that elongation at break values remained the same, regardless of pH when pH was adjusted from 7 to 12, while tensile strength increased as pH was highly alkaline (Shiku and others 2003).

There are several studies developing protein films using the acid extraction method. Films were successfully produced from fish myofibrillar protein at very acidic conditions (pH 2-3) (Shiku and others 2003, Chinabhark and others 2007, Weng and others 2009). Shiku and others (2003) discovered that myofibrillar protein films from blue marlin were formed at pH 2-3, but not at pH 4-6. This could be caused by decreased myofibrillar protein solubility at pH 4-6.

Light transmission, water vapor permeability, films solubility, and enzymatic hydrolysis of the films did not change as pH changed (Shiku and others 2003). Prodpran and Benjakul (2005) suggested that surimi films made from threadfin bream from acid and alkaline film forming solution did not show any difference in light transmission,

tensile strength, and elongation at break. However, films prepared from acid surimi solution (pH 3) had higher water vapor permeability and were more yellowish.

In addition, acidic condition enhanced acid proteinase activity. According to Weng and others (2007), films made from surimi solutions at pH 3 showed decreased film strength due to degradation of myosin heavy chain (MHC) caused by endogenous cathepsin D and cysteine proteinase. Thus, to avoid degradation and improve film characteristics, pepstatin A and cysteine protease inhibitor were added into the film-forming solutions (Weng and others 2007). Enzyme activity was also deactivated by heating. Weng and others (2007) found that heating film-forming solution (pH 3) at 70 ºC for 20 min gave optimum improvement in the mechanical properties of the films.

Generally, protein films possess gas barrier properties (Bourtoom 2009), but apparently have lower tensile strength than most polysaccharide and synthetic films, as well as lower elongation at break than their synthetic counterparts (Krochta 2002). In addition, protein films have poor water vapor permeability due to the existence of hydrophilic side chains (Bourtoom 2009). Many studies have focused on methods to improve film properties, for instance introduction of cross-linking agents and enzyme addition. Cross-linking agents that have been reported to improve tensile strength were formaldehyde, glutaraldehyde, glyoxal (Hernandez-Munoz and others 2004), citric acid (Reddy and others 2012), and phenolic compounds (tannic acid, catheic acid, catechin, ferrulic acid) (Prodpran and others 2012). However, elongation at break decreased as

cross-linking agents were introduced (Hernandez-Munoz and others 2004, Reddy and others 2012).

Transglutaminase has been used to improve gel strength because this enzyme can induce covalent bonds between glutamyl and lysine residues. Various effects in film formulation have been reported from transglutaminase addition. Larre (2000) reported that transglutaminase improved tensile strength and elongation at break of deamidated gluten films. However, Tang and others (2005) showed that transglutaminase addition contributed to increased tensile strength but decreased elongation at break of soy protein isolate films. A study conducted by Patzsch (2010) revealed that transglutaminase effect depended on time and temperature of enzyme addition and also film drying. Film with the best mechanical properties was produced from treatment with transglutaminase addition at 50 ºC and drying at room temperature for 48 hr.

Type of plasticizer affected properties of protein films. Hydrophilic plasticizer addition induced lower glass transition temperature of fish myofibrillar protein films (Cuq and others 1997). Increasing glycerol concentration added to film-forming solution contributed to increased water vapor permeability of films produced from pea protein isolate films, while increasing sorbitol addition did not significantly change the parameter (Kowalczyk 2011). Based on a study of films produced from gelatin, decreased tensile strength was observed as concentration of hydrophobic plasticizer increased (Andreuccetti and others 2009).

Fish proteins have been utilized as raw materials for film formation, especially as biodegradable packaging (Cuq and others 1996, Cuq and others 1997, Iwata and others 2000, Prodpran and others 2005, Shiku and others 2003, Shiku and others 2004, Chinabark and others 2007, Prodpran and others 2007, Weng and others 2007, Weng and others 2009). Most of the studies used myofibrillar proteins due to its prominent gelling ability. However, several studies also used sarcoplasmic proteins. Films produced from fish myofibrillar proteins through the pH-shift method were slightly fishy; transparent; water-insoluble; possessed good mechanical properties; and showed poor water barrier ability (Cuq 2002). In the presence of sarcoplasmic proteins in the film-forming solution, tensile strength decreased. In addition, lowered elongation at break values were reported when the sarcoplasmic portion exceeded 30% (Artharn and others 2008). However, sarcoplasmic proteins alone could produce films with good mechanical properties (Iwata and others 2000).

2.4 VISCOSITY AND MECHANICAL PROPERTY MEASUREMENT

2.4.1 Viscosity measurement

Viscosity is defined as the internal friction of a fluid (Leblanc and others 2009). The basic principle of a viscometer is to provide either a controlled strain rate or controlled stress while measuring viscosity. If one of these two parameters is controlled, then the other parameter will depend on the viscosity of the fluid. The shear viscosity is

then easily obtained by dividing the shearing stress by the corresponding shear strain rate (Leblanc and others 2009). There are many types of viscometers, for instance concentric cylinders, cone and plate viscometers, parallel disks, and capillary viscometers. In addition, viscosity can also be measured by using falling body, oscillating, and ultrasonic methods (Leblanc and others 2009). In this study, a Cup and Bob (Coutte) viscometer was used, which is considered a concentric cylinder (Figure 2.1). This viscometer is able to effectively measure low viscosity materials and mobile suspensions (Rheosys 2011). The large surface area gives it greater sensitivity so it can produce good data at low shear rates and viscosities.

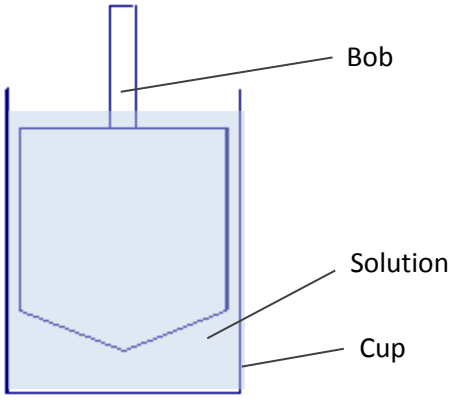

Figure 2.1-Cup and Bob viscometer

Many studies have used this parameter to help describe molecular configuration in solutions (Kinsella and others 2004, Eissa 2011, and Purwanti and others 2011). Kinsella and others (2004) showed that viscosity of a dispersion increased as molecular

dimension in solution increased. They observed that the viscosity of unfolded protein solution was higher than that of native proteins. Increase in molecular size caused by heat-induced aggregation could also increase the intrinsic viscosity of the dispersion (Purwanti and others 2011).

However, for a dilute protein solution, heating could lead to the opposite effect. Heating dilute (8% w/w) whey protein solution resulted in decreased viscosity that was later associated with shrinkage of the protein molecules (Eissa 2011). Protein concentration also affected viscosity of the solution. A study on monoclonal antibody indicated an exponential increase in viscosity as protein concentration increased (Kanai and others 2008).

Ionic strength of a protein solution is inversely related to viscosity. Increasing ionic strength resulted in a large decrease in viscosity that probably related to disruption of self-association between proteins (Kanai and others 2008). However, the extent of viscosity decrease was largely affected by the type of salt added. A study by Du and others (2010) showed that hydrophobic salt induced better viscosity reduction because it broke up protein-protein association through hydrophobic bonds.

2.4.2 Mechanical property measurement

Mechanical property measurement has been widely used for film and plastic characterization. Among this measurement, tensile strength and elongation at break are

mostly used (Shiku and others 2003, Artharn and others 2008, Chen and others 2012). However, some studies also use puncture strength and distance (Cuq and others 1996, Park and other 2004). Tensile strength is measured by either the amount of stress necessary to cause appreciable film deformation or the maximum stress that a material can withstand (Davis 2004). A tensile test involves mounting a specimen in a machine and then subjecting it to tension (Figure 2.2). The tension force is later recorded as a function of the increase in gage length. Tensile strength is calculated by dividing maximum load in Newton by the average original cross sectional area of the specimen in mm^2 (ASTM 2010). Elongation at break is also recorded.

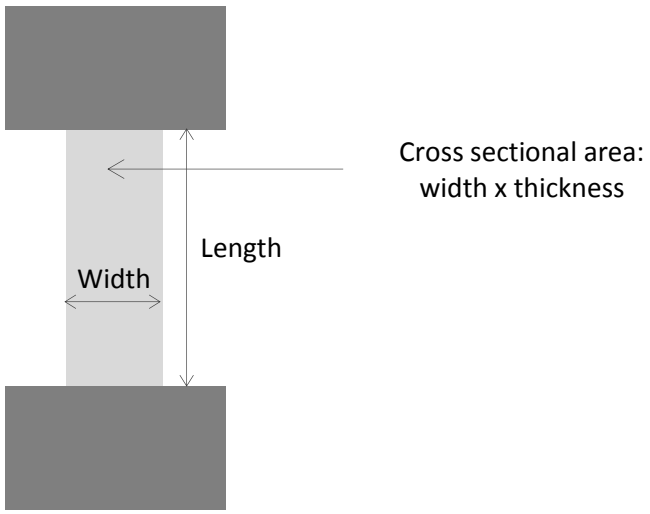

Figure 2.2-Specimen position in tensile testing apparatus

Puncture test is mainly conducted by clamping a specimen between circular plates, or plates with circular openings, and securing it in a compression and/or tensile

testing machine. A particular force is applied against the center of the unsupported portion of the test specimen by a steel plunger attached to the load indicator until the specimen ruptures (ASTM 2009) (Figure 2.3). The maximum force is reported as puncture strength. In order to compensate for variation in thickness, the maximum force can be divided by thickness to obtain puncture strength (Mezgheni and others 1998).

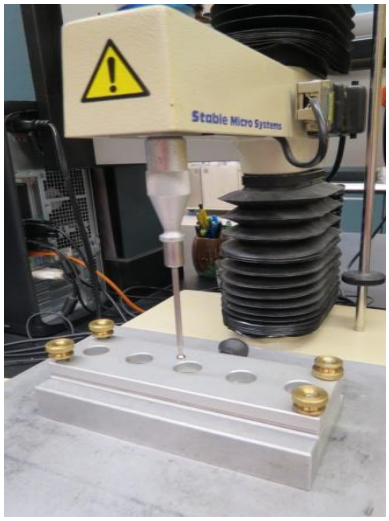

Figure 2.3-Apparatus used to measure puncture strength and distance

2.5 Evaluation of Protein Functionality by Analytical Biochemistry

2.5.1 Surface reactive sulfhydryl (SRSH)

Surface reactive sulfhydryl (SRSH) is one of the parameters used to determine protein configuration and interaction (Iwata and others 2000, Arzeni and others 2012). Arzeni and others (2012) suggested that increasing the number of SRSH could indicate

exposure of sulfhydryl groups from unfolded protein that were previously inside the three dimensional conformation of the native protein. SRSH values could also provide information regarding protein aggregation (Margoshes 1999).

Spectrophotometric assay has been used widely to quantify thiol groups. According to Riener and others (2002), it is simple and rapid. It does not need parallel standard series if a calibrated spectrophotometer is used. This method is based on the reaction of thiolate anion ($R\text{-}S^-$) with Ellman's reagent [5,5' dithio-bis-(2-nitrobenzoic acid)] or $DTNB^{2-}$, creating a mixed disulfide ($R\text{-}S\text{-}TNB^-$) and one equivalent of TNB^{2-} (Figure 2.4). TNB^{2-} exhibits intense light absorption at 412 nm, irrespective of pH of the solution for pH>7.3.

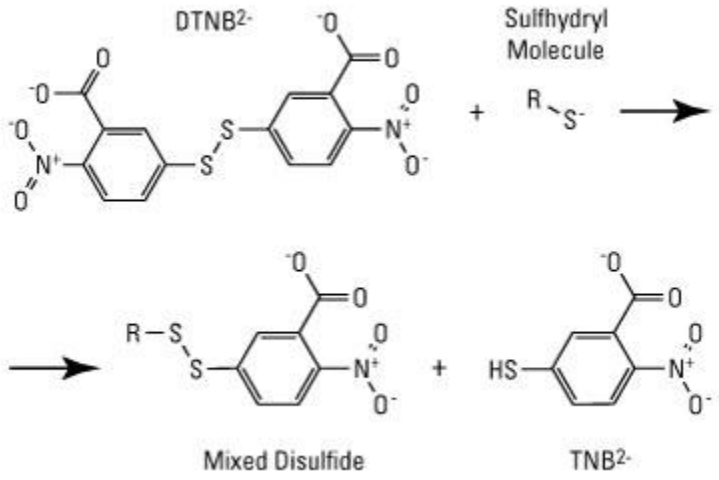

Figure 2.4-Reaction of a mercaptan with Ellman's reagent, yielding a mixed disulfide and one equivalent of TNB^{2-}

Surface and total sulfhydryl group assays are often used to describe molecular configuration of proteins. The difference between these two assays is the presence or absence of protein denaturing agents. Total reactive sulfhydryl analysis utilized 8 M urea solution (Reed and Park 2011), thus Ellman's reagent could access thiol groups that were previously hidden inside the protein structure, while the surface reactive sulfhydryl assay eliminates this step so it only quantifies thiol groups on the surface of the proteins.

Yongsawatdigul and other (2003) showed that surface reactive sulfhydryl of threadfin bream actomyosin started to increase at 30 ºC, indicating unfolding of actomyosin, and gradually decreased at temperature range of 40 to 80 ºC. A study on carp actomyosin showed a similar trend (Sano and other 1994). However, aggregation related to disulfide bond formation started to occur at a higher temperature, which is 60 ºC. Decreasing surface reactive sulfhydryl indicated formation of inter or intra molecular disulfide bonds. Ishioroshi and others (1981) showed that oxidation of SH groups only occurred on heavy meromyosin (HMM), not in light meromyosin, indicating that this binding only occurred on the globular head of myosin.

Besides heating, exposure of buried sulfhydryl groups or aggregation could also be induced by high pressure treatment and freezing. Hsu and others (2007) showed that surface reactive sulfhydryl of tilapia actomyosin increased as pressure increased greatly from 100 to 200 MPa, indicating that protein unfolded at this condition. Frozen storage, at -10 ºC for 10 days, induced protein aggregation by disulfide linkages (Sultanbawa and

other 2001). Because of its negative impact, this should be avoided by adding cryoprotectants prior to freezing.

2.5.2 Surface hydrophobicity

Hydrophobicity interaction is defined as the interaction between non-polar molecules, when they are placed in a polar solvent (Chandler 2005). Similar to sulfhydryl analysis, the surface hydrophobicity assay is also used to help understand denaturation and/or aggregation of proteins. Increased surface hydrophobicity values indicate protein denaturation (Arzeni and others 2012), while a decreased value indicates aggregation (Farouk and others 2003).

ANS (1-anilinonaphtalene 8-sulphonate) is a common fluorescent probe used to measure surface hydrophobicity of protein. According to Alizadeh-Pasdar (2000), ANS exhibited a low quantum yield of fluorescence in aqueous solution. When ANS binds to the hydrophobic sites of proteins, fluorescence intensity increased and this can be used to quantify surface hydrophobicity of protein. The ANS method offers several advantages, which are simplicity, rapidity, and requires only a small amount of sample (Cardamone and others 1992).

Surface hydrophobicity values could increase under heating (Sano and others 1994, Yongsawatdigul and other 2003). Heating threadfin bream actomyosin resulted in

increasing exposure of hydrophobic amino acids at above 30 ºC and the maximum value was reached at 70 ºC (Yongsawatdigul and other 2003). A similar study on carp actomyosin showed that substantial increase in surface hydrophobicity was observed at temperature range of 30 to 50 ºC (Sano and others 1994). Above 50 ºC, the increase became slower.

CHAPTER 3

FILM-FORMING ABILITY OF ALASKA POLLOCK PROTEINS OBTAINED FROM SODIUM CHLORIDE AND ALKALI SOLUBILIZATION

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3.1 ABSTRACT

Fried surimi seafoods have significantly lower fat content (2-3%) compared to other fried foods served in fast food restaurants (12-18%). We hypothesized that fish protein is able to form a film that minimizes fat uptake and moisture release during frying. Consequently, applying appropriately prepared surimi slurries to seafoods prior to frying might reduce its fat uptake. Therefore, our objective was to determine preparation methods for Alaska pollock surimi slurry to optimize mechanical properties based on the film-forming ability of fish protein. Surimi concentration (15 and 20%), sodium chloride concentration (0, 2, and 5%), and pH (7, 9, and 11) were varied. Surimi slurries were homogenized, pH-adjusted, centrifuged followed by vacuum to remove air bubbles, then heated in a 90 ºC water bath, casted into Teflon plates, and finally dried at 50 ºC. Films were analyzed for their mechanical (puncture and tensile) properties. Viscosity was also measured after homogenization and after heating during preparation. Heated surimi slurries were subjected to surface reactive sulfhydryl content and surface hydrophobicity analysis. For both surimi concentrations, surimi films with 0% NaCl, pH 11 showed the highest elasticity, as indicated by high puncture distance, tensile elongation, and puncture strength. In most treatments, sodium chloride addition contributed to higher tensile strength and decreased puncture strength, puncture distance, and elongation. Our results suggested that surimi slurry with 15 and 20% surimi, 0% NaCl, and pH 11 improved film forming ability and could be applied in our future study to reduce fat uptake in fried seafoods.

Keywords: film-forming, Alaska pollock surimi, alkali, sodium chloride, solubilization

Practical Application: Surimi slurries that produce films with optimum properties could be used as a coating solution for seafood prior to frying. The film is expected to block oil migration into foods while also minimizing moisture loss and subsequently reducing fat content, which could increase overall yield.

3.2 INTRODUCTION

Deep frying is a common way to prepare foods. Fried foods have unique characteristics; being crispy outside but moist inside; enticing aroma, and visual appeal (Mallikarjunan and others 2010). However, consuming fried foods contributes to high dietary fat consumption thus increasing prevalence of obesity (Bray and other 1998). A recent report showed that average daily total fat uptake in US adult population was 93.3 g and 66 g for males and females, respectively (CDC 2010). The numbers are close or similar to maximum limits suggested by Centers for Disease Control and Prevention, which are 93.3 g for males and 70 g for females, assuming sedentary lifestyle (CDC 2012).

To reduce total fat consumed, developing ways to reduce fat content in fried foods have been proposed. Our previous study showed that fried surimi seafoods have significantly lower fat content than other fried foods (2.3%) (Hunt and Park 2012). It was predicted that during frying, fish proteins in surimi seafood form films that act as a

barrier to reduce fat migration. To maximize the reduction of fat uptake by fish protein films, an understanding regarding the ability of fish protein to produce films is therefore needed.

According to Park and Lin (2005), surimi is stabilized myofibrillar proteins obtained from mechanically deboned fish flesh that is washed with water and blended with cryoprotectants. Surimi is used to produce numerous types of products. The popular surimi-based product in the US is crabstick. However, various surimi seafoods are manufactured and consumed around the world: Japan (kamaboko, chikuwa, tempura, and hanpen), SE Asia and China (fish ball and fish cake), Korea (ahmook), and Spain (baby eel).

Fish myofibrillar proteins can form films by creating three-dimensional gel networks. This property is associated with reactive surfaces mediating chemical bonds between each protein unit (Lanier and others 2005, Shiku and others 2004). Because most of the reactive groups are buried inside the protein structure, protein unfolding is a necessary step to enhance chemical binding. Protein unfolding can be obtained chemically by adjusting pH to acid or alkali or physically by chopping with salt and heating.

The chemical bonds involved in intermolecular protein interaction are hydrogen, ionic, hydrophobic, and covalent bonds. To obtain proper gelation, myofibrillar proteins should be solubilized and dispersed in solution. Myofibrillar protein solubilization can either be aided by adjusting pH or chopping surimi with a certain concentration of

sodium chloride (NaCl). In alkaline or acidic solution, charged proteins repel each other, enhancing their solubility (Prodpran and Benjakul 2005, Chinabhark and others 2007). NaCl, commonly known as salt, helps dissociate actomyosin for improved gelling (Kim and Park 2008; Lanier and others 2005). In addition, NaCl, within certain ranges of ionic strength, also enhances protein solubilization by causing protein-protein repulsion, which is called a salting-in effect (Kim and Park 2008).

Other studies have been conducted to produce films from fish protein (Shiku and others 2004; Prodpran and Benjakul 2005, Weng and others 2007). However, the effect of NaCl solubilization and alkaline treatment on film characteristics was not thoroughly discussed. Therefore, the objective of this study was to investigate characteristics of films produced from Alaska pollock surimi by alkaline and/or NaCl solubilization processes to extract myofibrillar proteins.

3.3 MATERIALS AND METHODS

3.3.1 Preparation of fish protein-based films

Alaska pollock surimi, grade FA, was obtained from Glacier Fish Company, Seattle, WA. Surimi contains moisture (74-75%), protein (16-17%), and cryoprotectants (9.0-9.3%). Surimi (15 and 20% w/w) was homogenized with certain concentrations of water and NaCl (0, 2, and 5% w/w) for 30 min in a cold room, then pH of the slurry was adjusted to 7, 9, or 11 by adding 1 N HCl or 5 N NaOH. Decision on using certain

concentration of surimi and salt, and also pH was based on trial and previous studies. Our trial suggested that slurries with 2, 5, and 10% surimi could not produce films because protein amount was not adequate to induce aggregation. We did not want to use concentration higher than 20% because our previous study on frying of seafoods coated with surimi slurry indicated that higher protein concentration in coating solutions induced higher fat uptake of fried foods. We varied surimi concentration to 0, 2, and 5%, considering the fact that 2% is the common concentration of salt used in surimi seafood production, while 5% salt gave the highest myofibrillar protein extractability (Lanier and others 2005). In this study we did not employ acid solubilization because in initial study we could not produce films when we set pH at 3. To remove air bubbles, surimi slurries were centrifuged in a Beckman J6-M1 (Beckman Coulter, USA) for 20 min at 1,780 g and 5 ºC, followed by evacuation of all air up to 2 hours using a high vacuum pump (Edwards High Vacuum Pump E2M2, Edwards High Vacuum Intl., Sussex, UK). The slurry was additionally centrifuged at 1,780 g, 5 ºC for 5 min. The slurries were then heated in a water bath at 90 ºC for 25 min. The purpose of heating was to promote protein unfolding and also to deactivate protease. Subsequently, the slurries were kept in an ice bath for about 15 min to avoid further heating. Finally, the slurries were casted in polytetrafluoroethylene plates (5 g slurry per each 25.4 x 101.6 mm^2 well) and dried in a shaker (Lab-Line Orbiz Environ-Shaker, Lab-Line Instruments, Inc., Melrose Park, ILL, USA) at 50 ºC for 2.5 hours.

3.3.2 Mechanical tests

Films were conditioned at 50% RH and 25 ºC for 2 days prior to mechanical tests in order to make a reliable comparison between different materials and different laboratories (ASTM 2008). Both puncture and tensile properties were measured using a texture analyzer (TA-XT plus, Texture Technologies Corp, N.Y., USA). Puncture tests were conducted following the procedures described in Park and other (2004) and ASTM D6241 (ASTM 2009) with slight modification. Films were placed in an extensibility fixture with 10 mm openings (TA-108S-1) (Figure 3.1a). A spherical probe with 5 mm diameter penetrated the samples at a speed of 1 mm/s. Puncture strength was the maximum force a film could withstand before rupture while puncture distance was the probe's travel distance from the point where the probe first touched the film to the point where the film ruptured. To eliminate effect of thickness variation among treatments, puncture strength was calculated by dividing puncture force (N) by thickness of film (mm) (Mezgheni and others 1998). Film thickness was measured using a hand-held micrometer (Browne and Sharpe Mfg., Providence, RI, USA). The average of four measurements for thickness was recorded for each film. For puncture strength and distance, eight measurements were collected for each treatment.

Tensile determination was based on ASTM D882 (ASTM 2010) with slight modification. Films with a dimension of 10x160 mm were loaded into tensile grips (TA-96B) (Figure 3.1b). Initial grip separation was set at 30 mm and crosshead speed was 0.5 mm/s. Eight films obtained from two preparations were prepared for each treatment.

Tensile strength was the tensile force (N) recorded at the point of rupture divided by the film's cross sectional area (mm^2). The values were recorded in MPa. Cross sectional area was obtained from multiplying thickness and width of the film. Width of the film was set at 10 mm. Elongation at break values (mm) at the rupture point was also recorded.

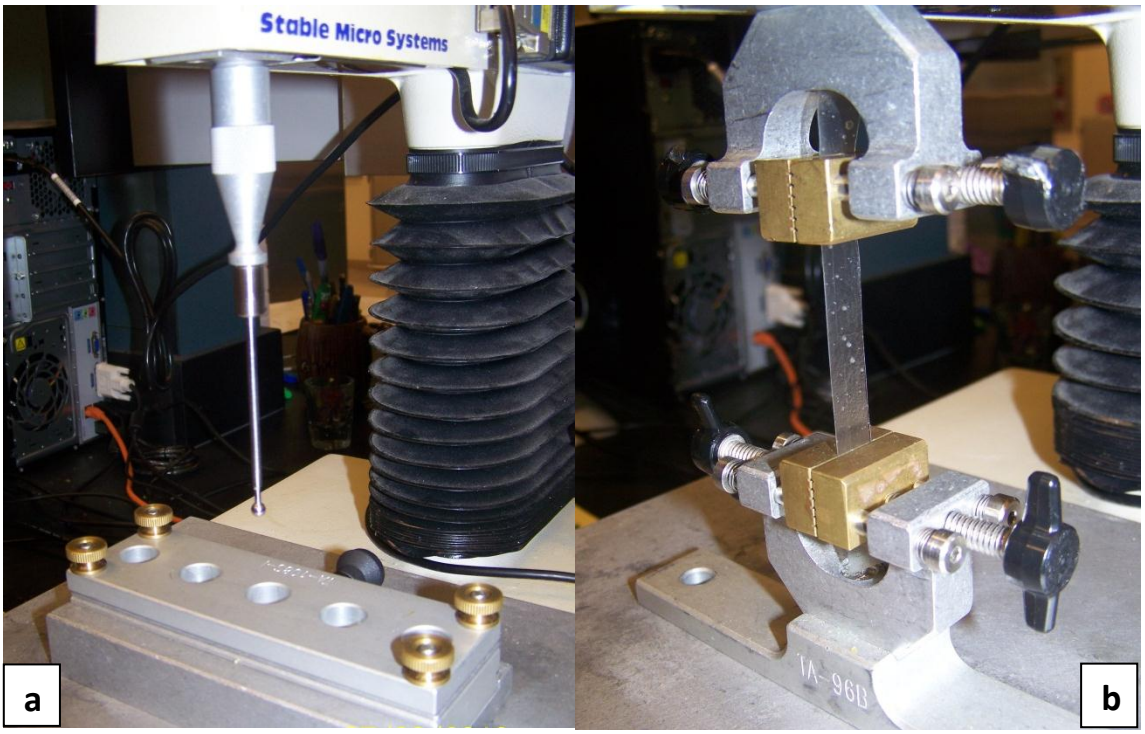


Figure 3.1-Mechanical test units to measure puncture (a) and tensile (b) properties of films

3.3.3 Viscosity

Viscosity of surimi slurries was measured with a CVO Rheometer (Malvern Instruments Ltd., Worcestershire, UK) at a shear value of 5.45 Hz (Shaviklo 2008). For

each treatment, viscosity was measured twice: the first was after homogenization (5 ºC) and the second was after heating (25 ºC). Four measurements were obtained for each sample.

3.3.4 Surface reactive sulfhydryl content

Heated surimi slurries were added with 0.6 M KCl in tris buffer at pH 7 then centrifuged at 20,000 g for 30 min at 5 ºC. The supernatants were used for sulfhydryl and surface hydrophobicity analysis. Surface reactive sulfhydryl was conducted using the procedure described in Hamada and others (1994) and Reed and others (2011) with slight modification. Two ml of 0.6 M KCl in 20 mM tris buffer, pH 7 and 50 µl of 10 mm DTNB (5,5'-dithiobis (2-nitrobenzoic acid)) were added into 0.5 ml of sample solution. Samples were then vortexed and incubated at 5 ºC for 15 min. After incubation, absorbance was measured at 412 nm using a UV-VIS spectrophotometer.

3.3.5 Surface hydrophobicity

Surface hydrophobicity of heated surimi slurries was determined using the method described by Alizadeh-Pasdar and Li-Chan (2000) with slight modification. Supernatants were diluted to 0, 0.05, 0.1, 0.15, and 0.2 mg protein/mL using 0.6 M KCl in 20 mM tris buffer, pH 7. Four ml of diluted sample were mixed with 20 µL of $8x10^{-3}$ ANS in 0.1 M phosphate buffer pH 7.4. Blanks were prepared using four ml of diluted

samples without ANS. Intensity of dilutions (with and without ANS) was recorded at an excitation wave length of 390 nm and emission wave length of 470 nm using luminescence spectrophotometer LS 50B (Perkin Elmer, Norwalk, CT, USA). Net intensity values were plotted against protein concentration. The initial slope value was used to describe surface hydrophobicity of heated surimi slurries.

3.3.6 Statistical Analysis

Statistical analysis was conducted using R software (R Development Core Team, Auckland, New Zealand). Data were subjected to analysis of variance (ANOVA) and TukeyHSD test was used for determining statistical significance of mean comparisons ($p<0.05$).

3.4 RESULTS AND DISCUSSION

3.4.1 Mechanical property measurement

Puncture strength, which is also known as breaking force, generally denotes hardness in sensory and is the force required at the time of fracture. This parameter is also used to describe quality of surimi gels (Poowakanjana and others 2012, Panpipat and others 2010, Chanarat and others 2013) and hardness of films (Park and other 2004). Whereas puncture distance gives information about elasticity of films.

Puncture strength achieved its maximum point at 15% surimi with 2% salt and pH 9. It was predicted that at this condition, proteins were highly soluble because the pH was actually futher away from its isoelectric point, considering capability of salt on lowering isoelectric point of proteins. In the absence of NaCl, puncture strength values increased as pH increased from 7 to 11 (Table 3.1). Shifting pH to an alkaline condition likely induced repulsion forces among proteins and improved solubilization (Prodpran and Benjakul 2005, Chinabhark and others 2007). Solubilization is an important step for film formation (Brandenburg and others 1993). At the time of drying, as moisture evaporates, proteins become more concentrated, resulting in film formation. NaCl addition ~~more~~ likely decreased puncture strength (Table 3.1), as a result of protein precipitation caused by salting-out.

Alkali condition also increased puncture distance (Table 3.2). Puncture distance tended to increase as slurries became more alkaline, especially at 0% salt. Salt addition contributed to decreased puncture distance and this probably caused by salting-out: salt concentration became significantly higher as most moisture was evaporated during drying. According to Damodaran (1994), molecular configuration contributed to film elasticity. Films with high elasticity should consist of more rigid proteins, compared to those of poor elasticity. Alkali condition promotes protein unfolding, thus enhacing protein aggregation and creating rigid structures.

According to Park and other (2004), tensile strength is defined as the maximum strength that a film can withstand for a particular tensile stress applied. The effect of pH

was more obvious with 15% slurries than 20% slurries. Slurries with 15% surimi with 2% salt and pH 9 demonstrated the highest tensile strength.

Tensile strength data were not not in agreement with puncture strength values. However, it is common to see this type of conflicting relationship between these two parameters (puncture strength and tensile strength) (Banerjee and others 1995, Park and other 2004). Our results suggested that precipitation caused by salting out gave increase in tensile strength but it gave otherwise effect for puncure strength.

Elongation at break, which describes stretchability of films (Park and other 2004), mostly decreased as NaCl addition increased (Table 3.4); showing a similar trend with puncture distance. Nonetheless, exceptions were observed for pH 7Addition of salt up to 2% maximized elongation. However, at pH 9 and 11, salt addition resulted in decreasedelongation. Maximum elongation occurred that pH 11 and 0% salt, for 15% surimi and pH 9 and 2% salt for 20% surimi. It was assumed that myofibrillar protein was less solubilized at pH 7 and 0% NaCl, therefore increasing NaCl content to 2% resulted in improved protein dispersion. However, 5% NaCl caused decreased elongation at break, indicating that elasticity of the films was reduced. This could be caused by a salting out effect of high concentration NaCl during drying.

Table 3.1-Puncture strength (N/mm) of Alaska pollock protein-based films obtained from certain surimi concentration, NaCl content, and pH

NaCl content	Puncture strength (N/mm)*					
	15-pH 7	**15-pH 9**	**15-pH 11**	**20-pH 7**	**20-pH 9**	**20-pH 11**
0%	87.99 ± 33.99 Ba	127.24 ± 45.46 Ba	175.16 ± 29.19 Cb	93.54 ± 33.22 Ba	180.47 ± 68.32 Bb	**208.50 ± 58.80** Cb
2%	120.61 ± 14.23 Ca	**362.62 ± 49.57** Cb	105.50 ± 13.68 Ba	63.84 ± 26.87 Ba	129.64 ± 19.98 Bb	144.34 ± 23.66 Bb
5%	36.61 ± 12.45 Ab	22.24 ± 5.07 Aa	28.23 ± 10.24 Aab	29.30 ± 7.11 Ab	18.52 ± 3.13 Aa	25.03 ± 5.93 Aab

15 and 20 on the second row denote the concentration of surimi in protein slurry. *Means and standard deviations of 8 replicates. [a]Mean values followed by different letters in the same row and same surimi content are statistically significant ($p<0.05$). [A]Mean values followed by different letters in the same column are statistically significant ($p<0.05$). Numbers in bold letters showed the maximum values for certain surimi concentration.

Table 3.2-Puncture distance (mm) of Alaska pollock protein-based films obtained from certain surimi and NaCl concentration and pH

NaCl content	Puncture distance (mm)*					
	15-pH 7	**15-pH 9**	**15-pH 11**	**20-pH 7**	**20-pH 9**	**20-pH 11**
0%	3.45 ± 1.45 Ba	5.78 ± 2.55 Ca	**9.19 ± 1.80** Cb	2.43 ± 0.85 Ba	6.37 ± 1.79 Bb	**7.98 ± 1.31** Cb
2%	3.41 ± 0.68 Ba	3.80 ± 0.33 Ba	4.11 ± 0.80 Ba	2.35 ± 0.76 Ba	5.71 ± 1.20 Bc	3.83 ± 0.45 Bb
5%	0.36 ± 0.14 Aa	0.25 ± 0.08 Aa	0.42 ± 0.20 Aa	0.48 ± 0.15 Aa	0.31 ± 0.19 Aa	0.50 ± 0.21 Aa

15 and 20 on the second row denote the concentration of surimi in protein slurry. *Means and standard deviations of 8 replicates. [a]Mean values followed by different letters in the same row and same surimi content are statistically significant ($p<0.05$). [A]Mean values followed by different letters in the same column are statistically significant ($p<0.05$). Numbers in bold letters showed the maximum values for certain surimi concentration.

Table 3.3-Tensile strength (MPa) of Alaska pollock protein-based films obtained from certain surimi concentration, NaCl con pH

NaCl content	Tensile strength (MPa)*					
	15-pH 7	**15-pH 9**	**15-pH 11**	**20-pH 7**	**20-pH 9**	**20-pH 11**
0%	3.5 ± 0.7 Aa	4.1 ± 0.7 Aa	4.6 ± 1.3 Aa	3.3 ± 1.0 Aa	4.7 ± 1.5 Aab	6.3 ± 1.3 Ab
2%	5.6 ± 0.7 ABa	**13.5 ± 1.9** Bb	4.5 ± 1.8 Aa	3.5 ± 1.1 Aa	6.0 ± 1.3 Ab	5.2 ± 1.0 Ab
5%	7.6 ± 3.0 Ba	12.4 ± 5.0 Ba	9.9 ± 3.3 Ba	10.4 ± 3.2 Ba	10.3 ± 1.5 Ba	**10.9 ± 2.6** Ba

15 and 20 on the second row denote the concentration of surimi in protein slurry. *Means and standard deviations of 8 replicates. [a]Mean values followed by different letters in the same row and same surimi content are statistically significant ($p<0.05$). [A]Mean values followed by different letters in the same column are statistically significant ($p<0.05$). Numbers in bold letters showed the maximum values for certain surimi concentration.

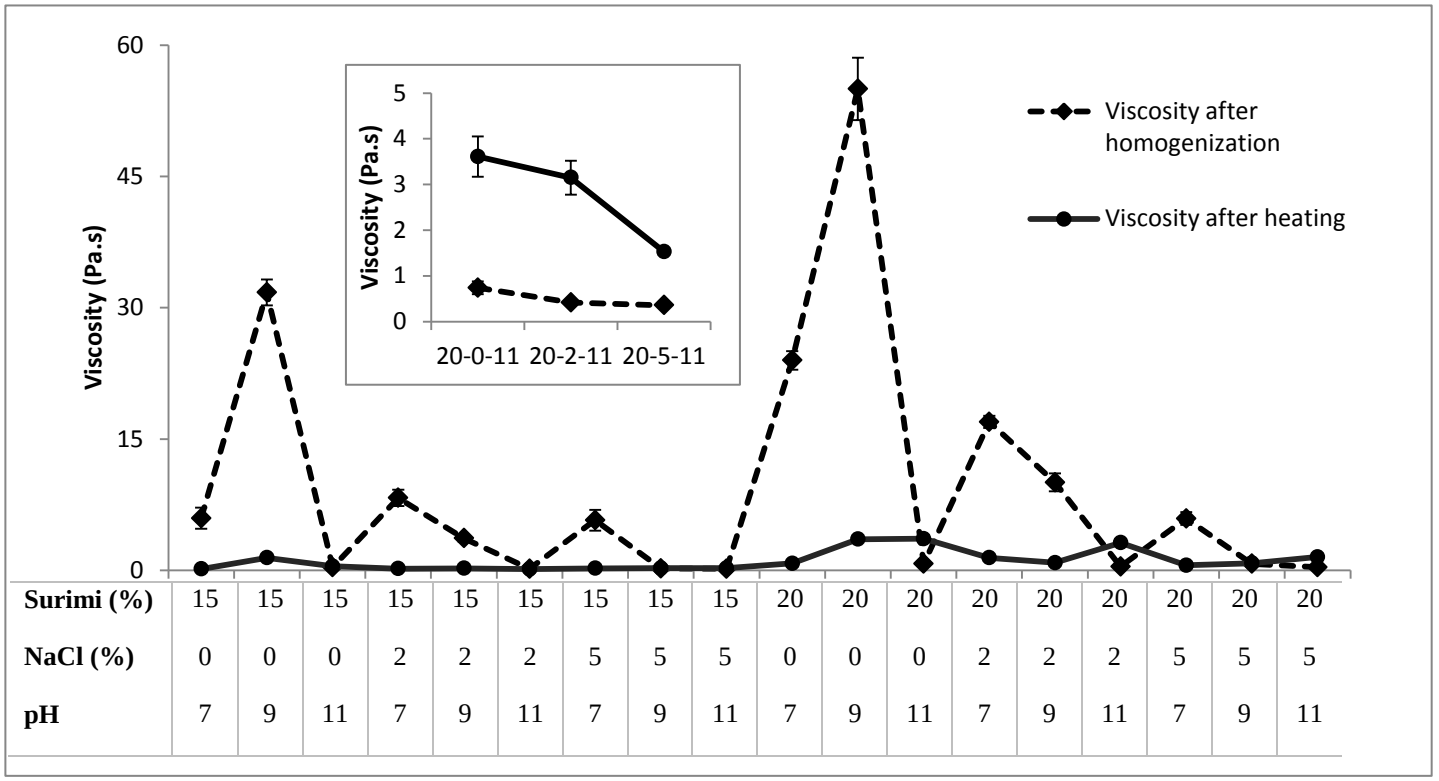
Table 3.4-Elongation at break (mm) of Alaska pollock protein-based films obtained from certain surimi concentration, NaCl content, and pH

NaCl content	Elongation at break (mm)*					
	15-pH 7	**15-pH 9**	**15-pH 11**	**20-pH 7**	**20-pH 9**	**20-pH 11**
0%	9.81 ± 4.02 Ba	43.03 ± 14.85 Cb	**52.63 ± 10.05** Cb	2.12 ± 0.86 Aa	33.07 ± 15.53 Bb	74.24 ± 21.75 Bc
2%	36.22 ± 7.75 Cb	22.02 ± 5.90 Ba	22.83 ± 11.17 Ba	32.26 ± 14.67 Ba	**104.38 ± 49.55** Bb	98.21 ± 37.24 Cb
5%	0.31 ± 0.09 Aa	0.39 ± 0.17 Aa	0.29 ± 0.05 Aa	1.12 ± 0.58 Aa	1.50 ± 0.30 Aa	1.08 ± 0.56 Aa

15 and 20 on the second row denote the concentration of surimi in protein slurry. *Means and standard deviations of 8 replicates. [a]Mean values followed by different letters in the same row and same surimi content are statistically significant ($p<0.05$). [A]Mean values followed by different letters in the same column are statistically significant ($p<0.05$). Numbers in bold letters showed the maximum values for certain surimi concentration.

3.4.2 Viscosity

Viscosity is defined as a resistance to flow. This parameter has been used to help explain molecular configuration in solutions (Kinsella and others 2004, Eissa 2011, and Purwanti and others 2011). According to Kinsella and others (2004), viscosity of a dispersion increased with increasing molecular dimension; for instance viscosity of unfolded proteins is higher than native proteins. This observation was similar to our findings. In the absence of NaCl, increasing pH to 9 induced protein denaturation and yielded higher viscosity, compared to that at pH 7 (Figure 3.2). However, surimi slurries at pH 11 had very low viscosity because proteins are intensely dispersed in the slurries due to repulsion effects among protein molecules. NaCl addition typically generated lower viscosity of surimi slurries (Figure 3.2). This could be explained by the repulsion effect among proteins and also dissociation of actomyosin induced by NaCl that created lower molecular weight proteins (Kim and Park 2008).



Surimi (%)	15	15	15	15	15	15	15	15	15	20	20	20	20	20	20	20	20	20
NaCl (%)	0	0	0	2	2	2	5	5	5	0	0	0	2	2	2	5	5	5
pH	7	9	11	7	9	11	7	9	11	7	9	11	7	9	11	7	9	11

Figure 3.2-Viscosity after homogenization and after heating of surimi slurries at various conditions of surimi content, NaCl content, and pH

Viscosity of the heated surimi slurries was also investigated. Heating decreased viscosity of the slurries, except for 20% surimi, 0% salt at pH 9 and 11, and 20% surimi, 2% salt at pH 11 (Figure 3.2). The decreased viscosity was apparently caused by the lack of hydrogen bonds. Prior to heating, the surimi slurries were kept at refrigeration temperature. At this condition for the dispersed solution, the main chemical interaction was hydrogen bonding and this bond subsided as the slurries were heated. The lower viscosity could also be caused by shrinkage of protein molecules, as observed in dilute whey protein solutions upon heating, as suggested by intrinsic and voluminosity data (Eissa 2011). A slight increase in viscosity upon heating of surimi slurries with 20% surimi and pH 9 and 11 (Figure 3.2) was an indication of aggregation that occurred during heating. Aggregation of protein can induce polymerization, yielding a larger molecular dimension of protein and result in higher viscosity of the solution (Purwanti and others 2011).

3.4.2 Sulfhydryl content

Surface reactive sulfhydryl (SRSH) is another parameter to determine protein configuration and interaction (Iwata and others 2000, Arzeni and others 2012). Increasing SRSH values as pH was shifted from 7 to 9 (Figure 3.3) indicated exposure of previously buried sulfhydryl groups (Arzeni and others 2012). However, an exception was observed for 15% surimi and 2% NaCl. NaCl incorporation also caused protein denaturation and exposed free sulfhydryl sites. At pH 7, SRSH content increased as NaCl content

increased to 2% (Figure 3.3). Nonetheless, combining NaCl and alkaline treatment appeared to give specific effects, depending on the concentration of surimi, NaCl, and pH. For 15% surimi-5% NaCl, 20% surimi-2% NaCl, and 20% surimi-5% NaCl, maximum SRSH values were observed at pH 9. Increasing pH from 7 to 9 resulted in protein unfolding and subsequent exposure of sulfhydryl group. However, when pH increased continuously to 11, proteins aggregated resulting in reduced surface sulfhydryl content.

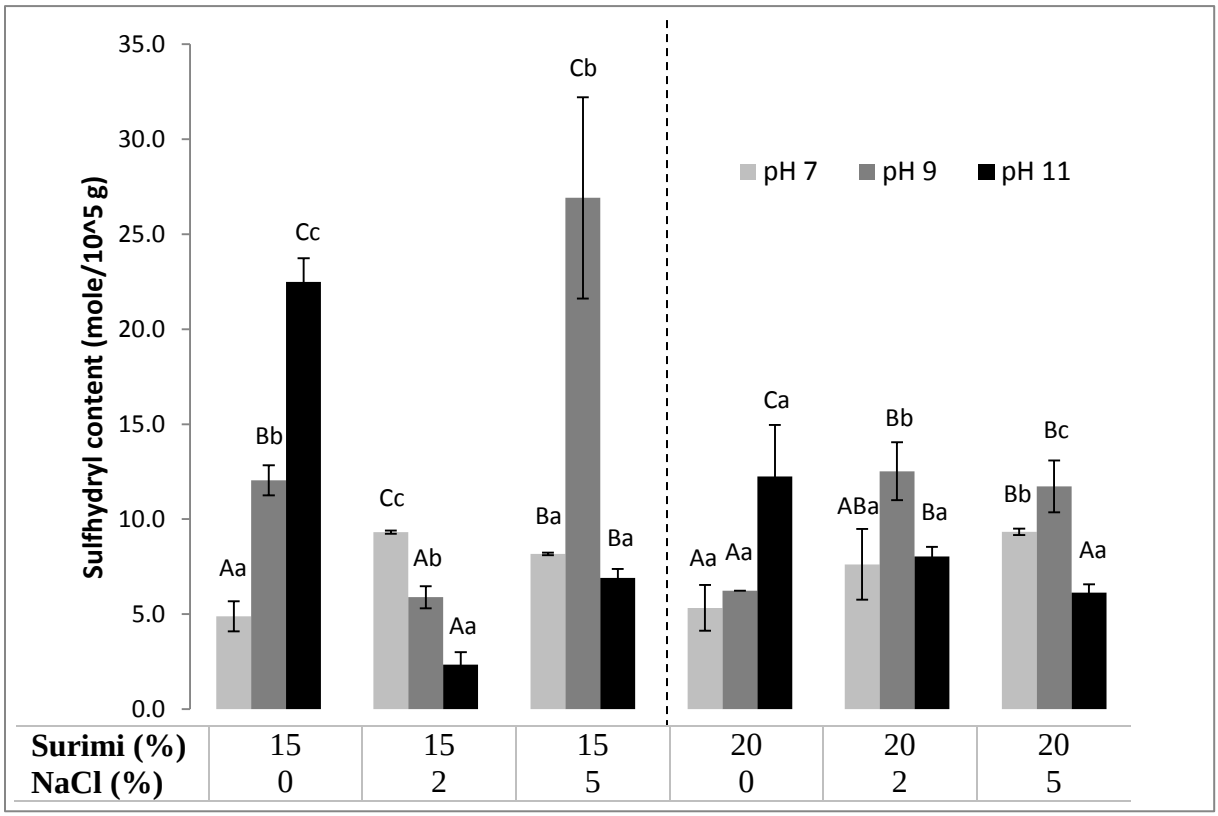


Figure 3.3- Surface reactive sulfhydryl content of surimi slurries at certain combination of surimi content, NaCl content and pH. [a]Mean values with different letters for the same surimi and salt content are statistically significant ($p<0.05$). [A]Mean values with different letters for the same surimi content and pH are statistically significant ($p<0.05$)

3.4.3 Surface hydrophobicity

Surface hydrophobicity values are also used to estimate protein denaturation and aggregation. According to Arzeni and others (2012), exposure of aromatic residues, as a consequence of denaturation, increased surface hydrophobicity. This was in agreement with our findings, as described in Figure 3.4. Surface hydrophobicity, in general, increased as pH continued to increase to 11 for 15% surimi with 0 and 5% NaCl and 20% surimi and 0% NaCl.Effect of NaCl on surface hydrophobicity highly depended on pH of the slurries. Addition of NaCl, from 2 to 5%, could increase surface hydrophobicity by promoting protein unfolding, particularly at pH 9 and 11 (Figure 3.4). However, the pH change from 9 to 11 did not affect surface hydrophobicity when 2% NaCl for both surimi contents and 20% surimi and 5% NaCl.

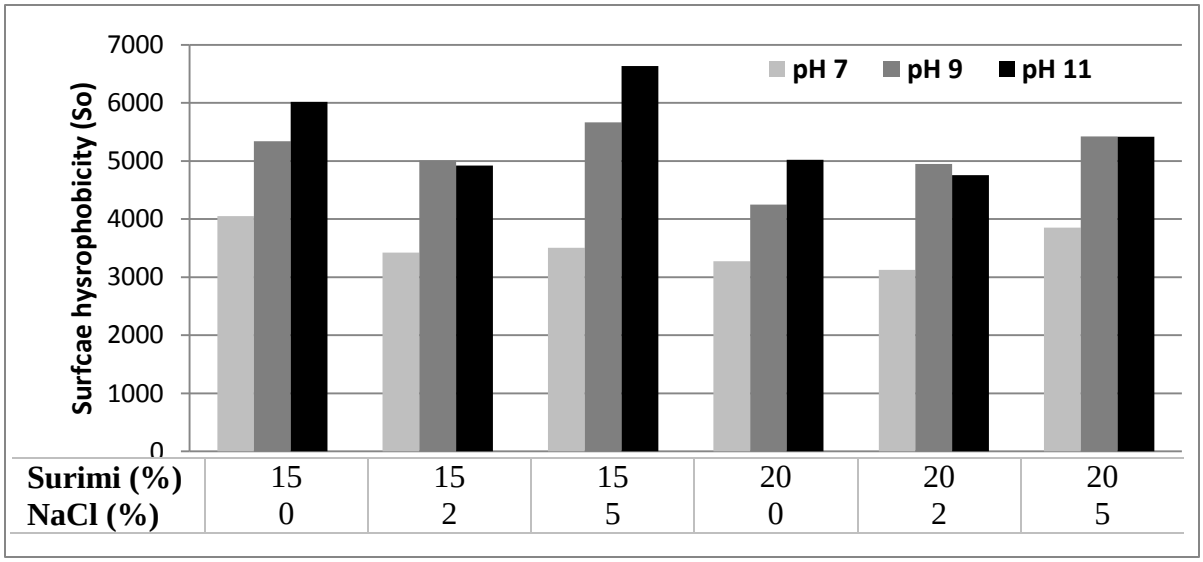


Figure 3.4-Surface hydrophobicity of surimi slurries at certain combination of surimi content, NaCl content and pH

3.5 CONCLUSION

Films prepared with high NaCl content (5%) showed high tensile strength, but low puncture distance, elongation at break, and puncture strength. Films made from highly alkaline slurries produced films with high puncture distance, elongation at break, and breaking force, as a result of very good dispersion of proteins in the matrix. Generally, the pH increase from 7 to 11 improved puncture strength, but did not significantly changed tensile strength. In the presence of salt, the viscosity of surimi slurries decreased as pH increased because the repulsion effect among proteins was more prominent. Heating surimi slurries at 90 °C for 25 min resulted in decreased viscosity due to disruption of hydrogen bonds. However, slurries containing 20% surimi at pH 11 showed a reverse effect that might be due to significant aggregation. Surface hydrophobicity and surface reactive sulfhydryl content data of heated surimi slurries confirmed that highly alkaline conditions promoted protein unfolding, while NaCl can either induce protein unfolding or aggregation during heating.

CHAPTER 4

CHARACTERIZATION OF FILMS MADE FROM FISH MYOFIBRILLAR PROTEINS SOLUBILIZED BY NACL AND STP

Running Head: Films from Fish Proteins

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4.1 ABSTRACT

Characteristics of films made from fish myofibrillar protein were investigated. Films were made from varied concentrations of Alaska Pollock surimi (15 and 20%), sodium tripolyphosphate (1.0, 1.5, and 2.0%), and sodium chloride (0, 2, and 5%). Films were subjected to mechanical property (tensile and puncture) measurement, while surimi slurries were tested for viscosity (after homogenization and after heating followed by direct cooling), surface hydrophobicity, and surface reactive sulfhydryl content. Puncture and tensile strength mainly increased as sodium tripolyphosphate concentration increased, especially with 0 and 2% salt. However, in general, puncture distance and elongation at break decreased. Salt addition, in the presence of STP, attributed to reduced puncture strength, puncture distance, and elongation at break. Heating reduced viscosity of surimi slurries. Surface reactive sulfhydryl content and surface hydrophobicity evaluation confirmed that salt addition promoted gelation of fish myofibrillar proteins.

K**eywords:** myofibrillar protein films, Alaska pollock surimi, sodium chloride, sodium tripolyphosphate, solubilization

Practical Application: With successful film-forming ability, fish proteins can be used as a coating material to reduce fat uptake and moisture loss for fried seafood.

4.2 INTRODUCTION

Fried food consumption contributes significantly to high dietary fat consumption. However, avoiding these foods is a challenge due to high consumer preference. This fact has resulted in studies to find strategies to reduce fat uptake while still preserving the appealing sensory attributes.

According to Mellema (2003), fried foods absorb oil by two methods, condensation and capillary mechanisms. These two mechanisms highly depend on the characteristics of food surface and moisture loss. Modifying the food surface, such as by applying edible coatings, has been utilized to reduce oil uptake (Mallikarjunan and others 2003, Albert and others 2002, Han and others 2009). A study by Pinthus and others (1992) found that oil uptake can be reduced by decreasing deformability while increasing yield strength of crust. Therefore, films with improved mechanical properties can be used for our further study in fat uptake reduction.

Fish proteins have been used to produce films, especially as bio-packaging (Cuq and others 1996, Cuq and others 1997, Iwata and others 2000, Prodpran and others 2005, Shiku and others 2003, Shiku and others 2004, Chinabark and others 2007, Prodpran and others 2007, Weng and others 2007, Weng and others 2009). Most of the studies used myofibrillar proteins, which are utilized for its gelling ability (Lanier and others 2005, Shiku and others 2004). Fish myofibrillar protein-based films obtained from pH-shift method have been reported to be slightly fishy;

transparent; water-insoluble; possess good mechanical properties; have poor water barrier ability (Cuq 2002), and have antioxidant property (Weng and others 2009).

According to Cuq (2002), in order to produce films from proteins, three essential steps must be followed: 1) break intermolecular bonds that stabilize native protein configuration, 2) arrangement of protein molecules, and 3) formation of new bonds that promote three-dimensional network formation. The first step can be mediated by shifting pH of film-forming solution to alkali or acid (Prodpran and others 2005, Weng and others 2009), adding salt, or incorporating sodium tripolyphosphate (STP). Salt, especially NaCl, helps dissociate myofibrils and extract actomyosin, myosin, actin, and other myofibrillar proteins and thus improves gelation (Nielsen and others 2004, Xiong and others 2009). Moreover, it also increases protein-protein repulsion (Xiong and others 2009). STP also dissociates actomyosin, starting at both ends of the A-band (Xiong and others 2000). STP also increases pH (Shults and Wierbicki 1973), which induces more repulsion force among proteins.

The second step, which is protein arrangement, can be achieved by heating film-forming solutions. Protein molecules unfold upon heating and expose active sites that play important roles for the next step (Lanier and others 2005), creating a three dimensional network. This step is indicated by extensive interaction between protein molecules. The bonds involved are hydrogen, ionic, hydrophobic, and covalent (Shiku and others 2003, Weng and others 2007).

Surimi is a good source of fish myofibrillar proteins. According to Park and others (2005), surimi is produced by mincing fish fillet then the fish mince is subjected to the washing and dewatering process, which removes sarcoplasmic proteins in order to concentrate myofibrillar proteins. Furthermore, the fish mince is refined, pressed by screw press, added with cryoprotectants, and finally frozen. Alaska pollock (*Theragra chalcogramma*) has been widely used as a surimi resource and produces high quality gel (Guenneugues and others 2005).

Our objective was to determine the characteristics of films prepared from Alaska pollock surimi by sodium chloride and/or sodium tripolyphosphate solubilization.

4.3 MATERIALS AND METHODS

4.3.1 Preparation of fish protein-based films

FA grade Alaska pollock surimi was obtained from Glacier Fish Company, Seattle, WA. Surimi (15 and 20%) was homogenized in water with NaCl (0, 2, and 5%) and STP (1.0, 1.5, and 2.0%) using a homogenizer (PowerGen 700, Fisher Scientific, Pittsburgh, PA, USA) at speed 2 for 30 min in a cold room (5 ± 1 ºC). In order to remove air pockets, surimi slurries were centrifuged in a Beckman J6-M1 (Beckman Coulter, USA) at 5 ºC for 20 min at 1,780 g followed by air removal for up to 2 hours using a high vacuum pump (E2M2, Edwards High Vacuum Pump,

Edwards High Vacuum Intl., Sussex, UK), and subjected to centrifugation again at 5 ºC at 1,780 g for 5 min. The slurries were then heated in a water bath for at 90 ºC for 25 min to promote more protein unfolding and also to deactivate protease.

Subsequently, the slurries were kept in an ice bath for about 15 min to cool down. Finally, aliquots of the slurries (5 g) were casted in each well (2.54x10.16 cm^2) on polytetrafluoroethylene plates and dried in a shaker (Lab-Line Orbiz Environ-Shaker, Lab-Line Instruments, Inc., Melrose Park, ILL, USA) at 50 ºC for 2.5 hr.

4.3.2 pH measurement

The pH of surimi slurries with various concentrations of NaCl and STP was measured using an Accumet Research AR 15 pH-meter (Fisher Scientific, USA) in a cold room.

4.3.3 Mechanical tests

Films were conditioned at 50% relative humidity (RH) and 25 ºC for 2 days prior to mechanical tests (ASTM 2008). RH was set up by using a magnesium nitrate hexahydrate (Spectrum, Gardena, CA, USA) solution. Both puncture and tensile properties were measured using a texture analyzer (TA-XT plus, Texture Technologies Corp, NY, USA). Puncture tests were conducted following the

procedures described in Park and other (2004) and ASTM D6241 (ASTM 2009) with slight modification. Films were placed in an extensibility fixture with 10 mm openings (TA-108S-1) (4.1a). A spherical probe (5 mm diameter) penetrated the samples with a crosshead speed of 1 mm/s. Puncture strength was the maximum force a film could withstand before rupture while puncture distance was the distance of the probe moved to the point where the film ruptured. To eliminate the effect of thickness variation among treatments, puncture strength was calculated by puncture force (N) divided by film thickness (mm) (Mezgheni and others 1998). Film thickness was measured using a hand-held micrometer (Browne and Sharpe Mfg., Providence, RI, USA). The average of four measurements was recorded for each film. For puncture strength and distance, eight measurements were collected for each treatment.

Tensile determination was based on ASTM D882 (ASTM 2010) with slight modification. Films prepared with the dimension 10x160 mm were loaded into tensile grips (TA-96B) (4.1b). Initial grip separation was set at 30 mm and crosshead speed was 0.5 mm/s. Eight films obtained from two preparations were measured for each treatment. Tensile strength (MPa) was the tensile force (N) recorded at the point of rupture divided by cross sectional area of the film (mm^2). Cross sectional area was obtained by multiplying thickness and width of the film. Width of the film was set at 10 mm. Elongation values (mm) at break were also recorded at the rupture point.

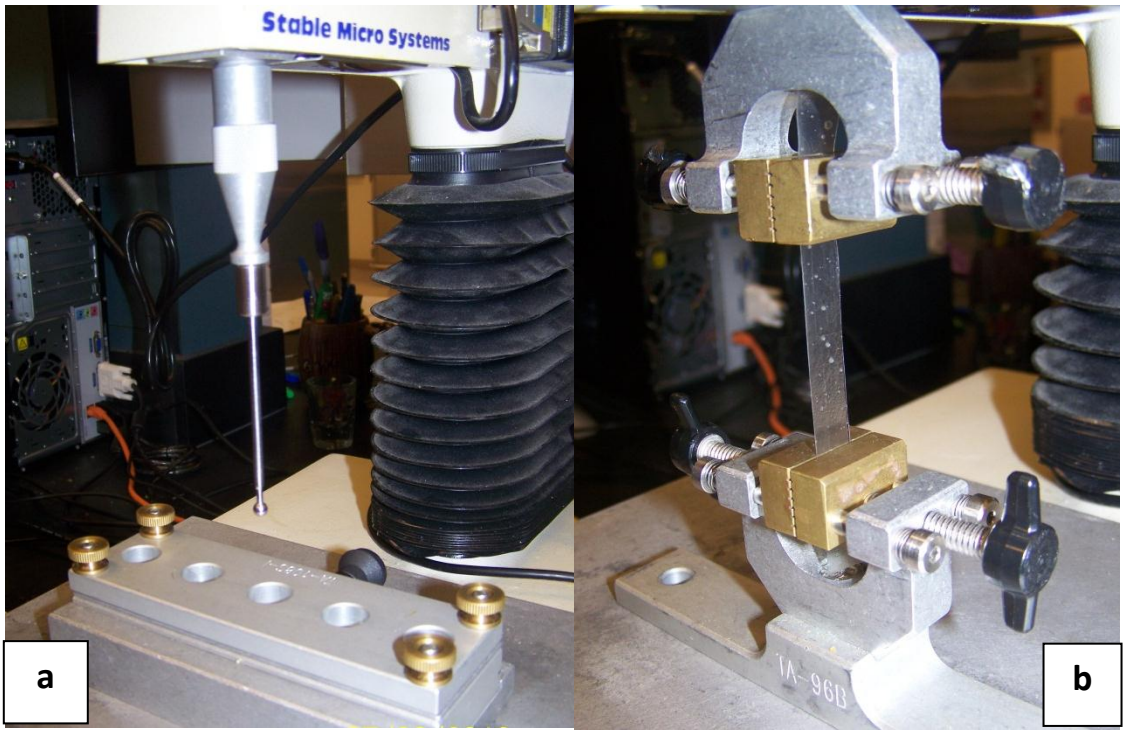

Figure 4.1-Mechanical test units to measure puncture (a) and tensile (b) properties of films

4.3.4 Viscosity

Viscosity of surimi slurries were measured with a CVO Rheometer (Malvern Instruments, Ltd., UK) at a shear value of 5.45 Hz (Shaviklo 2008). Viscosity was measured after homogenization (5 ºC) and after heating followed by direct cooling (25 ºC). Four replicates were obtained for each sample at both stages.

4.3.5 Surface reactive sulfhydryl content

Heated surimi slurries were added with 0.6 M KCl in tris buffer at pH 7 before subjecting to centrifugation (5 ºC) at 20,000 g for 30 min (Sorvall RC-5B refrigerated super-speed centrifuge, DuPont Instruments, Newton, CT, USA) . The supernatants were used for sulfhydryl analysis.

Surface reactive sulfhydryl was conducted using the procedure described in Reed and others (2011) with slight modification. Two mL of 0.6 M KCl in 20 mM tris buffer pH 7 and 50 µL of 10 mm DTNB (5,5'-dithiobis (2-nitrobenzoic acid)) were added into 0.5 mL samples. Samples were mixed using vortex and incubated at 5 ºC for 15 min before measuring their absorbance at 412 nm using UV-VIS spectrophotometer.

4.3.6 Surface hydrophobicity

Surface hydrophobicity of heated surimi slurries was determined using a method developed by Alizadeh-Pasdar and other (2000) with slight modification. Supernatants, which were prepared above for the measurement of surface reactive sulfhydryl group, were diluted to 0, 0.05, 0.1, 0.15, and 0.2 mg protein/mL using 0.6 M KCl in 20 mM tris buffer pH 7. Four mL of the diluted samples were mixed with 20 µL of $8x10^{-3}$ ANS in 0.1 M phosphate buffer (pH 7.4) and incubated at room temperature for 10 min. A duplicate set of the diluted samples (4 mL of each) was also prepared with no ANS added. The intensity of dilutions (with and without ANS) was recorded at excitation length 390 nm and emission length 470 nm using luminescence spectrophotometer LS 50B (Perkin Elmer, Norwalk, CT, USA). Finally, the net intensity values were plotted against protein concentration. The initial slope value describes the surface hydrophobicity of the surimi solutions.

4.3.7 Statistical Analysis

Statistical analysis was conducted using R software (R Development Core Team, Auckland, New Zealand). Data were subjected to analysis of variance (ANOVA) and TukeyHSD test for determining statistical significance of mean comparisons ($p \leq 0.05$).

4.4 RESULTS AND DISCUSSION

4.4.1 pH of surimi slurries

The pH of surimi slurries increased as STP concentration increased (Table 4.1). It was in agreement with Shults and Wierbicki (1973) and Puolanne and others (2001). Tripolyphosphate has multiple negative charges and can bind to positively charged groups of myofibrillar proteins through ionic interaction, resulting in more net negatively-charged protein groups. This could lead to increasing pH of the solutions because more hydroxyl (OH^-) ions are available in the system. Besides, adding more sodium tripolyphosphate increased buffering capacity of the sodium tripolyphosphate solution.

Table 4.1-pH of surimi slurries with 15% surimi

NaCl Content (%)	STP Content (%)	pH
0	1.0	8.59
0	1.5	8.72
0	2.0	8.84
2	1.0	8.30
2	1.5	8.43
2	2.0	8.55
5	1.0	7.87
5	1.5	8.03
5	2.0	8.12

The reduction of pH was observed as salt concentration increased. The pH values were from 8.59-8.84 at 0% NaCl and reduced to 8.30-8.55 at 2% NaCl. At 5% NaCl, pH values dropped further to 7.87-8.12 (5% NaCl). This was probably accomplished by chlorine ion (Cl^-) binding with the positive charges of proteins to a stronger degree than the sodium ion (Na^+) combined with negative charges, resulting in more free H^+ ions.

4.4.2 Mechanical property measurement

Mechanical property tests that are commonly used to characterize films are tensile strength and elongation at break (Cuq 2002). Tensile strength is maximum force per cross-sectional area that a film can withstand without rupture against an applied tensile stress, while elongation at break is defined as a degree to which a film can stretch before breaking (Cuq 2002, Park and other 2004). For 0 and 2% NaCl content, tensile strength slightly increased as STP content increased from 1 to 1.5% (Table 4.2). This should be credited to the ability of STP to increase pH (Shults and Wierbicki 1973) and also extract myofibrillar proteins (Xiong and others 2000). More myofibrillar proteins extracted contributed to better gelation. At 5% NaCl, 1.0% STP gave the highest tensile strength for both surimi contents evaluated but the value was not statistically different from that of 1.5% ($p>0.05$).

Increase of tensile strength observed for films prepared from solutions with 5% salt might be caused by a salting out effect during the drying process. Films prepared from this 5% treatment showed many crystals that are possibly proteins that precipitated, while films made with less NaCl content were translucent (2%) or transparent (0%) without any macroscopic crystals (Figure 4.2).

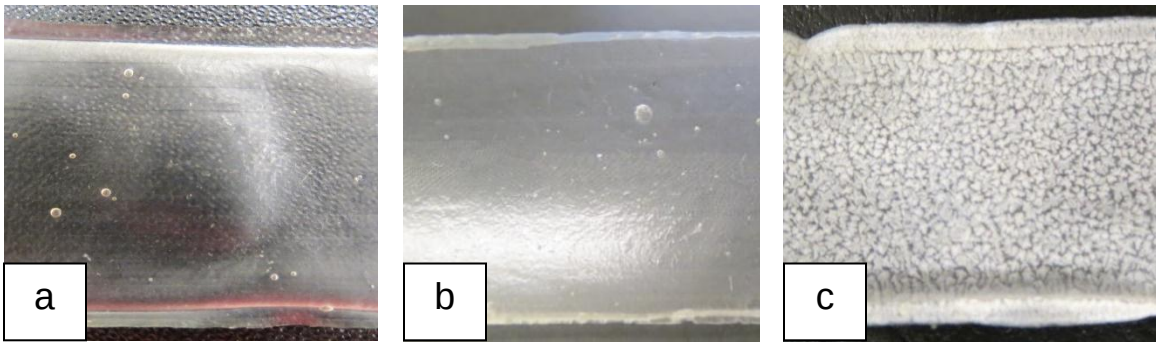

Figure 4.2-Films prepared from surimi slurry with 15% surimi and different salt content. Film made from 0% salt treatment was transparent (a), while 2% salt-film was translucent (b), and 5% salt treatment showed crystallization (c)

Elongation values at break decreased as STP content increased from 1 to 1.5%. No significant changes ($p < 0.05$) were observed between 1.5% and 2.0% STP (Table 4.3).

According to Park and other (2004), puncture strength describes the extent of crystallinity in films. Our observations showed that for films prepared from solutions with 15% surimi, 0 and 2% salt, puncture strength increased as STP was added up to 1.5%, for 15% surimi. Further STP addition did not give significant puncture strength

improvement (Table 4.4). On the contrary, at 5% NaCl, puncture strength decreased as STP increased. At this high level salt concentration (5%), large protein aggregates can be formed due to the salting-out effect during drying, which affected puncture strength. Salt addition was attributed to decreased puncture strength of films, regardless of STP concentration. This finding confirmed the negative effect of STP if NaCl concentration is above 0.5 M (Robe and other 1993). After drying, NaCl concentration in films exceeded this point.

Puncture distance relates to the elasticity of films (Park and other 2004). Lower elasticity values were observed as salt content increased, for the same combination of surimi and STP proportion. A smilar trend was found with STP concentration, where elasticity values decreased as STP concentration increased, especially when STP was added from 1.0 to 1.5%. This could result from the ability of STP to dissociate myofibrillar proteins.

Table 4.2-Tensile strength (MPa) of Alaska pollock protein-based films obtained from various surimi, NaCl, and STP concentrations

NaCl content	Tensile strength (MPa)*					
	15-1	**15-1.5**	**15-2**	**20-1**	**20-1.5**	**20-2**
0%	4.06 ± 0.63 Aa	5.30 ± 1.19 Ab	6.18 ± 0.88 Ab	5.86 ± 1.44 Aa	7.80 ± 1.89 Bb	8.09 ± 1.14 Bb
2%	3.96 ± 0.81 Aa	4.48 ± 1.06 Aa	4.72 ± 0.91 Aa	4.21 ± 0.35 Aa	5.24 ± 0.69 Ab	5.62 ± 0.67 Ab
5%	5.63 ± 3.32 Aa	**7.79 ± 1.74** Ba	5.91 ± 2.73 Aa	**10.76** ± 1.86 Bb	9.44 ± 2.49 Bab	7.86 ± 1.99 Ba

*Means and standard deviations of 8 replicates. [a]Mean values followed by different letters in the same row and same surimi content are statistically significant ($p<0.05$). [A]Mean values followed by different letters in the same column are statistically significant ($p<0.05$). Numbers in bold letters showed the maximum values for certain surimi concentration.

Table 4.3-Elongation at break (mm) of Alaska pollock protein-based films obtained from various surimi, NaCl, and STP concentration

NaCl content	Elongation at break (mm)*					
	15-1	**15-1.5**	**15-2**	**20-1**	**20-1.5**	**20-2**
0%	**49.55 ± 18.53** Ca	32.67 ± 16.20 Ca	32.21 ± 14.39 Ca	**56.51 ± 21.12** Cb	27.71 ± 10.74 Ca	18.88 ± 8.48 Ca
2%	26.68 ± 7.87 Bb	18.88 ± 6.02 Bab	14.24 ± 6.44 Ba	14.44 ± 6.06 Bb	7.40 ± 3.56 Ba	8.26 ± 2.17 Ba
5%	0.48 ± 0.21 Ab	0.16 ± 0.06 Aa	0.06 ± 0.02 Aa	0.51 ± 0.13 Ab	0.39 ± 0.14 Aab	0.29 ± 0.07 Aa

*Means and standard deviations of 8 replicates. [a]Mean values followed by different letters in the same row and same surimi content are statistically significant ($p<0.05$). [A]Mean values followed by different letters in the same column are statistically significant ($p<0.05$). Numbers in bold letters showed the maximum values for certain surimi concentration.

Table 4.4-Puncture strength (N/mm) of Alaska pollock protein-based films obtained from various surimi, NaCl, and STP concentrations

NaCl content	Puncture strength (N/mm)*					
	15-1	**15-1.5**	**15-2**	**20-1**	**20-1.5**	**20-2**
0%	137.53 ± 18.08 Ca	175.21 ± 34.87 Cab	**197.62 ± 59.45** Cb	**222.78 ± 26.40** Ca	210.01 ± 29.35 Ca	211.22 ± 23.01 Ca
2%	86.52 ± 7.45 Ba	102.27 ± 14.94 Bb	94.22 ± 9.97 Bab	91.49 ± 14.74 Ba	75.92 ± 8.26 Ba	83.38 ± 17.82 Ba
5%	17.48 ± 7.32 Ab	7.92 ± 1.09 Aa	6.99 ± 2.05 Aa	22.66 ± 3.93 Aa	23.31 ± 4.28 Aa	14.34 ± 3.27 Ab

*Means and standard deviations of 8 replicates. [a]Mean values followed by different letters in the same row and same surimi content are statistically significant ($p<0.05$). [A]Mean values followed by different letters in the same column are statistically significant ($p<0.05$). Numbers in bold letters showed the maximum values for certain surimi concentration.

Table 4.5-Puncture distance (mm) of Alaska pollock protein-based films obtained from various surimi, NaCl, and STP concentrations

NaCl content	Puncture distance (mm)*					
	15-1	**15-1.5**	**15-2**	**20-1**	**20-1.5**	**20-2**
0%	**7.17 ± 1.17** Cb	5.59 ± 0.96 Ca	6.26 ± 1.33 Cab	**7.58 ± 2.18** Cb	4.72 ± 0.79 Ca	3.81 ± 0.70 Ca
2%	4.04 ± 0.79 Bb	3.24 ± 0.56 Ba	2.78 ± 0.26 Ba	2.76 ± 0.66 Bb	2.01 ± 0.21 Ba	2.12 ± 0.39 Ba
5%	0.90 ± 0.25 Aa	0.79 ± 0.27 Aa	0.79 ± 0.28 Aa	0.66 ± 0.15 Aa	1.11 ± 0.29 Ab	1.02 ± 0.34 Aab

*Means and standard deviations of 8 replicates. [a]Mean values followed by different letters in the same row and same surimi content are statistically significant ($p<0.05$). [A]Mean values followed by different letters in the same column are statistically significant ($p<0.05$). Numbers in bold letters showed the maximum values for certain surimi concentration.

4.4.3 Viscosity

Viscosity of surimi slurries decreased after heating (Figure 4.3). It was predicted that viscosity decreased after heating due to the loss of interaction (hydrogen bond) between proteins and the solvent (water). Hydrogen bonds were stable at low temperature, but dissipated during heating. The changes in viscosity, while STP contents increased, were not consistent. Generally, though, solution viscosity with 2% STP was lower than 1% STP after homogenization. The only exception was found on 20% surimi and 5% salt. This finding might be related to the capability of STP to dissociate myofibrillar proteins. The viscosity of a dispersion increased as molecular dimension of solutes increased, and vice versa (Kinsella and others 2004).

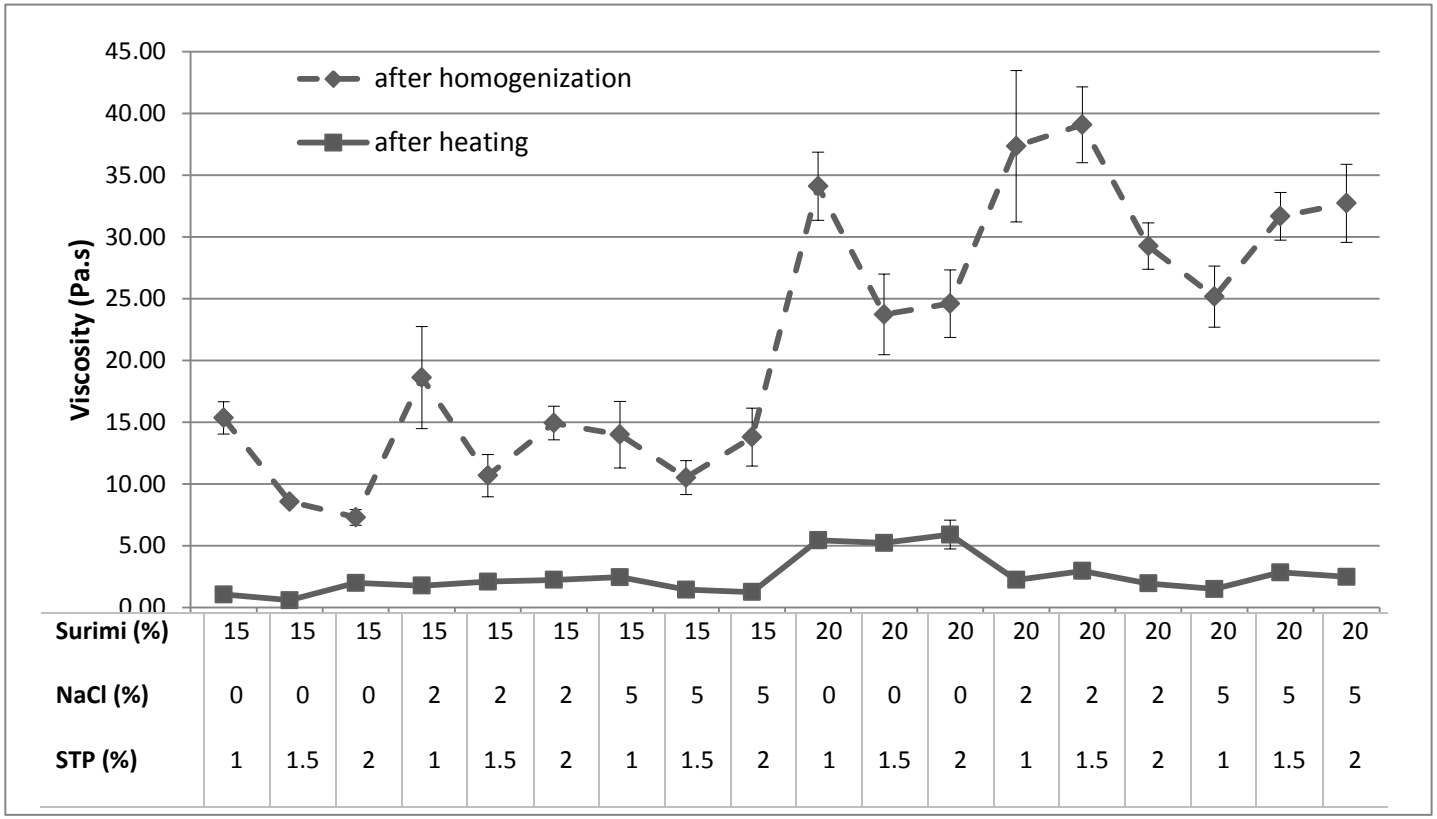

Figure 4.3-Viscosity of surimi slurries after homogenization and after heating at various combinations of surimi, NaCl, and STP concentrations

4.4.4 Surface reactive sulfhydryl content

Surface reactive sulfhydryl content of 20% surimi slurries were higher than 15% surimi because more protein molecules were present in the 20% surimi slurries. However, in the presence of salt, the difference between these two surimi contents evaluated at the same concentration of NaCl and STP became smaller (Figure 4.4). Reduced surface reactive sulfhydryl could indicate protein aggregation. The decreasing values, as salt was added, showed that salt helped promote aggregation via disulfide interactions, especially at 20% surimi. Salt has been widely used to extract proteins and induce gelation (Nielsen and other 2004).

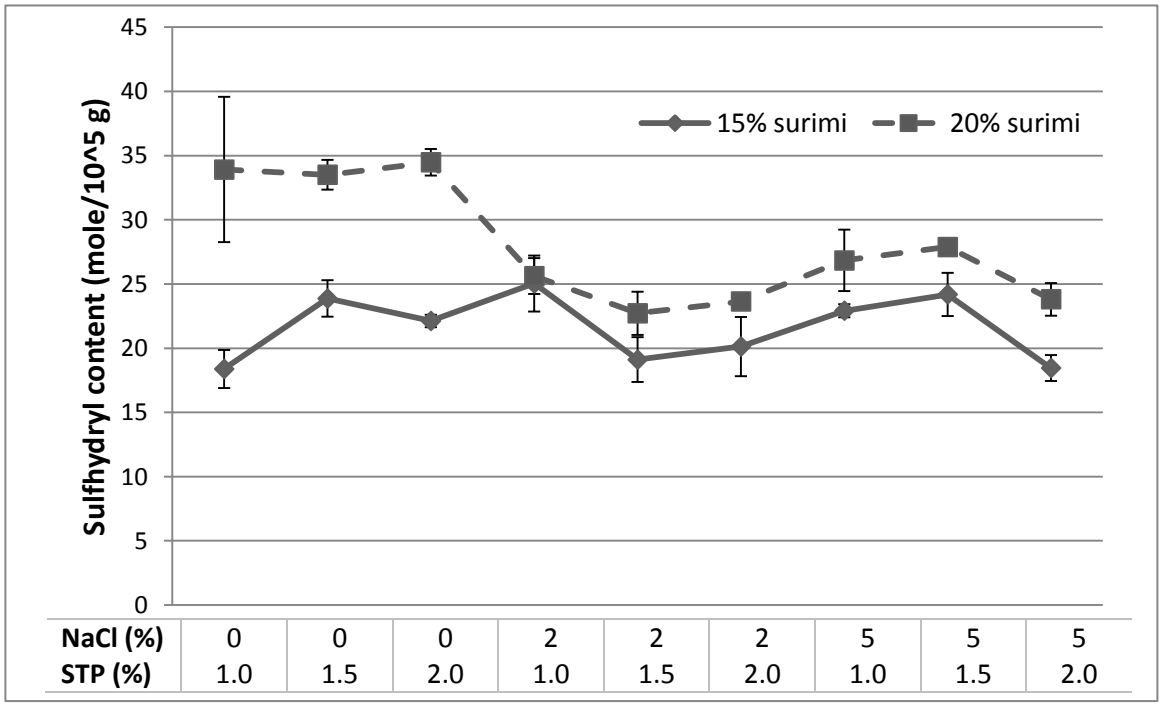

Figure 4.4-Surface reactive sulfhydryl content of heated surimi slurries with various surimi, NaCl, and STP concentrations

4.4.4 Surface hydrophobicity

Surface hydrophobicity of heated surimi slurries among different STP levels, for the same surimi and salt content, was not clearly different (Figure 4.5). During the preparation of surimi slurries, STP was added to shift the pH of protein solutions. Ideally, shifting pH to alkali condition exposed more hydrophobic groups (Arzeni and others 2012) that later played an important role in film formation. However, surface hydrophobicity of heated surimi slurries among different STP levels did not show obvious change. It could be because STP only gave minimal alteration to solution pH (Table 4.1).

In general, salt addition contributed to lower surface hydrophobicity of the heated surimi slurries (Figure 4.5). The obvious surface hydrophobicity reduction was observed as salt concentration increased from 0 to 2% (Figure 4.5). We assumed that the reduction was related to the salt effect on gelation (Nielsen and other 2004), which also involved hydrophobic interactions. Decreased surface hydrophobicity values might confirm the occurrence of aggregation during heating.

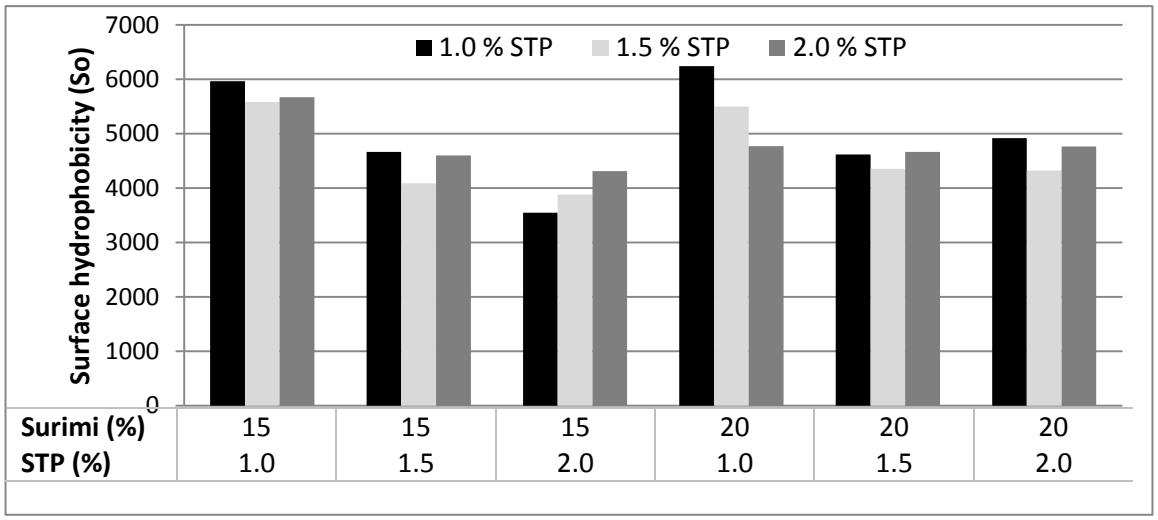

Figure 4.5-Surface hydrophobicity of heated surimi slurries with various surimi, NaCl, and STP concentration

4.5 CONCLUSION

As STP concentration in the surimi slurries increased up to 2%, puncture and tensile strength increased, but puncture distance and elongation at break decreased. This property should be related to the effect of STP on myofibrillar protein extraction and pH shifting. Salt addition, in the presence of STP, attributed to reduced puncture strength, puncture distance, and elongation at break. Regardless of surimi, NaCl, and STP concentrations, heating reduced the viscosity of surimi slurries. Surface reactive sulfhydryl content and surface hydrophobicity confirmed that salt addition promoted heat-induced gelation of fish myofibrillar proteins.

CHAPTER 5

GENERAL CONCLUSION

Surimi slurry is intended to be applied as a fat blocker solution to reduce fat content in fried seafoods. In order to obtain surimi slurries with high performance of film formation, a study on mechanical properties of films prepared from surimi slurries was conducted. We assume slurries that produced films with improved mechanical properties could be utilized as fat blocker solutions for our further study. In this study we used three agents to solubilize fish myofibrillar proteins, which are sodium chloride, sodium tripolyphosphate (STP), and alkaline condition.

Surimi slurries are proven to be able to form films at 15% and 20% surimi concentration. Sodium chloride, either in absence or presence of sodium tripolyphospate addition, contributed to higher tensile strength but decreased puncture strength, puncture distance, and elongation at break of films. Highly alkaline condition, in absence of salt, attributed to increased puncture strength, puncture distance, tensile strength, and also elongation at break. While sodium tripolyphosphate addition resulted in increased tensile strength, decreased elongation at break, and mostly lowered puncture distance and inconsistent effect on puncture strength. Bases on our findings, we concluded that highly alkaline (pH 11) surimi slurry and surimi slurry with 1% STP produced films with good mechanical properties and therefore will be used for our fat blocking study.

Viscosity data suggested that highly alkaline (pH 11) surimi slurries had very low viscosity caused by intense repulsion force among proteins. Addition of STP, in absence of salt, also reduced viscosity of the slurries. Heating step involved in film formation contributed to reduced viscosity associated with loss of hydrogen bond.

Information obtained from biochemical assay suggested that alkaline solubilization promoted protein unfolding that exposed sulfhydryl groups and hydrophobic side chains, while salt addition caused protein aggregation during heating indicated by reduced surface reactive sulfhydryl content and surface hydrophobicity as well. For this study, we did not see significant change in sulfhydryl and surface hydrophobicity of surimi slurries added at three level of STP concentrations evaluated.

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