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Title: Characterization of Rheological Properties and Thermal Stability of Fish Myofibrillar Proteins

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Jae W. Park

Effects of moisture content, pH, and salt concentration on dynamic rheological properties and gel fracture quality of Pacific whiting surimi were investigated. Torsion tests showed that shear stress decreased rapidly and strain values decreased gradually as moisture concentration increased. As pH increased, fracture shear stress and strain values increased, whereas lightness values (L*) decreased. Increasing salt concentration up to 1% increased fracture shear stress and strain values, but further increase affected negatively. A strong relationship was found between the G' and fracture stress values as affected by moisture or pH, but not by salt concentration. Linear regression analyses indicated that while

moisture concentration and pH can be used as an index estimating final gel quality, salt concentration cannot be used.

Thermal stability, proteolytic enzyme degradation, and thermal aggregation patterns of myofibrillar proteins from various fish species were also compared. There was a species effect for both optimum setting and chopping temperatures. While cold water fish species had the highest shear stress values at 5 °C or lower temperatures, warm water fish species had higher fracture shear stress values at 20-30 °C. Proteolytic enzyme activity increased linearly with incubation time when the test was conducted at the optimum autolysis temperature up to 240 min. SDS-PAGE analysis showed that myosin heavy chain was the major protein targeted by proteolytic enzymes. For all tested fish species, a 0.5 °C/min heating rate resulted in higher turbidity values followed by 1 °C/min, and then 2 °C/min. There was a species effect on the temperature where turbidity started to increase. The transition temperatures obtained from temperature sweep measurements were very close to those obtained from DSC, indicating that peaks obtained from the dynamic tests were related to the protein unfolding.

Characterization of Rheological Properties and Thermal Stability of Fish Myofibrillar Proteins

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Major Professor, representing Food Science and Technology

Redacted for privacy

Head of the Department of Food Science and Technology

Redacted for privacy

Dean of the Graduate School

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CONTRIBUTION OF AUTHORS

Dr. Jae W. Park was involved in the design, analysis and writing of each chapter. Dr. Byung Y. Kim was involved with the analysis and writing of Chapter 3 and Chapter 4.

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CHARACTERIZATION OF RHEOLOGICAL PROPERTIES AND THERMAL STABILITY OF FISH MYOFIBRILLAR PROTEINS

CHAPTER 1

INTRODUCTION

Surimi is refined fish myofibrillar proteins stabilized with cryoprotectants. It is an intermediate product used in the manufacturing of a variety of surimi seafood, such as crab legs and flakes.

Two major methods are used to study the rheological properties of fish proteins: small strain (deformation without fracture) and large strain (deformation at fracture) tests. These tests provide useful information regarding surimi gel structure (Kim and Park 2000). Small strain tests are nondestructive, which enables measurements while preserving sample structure. Therefore, they can be used to monitor the physical property changes that relate to the molecular structure of a material (Hamann and MacDonald 1992). Information obtained from dynamic rheological tests has been proven useful in product development (Rao 1999), and could therefore be used for product quality control, as well.

In addition to information on the characteristics of a surimi gel, rheological studies can provide useful information on surimi paste-gel transition. Various studies have suggested that the apparent viscosity of dilute extracts of fish proteins

correlates with the degree of protein denaturation, which in turn, determines the gelation properties (Matsumoto 1980; Jimenez-Colmenero and Borderias 1983; Borderias and others 1985). Dynamic rheological tests, for instance, provide valuable information on the viscoelastic nature of foods. From dynamic viscoelastic tests in the linear viscoelastic range, the storage modulus (G'), loss modulus (G"), and phase angle (δ) can be obtained (Rao 1992).

In commercial practices, the punch or torsion test is used to measure the fracture properties of surimi and surimi seafood. The torsion test measures fracture stress and fracture strain independently (Hamann and MacDonald 1992). Deformation, however, is greatly influenced by force if the measurement is done by the punch test. Therefore, the torsion test was used in this project. However, regardless of whether the punch or torsion is used, testing is time consuming. As a result, there is a great need to develop a rapid analysis for estimating the gelation properties of surimi.

The overall objective was to determine the effect of various processing conditions on the rheological properties of surimi and the thermal stability of fish proteins. The specific objectives were 1) to characterize fish myofibrillar proteins using rheological and biochemical approaches and 2) to investigate if oscillatory dynamic data could be used to predict surimi gel quality.

CHAPTER 2

LITERATURE REVIEW

2.1. GELATION OF FISH MYOFIBRILLAR PROTEINS

Surimi can be described as refined fish myofibrillar proteins stabilized with cryoprotectants. Cryoprotectants, such as sugar and sorbitol, are added to minimize protein denaturation during frozen storage. Preventing protein denaturation during frozen storage is important because freezing and frozen storage without cryoprotectants reduces gel strength (MacDonald and Lanier 1991) and causes undesirable gel colors in the surimi products (Park 1995b). After heading, deboning, washing, and dewatering, myofibrillar proteins are isolated from the undesirable materials, such as bone, blood, fat, skin, and soluble pigments. Myoglobin and hemoglobin, which are responsible for the red hue of fish, are removed during the dewatering step of surimi production. The washing process removes other sarcoplasmic proteins, as well.

Heat-induced muscle protein gel, an important technological property of surimi, can hold a large amount of water and other food components under appropriate conditions (Kocher and Foegeding 1993). The hydration of protein

prior to heat induced gelation is an important step in forming a gel (Oakenfull 1987).

Thermal denaturation is a prerequisite for heat induced gelation of food proteins. Denaturation and aggregation are the two major steps in heat-induced gelation. When aggregation is slow, with respect to denaturation, heat-denatured proteins align in an orderly fashion to form a fine gel network (Hermansson 1979). It is the gel structure that accounts for the textural strength and elasticity of the food as well as the entrapment of water, fat, and other food constituents (Lanier 1986).

Partial solubilization of myofibrillar proteins (myosin and actin), which results in a variety of desired functional properties (Stone and Stanley 1992), can be achieved using relatively high salt concentrations (0.3 - 0.6M NaCl) (Suziki 1981; Lee 1984, 1986) during comminution. The solubilized myofibrillar proteins form an irreversible gel upon heating. Both myosin and actomyosin have important roles in surimi gelation (Niwa 1992) and the gelling characteristics of actomyosin are largely derived from the myosin molecule. There are several factors affecting the gel forming ability of fish muscle proteins, such as freshness, species (Shimizu 1985), hydrogen bonds (Niwa and others 1982; Park and others 1994), hydrophobic interactions (Hamada 1992; Niwa 1975; Niwa and others 1983, 1986a), and covalent cross-linking (Niwa and others 1986b; Lee and others 1997a, 1997b, 1997c).

Studies indicated that myosin is responsible for the gel forming ability of fish proteins during low temperature setting (<40 °C) (Gill and Conway 1989;

Niwa and others 1980). A more ordered and stronger three dimensional network is formed (Foegeding and others 1986) during the setting process as a result of hydrophobic interactions (Stone and Stanley 1992). This protein-protein interaction during the setting process induces greater elasticity and higher water holding capacity in the surimi gels (Kimura and others 1991). This is thought to be due to the formation of a covalent bond between the lysine and glutamic acid by the function of transglutaminase (Lanier 2000).

Pacific whiting represents the third largest fishery biomass used in commercial surimi production. It is known for having a soft texture due to proteolytic activity (Morrissey and other 1995; Porter and others 1993). This enzyme activity limited the utilization of Pacific whiting in the US until 1991. However, the use of protease inhibitors and rapid heating by ohmic heating (Yongsawatdigul and others 1995) enabled the use of Pacific whiting for commercial surimi production.

An and others (1994a) reported that cathepsin L was the most predominant protease present in surimi, resulting in decreased surimi gel strength. While proteolytic degradation was highest at around 55 °C, the surimi paste was more stable at around 30 °C (Tsuyuki and others 1982; An and others 1994b; Chang-Lee and others 1989; Yongsawatdigul and others 1997). Proteolytic degradation, however, during surimi seafood manufacturing, can be solved by rapid heating to a minimum of 70 °C, which inactivates the proteases and prevents textural

degradation of the surimi gels (Patashnik and others 1982; Yongsawatdigul and others 1997).

The number and nature of cross-links formed per polymer chain determines the stability of the myofibrillar protein gel network against thermal and mechanical stresses (Damodaran 1997). A polymer chain is stable when its thermal kinetic energy is lower than the sum of its interaction energies at a given temperature. The thermal kinetic energy of a large polymer chain is lower than that of a small polymer chain. Therefore, gel structures formed with large polymers are more stable than their small polymer chain counterparts. In addition, in a given mass of gel network, gel structures formed with large polymers contain more crosslinks than that of a small polymer chain (Damodaran 1997). This is due to a reduced concentration of polymer chain "ends" or "tails", which often do not take part in cross-linking. Higher thermal kinetic energy and a lower number of crosslinks for short polymer chains could therefore be the reason for reduced surimi gel strength in Pacific whiting surimi; considering that proteolytic enzymes reduces the chain size of myosin and/or actomyosin molecules.

In the surimi industry, the quality of surimi is graded based on its gel forming ability and the chemical and visual parameters of the raw surimi. The texture of surimi gels is affected by the moisture content, salt concentration, myofibrillar protein solubilization, pH, and other processing parameters such as heating. Generally, as moisture concentration increases, the gel strength decreases (Lee and Toledo 1976; Reppond and Babbitt 1997, Yoon and others 1997).

It has been suggested that the presence of sarcoplasmic proteins, which reduces the concentration of actomyosin, decreases the gelling ability of myofibrillar proteins (Okada 1962; Okada and others 1973). Shimizu and Nishioka (1974) proposed a mechanism that water soluble sarcoplasmic proteins bind actomyosin and makes it less available for the cross-linking process. However, this information is now debated and it has been proposed that sarcoplasmic proteins do not interfere with gel formation of myofibrillar proteins (Morioka and Shimizu 1990; Ko and Hwang 1995; Hultin and Kelleher 2000).

There are four main types of chemical bonds that are involved during the formation of a network structure of surimi gels: hydrogen bonds, ionic linkages, covalent bonds, and hydrophobic interactions (Niwa 1992; Lanier 2000). Hydrogen bonds are important in the stabilization of bound water within the hydrogel. A large amount of water molecules are hydrogen bonded to the polar amino acid residues, which are abundantly exposed on the molecular surface of the proteins. The ionic linkages, which occur between negatively and positively charged amino acids of the protein chain, therefore, play an important role in the stabilization of the network structure of various food gels (Lanier 2000).

Hydrogen bonding and ionic linkages (electrostatic interactions) are exothermic (enthalpy driven) in nature. Therefore, they are stabilized at low temperatures and destabilized at high temperatures. Peptide hydrogen bonds in proteins, however, remain stable over a wide range of temperature since they are mostly buried in the interior (Damodaran 1996).

Covalent bonds are formed by electron sharing and once formed, they are not affected by temperature changes. An intermolecular disulfide (SS) bond is formed by the oxidation of two cysteine residues. In contrast, hydrophobic interactions are formed as a thermodynamic response to the exposure of hydrophobic sites to water as a result of protein denaturation (unfolding).

The mechanism of heat-induced denaturation is highly complex and primarily involves destabilization of the major noncovalent interactions. However, unlike hydrogen bonding and ionic linkages, hydrophobic interactions are endothermic (entropy driven). They are stabilized at high temperatures and destabilized at low temperatures. The stability of hydrophobic interactions, however, increases up to a certain point with increasing temperature, which will cause a gradual structural breakdown of water. This in return, will destabilize the hydrophobic interactions (Damodaran 1997). Consequently, the strength of hydrophobic interactions reaches a maximum at about 60-70 °C (Brandts 1967). Due to their inverse relationship with temperature, the changes in stability of hydrogen bonds and hydrophobic interactions consequently oppose each other as the temperature increases.

2.2. PHYSICAL ANALYSIS OF GELS

2.2.1. Rheology

Rheology is the science of deformation and flow of matter (Ferry 1980). It is concerned with how a material will respond when subjected to a force for long and short periods of time. Fluid, semi-solid, and solid foods exhibit a wide variety of rheological behavior ranging from Newtonian to time dependant and viscoelastic.

Understanding the rheology of a material is a prerequisite for understanding its application and processing. Rheological measurements of a surimi gel can be performed in two main categories: fracture and non-fracture gel analysis. In non-fracture gel analysis (small strain testing) samples are subjected to a deformation that is less than the deformation required for structural breakdown. On the other hand, in fracture gel analysis (large strain testing) samples are subjected to a deformation to the point where fracture occurs, causing permanent structural change (Kim and Park 2000).

Like all polymers, myofibrillar proteins exhibit a behavior between that of a purely viscous fluid and a purely elastic solid (Rao 1999). The term viscoelastic is used to describe this intermediate behavior. Viscoelastic materials, therefore, exhibit both an elastic and viscous response to deformation.

2.2.1.1. Non-fracture (small strain) gel analysis

Fracture analyses are often not sufficiently sensitive to explain the structure-property relationships of food materials. However, non-fracture tests permit evaluation of microstructural changes at the cellular level and facilitate characterizing time dependent or viscoelastic properties that food materials exhibit (Mohsenin 1986).

In oscillatory instruments, samples are subjected to harmonically varying stress or strain. When a sinusoidally varying stress is applied to a sample, a sinusoidally varying strain will be induced. Since it is a nondestructive technique, measurements can be done without changing the sample structure. This is the most common dynamic method for studying the viscoelastic behavior of food. In oscillatory dynamic testing, the linear viscoelastic region can be easily determined by changing the amplitude of input stress or strain function.

Results are very sensitive to chemical composition and physical structure. This enables us to relate dynamic rheological parameters to molecular changes. As a result, dynamic measurements can be used in a variety of applications including gel strength evaluation, monitoring starch gelatinization, and observing protein denaturation (Steffe 1996). Oscillatory dynamic testing instruments are therefore particularly useful for product development work.

2.2.1.2. Dynamic rheological tests

Dynamic rheological testing is commonly used to measure the linear viscoelastic properties of food polymers. This dynamic type of measurement is used to monitor changes in the elastic (G') and viscous (G") modulus of the food polymers with time, and in some cases with temperature at a selected frequency . Therefore, such a measurement provides a good understanding of the viscoelastic properties of the food polymers. There are two types of dynamic testing: controlled stress instruments, where the stress amplitude is fixed and the deformation (strain) is measured, and controlled rate instruments, where the deformation is fixed and stress is measured.

The elastic component G', often called the storage modulus, is a measurement of energy stored and subsequently released per cycle of deformation per unit volume since the strain is recoverable in an elastic solid (Ferry 1980). The viscous component, which is referred to as G", is also called the loss modulus to describe the viscous dissipation (loss) of energy as heat per cycle of deformation per unit volume.

The phase angle δ varies between 0 and 90°, depending upon the magnitudes of the G' and G". The phase angle marks the relative effects of the viscous and elastic components in a viscoelastic material. Since the G' and G" terms are related to the ability of the material to store or lose energy, the phase angle can be viewed as the ratio of energy lost to energy stored per cycle (Ak and

Gunasekaran 2001). The phase angle decreases as the elastic component gets stronger, but increases as the material becomes viscous.

Many different measuring systems can be used on a rheometer to study the viscoelastic properties of food polymers. These systems can be categorized into three major groups: (1) bob and cup (coaxial cylinder), (2) cone and plate, and (3) parallel plate. While mobile liquids, suspensions, and emulsions are used as application materials for a coaxial cylinder system, mobile liquids, suspensions, emulsions, dough, and pastes can be studied by the cone and plate and the parallel plate systems (CVO User Manual 1999). The cone and plate system is similar to the parallel plate configuration except that the top plate is replaced with a cone of small angle.

2.2.1.3. Fracture (large strain) gel analysis

Large strain (deformation) tests provide data related to the fracture properties of foods. A variety of instruments, principally utilizing compression, shear, or tension, are used to estimate the textural attributes of foods and most of them are usually designed to simulate jaw action. Consequently, large strain tests have been useful in determining macroscopic textural attributes of foods.

The Hamann torsion gelometer has been successfully adopted as a fundamental test method to measure the fracture properties of surimi gels (Kim and

Park 2000) (Figure 2.1). The test is used to measure shear stress (gel strength) and shear strain (cohesiveness). Mechanical advantages of the torsion test compared to other large strain testing, such as axial compression, are: (1) does not change sample volume, (2) does not change sample shape, and (3) no restriction for fracture: maximum shear, tension, and compression stresses all have the same magnitude, but act in different directions. However, the torsion test also has some disadvantages: (1) sample preparation is time consuming, (2) cross section diameters must be precisely 1.0 cm, considering that diameter accuracy has a significant effect on shear stress values, and (3) sample strain limit is 3.0 since after that the critical cross-section shape changes significantly (Hamann and MacDonald 1992). The first two disadvantages, however, can be overcome by using molded gels (Hoffman and Park 2000).

The punch test is not a fundamental, but an empirical method. It is the most popular measurement technique used in the surimi industry for evaluating gel strength. The force (g) at break and depth of penetration (cm) are used to describe the gel properties (Hamann and MacDonald 1992). However, the punch test has several disadvantages: (1) unlike the torsion test, breaking force and deformation from the punch test are not independent, (2) with too extreme characteristics (mushy or rubbery), samples can not be properly measured, (3) any change in test variables, such as probe diameter or speed can lead to different results for the same sample, and (4) results can not be compared with the results of other methods.

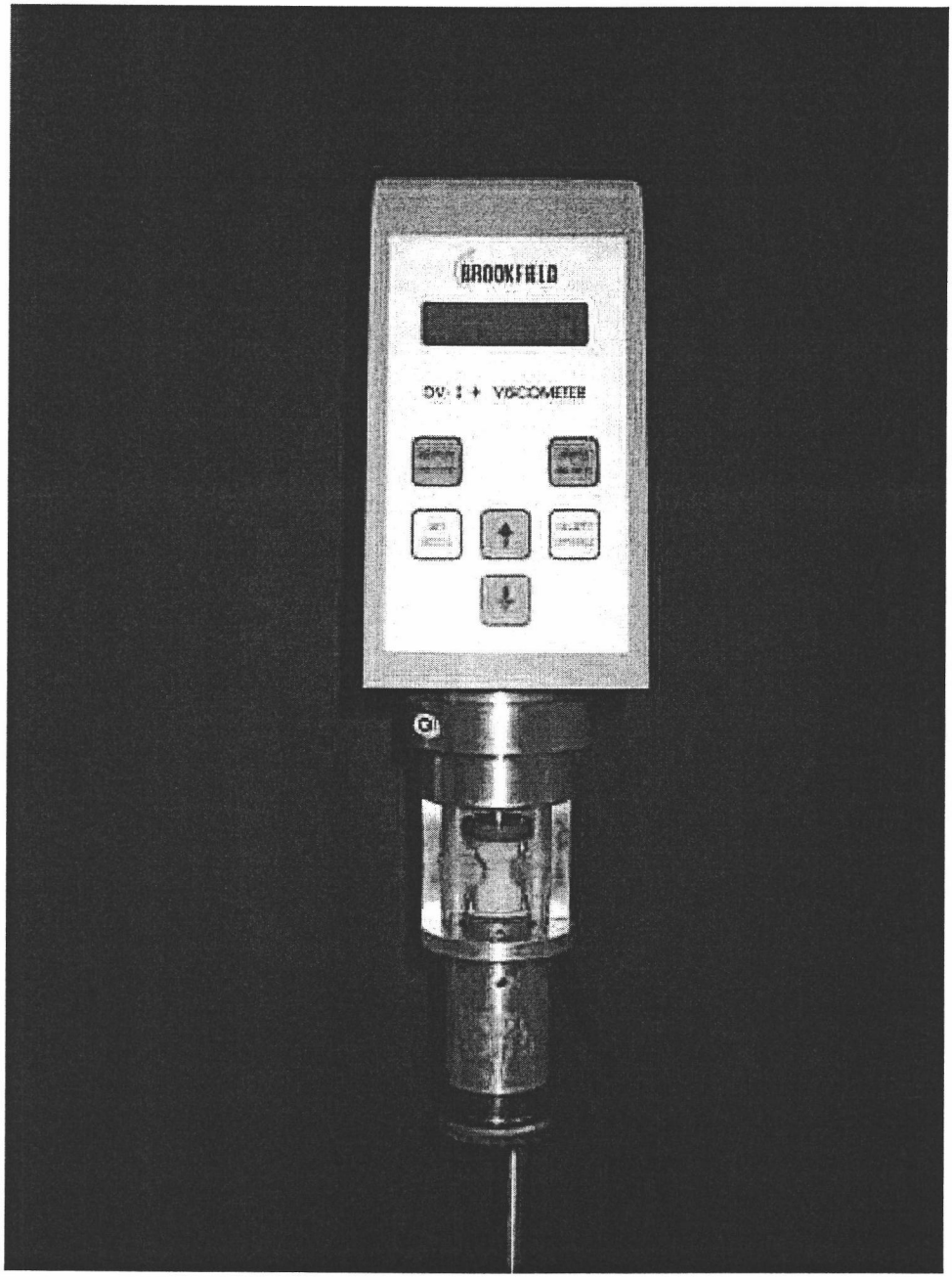

Figure 2.1. Torsion Gelometer.

2.2.2. Differential scanning calorimeter (DSC)

Differential scanning calorimetry (DSC) has become the most popular method to study the thermal transitions of foods in recent years. DSC is a technique in which the difference of energy input (heat flow) into a substance and a reference material is measured as a function of time or temperature when the temperature of the sample is scanned, in a controlled atmosphere (MacKenzie 1978). The reference crucible can be used empty or contain a thermally inert sample in the temperature range being studied.

Any thermally induced changes (for example protein denaturation) are recorded as a differential heat flow displayed as a peak on a thermogram. For proteins, the thermally induced process detectable by DSC is the structural melting or unfolding of a molecule. The transition of a protein from a native to a denatured conformation is accompanied by the rupture of inter and intramolecular bonds. Protein denaturation is recorded as an endothermic peak in the DSC thermogram, while aggregation is exothermic. Analysis of DSC thermograms reveals two important parameters: peak transition (denaturation) temperature (T_d) and enthalpy of denaturation (ΔH).

The ΔH value, calculated from the area under the transition peak, is a net value from a combination of endothermic reactions, such as the disruption of hydrogen bonds (Privalov and Khechinashvili 1974), and exothermic reactions, such as protein aggregation. T_d, in contrast, is related to the thermal stability of the

proteins. At T_d, the difference between the Gibbs free energy (ΔG) of an unfolded (native) protein structure (G_1) and the Gibbs free energy of a folded protein (denatured) structure (G_2) is equal to zero (Boye and others 1997).

Differential scanning calorimetry can also be used to measure thermal transitions in a single polymeric material or a polymer mixture. Although these temperatures are influenced by heating rate (Ruegg and others 1977) and protein concentration (Wright 1984), their determination under controlled conditions can provide a direct comparison of the thermal stability of different proteins. In addition, the information regarding the extent of reversibility of protein denaturation could be estimated using a DSC by rescanning the samples after heat denaturation and comparing the relative areas of transitions produced after the first and second heating (Relkin and others 1993).

2.2.3. Surimi color measurement

Like texture and flavor, color is an important quality factor for surimi and its related products (Park 1995b). CIE (Commission Internationale de l'Eclairage) Lab color scale is used to measure the color of surimi. In this system, the degree of lightness, redness, greenness, yellowness, or blueness represents L*, +a*, -a*, +b*, or -b*, respectively. From these L*, a*, and b* values, the whiteness value can be calculated using the whiteness I equation ($100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{0.5}$).

However, the whiteness II equation (L* - 3 | b* |) was used to calculate the whiteness of surimi gels because a* values are always constant regardless of surimi grade. It was also more effective than the whiteness I equation at differentiating the effect of protein additives (Park 1994).

2.3. EFFECT OF SALT CONCENTRATION, pH , AND SETTING ON SURIMI

2.3.1. Salt concentration

Solubility of a protein is fundamentally related to its hydrophilicity / hydrophobicity balance at the protein surface. Therefore, protein solubility characteristics are naturally affected by its amino acid composition (Damodaran 1997). Myofibrillar proteins have been classified as salt-soluble proteins. They are considered soluble in salt concentrations >0.3 M with or without pH adjustment (Hultin and Kelleher 2000). However, fish myofibrillar proteins in water have also been proven soluble in water (Stefansson and Hultin 1994; Lin and Park 1996).

Solubilization of myofibrillar proteins is related to many functional properties (Niwa 1992) such as surimi gel formation. Roussel and Cheftel (1990) stated that 1.7-3.5 % NaCl is required for surimi to form an adequate gel. Myosin molecules are released from the thick myofibril filaments and dispersed in solution

as monomers at this salt concentration (Ishioroshi and others 1979). Solubilized myofibrillar proteins subsequently form an organized three dimensional gel network upon heating (Niwa 1992; Lanier 1986).

Suzuki (1981), however, reported that high salt concentration (> 1 M) reduces the gel forming ability of myofibrillar proteins, probably due to the decreased solubility of myosin caused by the salting out effect (Regenstein and others 1984). At high salt concentrations, protein-protein interactions are favored over protein-solvent interactions due to competition for water molecules. This leads to aggregation and precipitation of the protein molecules (Morrissey and others 1987). In addition, Hennigar and others (1988) reported that high quality gels could be prepared from red hake muscle without NaCl, contrary to the theory that salt is required to solubilize myofibrillar proteins for the formation of gel network (Suzuki 1981; Lee 1984; Shimizu 1985; Niwa 1992).

Adding salt during the communition of fish mince reduces protein stability and causes protein denaturation (unfolding). As the protein unfolds, anionic groups in several amino acids are exposed, which increases the negative charges. These anionic groups can interact with water and increase the water-binding capacity of the myofibrillar proteins (Chung and Lee 1991). Cl^- ions also play an important role in protein solubility by selectively neutralizing positively charged sites on the protein molecule. This shifts the pI to a lower value resulting in increased solubility at the existing pH (Hamm 1986).

Lin and Park (1996) reported that minimal myofibrillar protein loss during extensive washing was observed at salt concentrations of 0.5-1.0%. The solubility of myofibrillar proteins increased when the salt concentration was below or above the reported range. Foegeding (1987) reported that increasing the NaCl concentration from 0.25 M to 0.5 M at pH 6.0 increased the solubility of salt-soluble proteins. However, there was no significant effect of NaCl at pH 5 and 7 on the solubility of salt soluble proteins. Alaska pollock gels with 0.5% salt had the lowest shear stress and strain values, however, stress values were similar at higher salt concentrations (Park 1995a). Gomez-Guillen and others (1997) studied the influence of salt on surimi gels with different protein additives and reported that the highest gel strength was obtained with gels containing soy protein and 2.5% salt.

2.3.2. pH

The pH is another important factor affecting the solubility of myofibrillar proteins (Turgeon and others 1992; Monahan and others 1995), which consequently affects the gelation properties of fish proteins. Surimi pH is around 7, and at neutral pH, most proteins, including fish proteins, are negatively charged.

Proteins are more stable against denaturation at the isoelectric point (pI) than at any other pH (Damodaran 1996). When the pH gets closer to the pI of fish

protein (pH = 5.5), the net charge of a protein molecule decreases and in return protein-protein interactions increase, a condition that favors aggregation through ionic linkages (Kinsella 1984). Myofibrillar proteins become insoluble since protein-water interactions are replaced by protein-protein interactions at the pI. Foegeding (1987) indicated that solubility of salt soluble proteins was lowest at pH 5.0. In addition, the gel forming ability of surimi increased drastically as the pH increased or decreased in the absence of salt (Torley and Lanier 1992; Nishino and others 1991).

At pH above or below the isoelectric point, the protein acquires net negative or positive charges, respectively. These net negative or positive charges affect the conformational state (unfolding) due to electrostatic repulsion (Biswas and Siddhartha 1995) providing more binding sites for water (Damodaran 1996). The proteins become soluble in water when the repulsive electrostatic forces are larger than the attractive hydrophobic interactions (Boye and others 1997).

Most proteins are stable over a certain pH range. However, extremely high or low pH causes protein denaturation (Morrissey and others 1987). Pour-El and Swenson (1976) stated that exposure to moderately high pH followed by readjustment to neutral pH improves protein functionality. This could be due to protein unfolding and/or buried sulfhydryl group activation. Extended exposure to extremely high pH, however, prevents aggregate formation (Schmidt and Illingworth 1978). The interaction between the carboxyl group (COOH) and the protonated amino (NH_3) group are inhibited at high pH values (Catsimpoolas and

Meyer 1970). This interferes with the cross-linking of aggregates, which in turn affects gel formation. However, highly deformable gels were obtained from Alaska pollock surimi at pH 11 when the ionic strength was lowered to 0.63 mM (Choi and others 2002). This indicates that low ionic strength might be responsible for gel formation at high pH rather than the pH effect alone.

2.3.3. Setting

Myosin heads tend to aggregate upon heating, even at low temperatures, such as 35 °C, while the tail shows helix-coil transitions at higher temperatures (Samejima and others 1981). Holding fish muscle proteins at temperatures below 40°C before subsequent heating at higher temperatures results in a slowly ordered structure of the protein molecules, which produces an elastic and translucent gel (Lanier and others 1982; Numakura and others 1985; Lee and others 1997a). The setting ability of fish pastes and the development of elasticity in the heat set gel are direct results of the thermodynamic requirement (Niwa 1992). In addition, Numakura and others (1989) reported a positive correlation between CaATPase activity and crosslink formation.

Calcium dependent endogenous TGase is thought to be the primary factor for the unique setting ability of surimi at low temperatures (5 - 40 °C) (Seki and others 1990; Kamath and others 1992; Lee and others 1997b). Imai and others

(1996), however, suggested that the content of nondisulfite crosslinks generally correlates with increased gel strength.

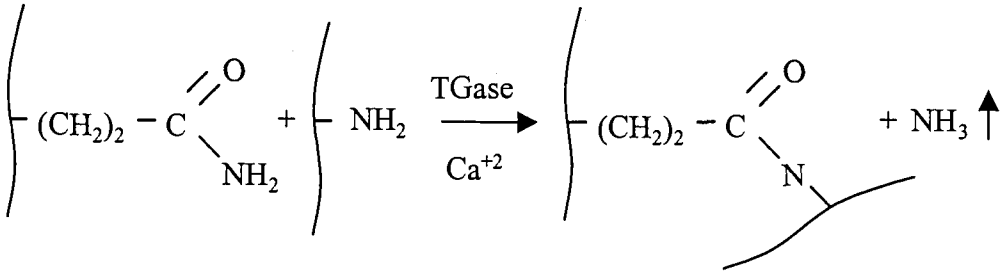

Figure 2.2. Formation of ε-(γ-glutamyl) lysine bonds by calcium-dependent transglutaminase (TGase).

Transglutaminase catalyses an acyl transfer reaction between γ-carboxamide groups of glutamine and primary amine residues resulting in the formation of covalent bonds (Figure 2.2) (Park 2000a). Endogenous TGase is water soluble and can be completely removed if washing is too extensive (Nowsad and others 1994). Therefore, TGase content can vary depending on the washing method used during surimi manufacture.

The optimum setting temperature is most likely dependent on the fish habitat temperature (Kamath and others 1992). Lee and Park (1998) reported that optimum setting temperatures were 5 °C for pollock and 25 °C for whiting. Atlantic croaker (Kamath and others 1992) and tilapia surimi (Yongsawatdigul and others 2000) both achieved maximum gel strength at 40 °C. All these results show

the variability in optimum setting conditions among species. In addition, the species specificity of myosin is inherent in the heavy chain portion of the molecule (Niwa and others 1980).

2.4. THERMAL STABILITY OF MYOFIBRILLAR PROTEINS OF VARIOUS FISH SPECIES

Knowing the thermal sensitivity of fish proteins during surimi production is very important for proper surimi manufacturing techniques. Processing guidelines should be specifically determined for each particular fish species by determining its respective thermal stability. Correct temperature control would prevent thermal damage of myofibrillar proteins, and thus, allow the manufacture of better quality product (Douglas-Schwartz and Lee 1988).

The native state of a protein is thermodynamically the most stable condition with the lowest achievable Gibbs free energy, which has a net stability of only about 10-20 kcal/mol. However, any change in the environment, such as pH, ionic strength, and/or temperature, can easily induce conformational changes in the protein to assume a new equilibrium structure (Damodaran 1997). The structural stability of proteins is primarily maintained by noncovalent interactions, such as hydrogen bonding, hydrophobic and van der Waals interactions, and also by interactions involving binding of the prosthetic group ligants (Damodaran 1997).

There have been several studies, however, suggesting that the thermal stability of proteins is affected by its amino acid composition. Ponnuswami and others (1982) reported that thermal denaturation temperatures of the same set proteins are negatively correlated to the number percent of Ala, Asp, Gly, Gln, Ser, Thr, Val, and Tyr. Other amino acid residues, however, have little effect on the peak transition temperature (T_d). Proteins that are high in hydrophobic amino acid residues, especially Val, Ile, Leu, and Phe, tend to be more stable than the more hydrophilic proteins (Zuber 1988). Ogawa and others (1993) studied the thermal stability of myosin of 10 fish species, and reported a positive correlation between the enthalpy of denaturation (ΔH) and a decrease in α-helical content (Δh).

A positive correlation between secondary structure content and thermostability was observed when several proteins from thermophilic and mesophilic organisms were examined (Merkler and others 1981). Proteins of thermophilic organisms usually contain large amounts of hydrophobic amino acid residues. An optimum distribution of polar or nonpolar amino acids may enhance thermostability by reducing chain flexibility due to intramolecular interactions. However, the thermostability of proteins can not be simply explained by its polar or nonpolar content. Thermostability is also inversely correlated with protein flexibility (Vihinen 1987). In addition, there are other factors, such as disulfide bonds and the presence of salt bridges buried in hydrophobic patches, which may contribute to thermostability.

Among noncovalent interactions, hydrophobic interactions have a huge impact on the conformational stability of proteins (Baldwin 1986; Dill and others 1989). Since hydrophobic interactions are endothermic and entropy driven, their strength increases as the temperature increases, up to 100 °C (Privalov and others 1986). This positive effect of temperature on hydrophobic interactions indicates that they play a very important role in the thermostability of proteins.

A relationship between thermal stability and the environmental habitat temperature has also been reported based on the thermodynamic analysis of myofibrillar ATPase activity (Jonston and others 1973, 1975; Hashimoto and others 1982; Tsuchimoto and others 1988). Fish myofibrillar proteins are more susceptible to thermal denaturation than mammalian or avian myofibrillar proteins (Connell 1961; Matsumoto 1980; Lanier 2000). Although this is not a desirable property for storage stability, it strongly effects to the gelation ability of surimi.

The temperature of a surimi paste after chopping should not exceed the temperature at which that fish protein becomes unstable, thus damaging its functionality (Lee 1984). Based on the study conducted by Arai and others (1973), regarding the relationship between species and thermostability of actomyosin ATPase, Lee (1986) suggested that warm water fish species could tolerate higher washing water temperatures than cold water fish species without a significant reduction in protein functionality. Park (2000b) reported that maintaining chopping temperatures between 0-5 °C provides maximum gelling functionality for Alaska

pollock. However, in industrial practices, the final surimi paste temperature of Alaska pollock is almost always higher than 5 °C when chopping is completed.

Gels prepared from Alaska pollock and lizard fish stored in ice for three days had much lower quality than those prepared from fresh fish. However, good quality gels were prepared from hoki even after several days in ice (MacDonald and others 1990). Hashimoto and others (1983) compared the thermal stability of Alaska pollock, tilapia, and white croaker surimi at 10 °C by measuring their gel hardness sampled at various storage times. While Alsaka pollock gel hardness gradually decreased with storage time, tilapia surimi showed no loss of gel hardness during 140 hr storage.

Holding salted fish muscle homogenates of certain fish species living in cold water, such as Alaska pollock and hoki, for a few hours at temperatures slightly above 0 °C yields a translucent gel. However, this low temperature setting takes only a few minutes for some Antarctic fish species, living in very cold waters. The thermostability of actomyosin from Atlantic krill is about 100 times lower than that of Alaska pollock (Nishita and others 1981). The results of Jiand and others (1989) suggested that actin may increase the thermal stability of myosin in solution, possibly by competing for SH groups with the myosin SH groups for oxidation.

Water content also has a significant effect on the thermal denaturation of proteins. Dry protein powders are very stable to thermal denaturation (Fujita and Noda 1981; Ruegg and others 1975). The effect of hydration, however, on the thermostability of a protein is related to protein dynamics. In the dry state, the

mobility of the polypeptide segment is restricted. As the water content increases, hydration causes swelling of the protein, which increases chain mobility and flexibility. As a result, the protein molecule assumes a more dynamic molten structure. During heating, the access of water to the salt bridges and peptide hydrogen bonds is much higher in the hydrated state than in the dry state, resulting in a lower T_d (Damodaran 1996).

Additives have a significant effect on the thermostability of food proteins, as well. Addition of 0.5 M NaCl to β-lactoglobulin, soy proteins, serum albumin, and oat glubulin significantly increased their respective peak transition temperatures (T_d) (Damodaran 1988, 1989; Harwalkar and Ma 1989). However, salt addition reduced the denaturation transition temperatures of tilapia myosin and actin to lower values and also lowered the enthalpies of heat denaturation (Park and Lanier 1989). This is probably due to increased solubility of fish myofibrillar proteins through unfolding, which increases their susceptibility to thermal denaturation. In addition, the type of salt used, along with fish species, affects the thermal stability of fish myofibrillar proteins. Saeki (1995) reported that addition of salt reduced the thermal stability of fish myofibrillar proteins and the effect of $CaCl_2$ was more pronounced than that of NaCl.

In addition, sugars, such as sucrose, lactose, glucose, and glycerol, are added to stabilize proteins against thermal denaturation (Arakawa and Thimasheff 1982; Harwalkar and Ma 1989). Sucrose and sorbitol are therefore widely used in

commercial surimi processing as cryoprotectants to extend the shelf life of frozen surimi.

CHAPTER 3

EARLY DETERMINATION OF RHEOLOGICAL PROPERTIES OF PACIFIC WHITING PROTEINS USING OSCILLATORY DYNAMIC TEST

O. ESTURK, J.W. PARK, and B.Y. KIM

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3.2. INTRODUCTION

Pacific whiting (*Merluccius productus*) represents the third most abundant fish resource used for surimi in the world. Surimi is refined fish myofibrillar proteins that are stabilized with cryoprotectants. Myofibrillar proteins can form a three dimensional network structure and bind a large amount of water when prepared at an optimum range of protein concentration, pH, ionic strength, and temperature.

The primary factors affecting gelation are hydrogen bonds (Niwa and others 1982; Park and others 1994), hydrophobic interactions (Hamada 1992; Niwa 1975; Niwa and others 1982, 1983, 1986a), covalent cross-linking (Niwa and others 1986b; Lee and others 1997a, 1997b, 1997c), and proteolytic enzyme activity (An and others 1994a, 1994b; Morrissey and others 1993). Gelation and textural properties of fish proteins are considered the most important parameters in surimi production. Therefore, accurate measurement of the rheological properties of surimi is critical to control product quality as well as for new product development.

Rheological information obtained from surimi paste represents the gelation properties of fish proteins. It has been reported that the apparent viscosity of dilute extracts of fish proteins correlates with the degree of protein denaturation, which in turn, determines the gelation properties (Matsumoto 1980; Jimenez-Colmenero and Borderias 1983; Borderias and others 1985). In addition to viscosity, other dynamic rheological parameters, such as storage modulus (G') and loss modulus

(G") obtained from temperature sweep and/or frequency sweep measurements, may rapidly estimate the quality of fish proteins.

Small strain (deformation without fracture) and large strain (deformation at fracture) tests are the main methods used for the rheological study of fish proteins. These tests give important information about the surimi gel structure (Kim and Park 2000). Small strain measurements can be used to monitor the physical property changes of the gel that relate to molecular changes (Hamann and MacDonald 1992). Information obtained from dynamic tests could be used during processing or while developing product formulations, assuming a relationship to the textural quality of the surimi gel can be established.

The rheological properties of fish proteins have been extensively characterized. However, limited information is available regarding the dynamic properties of Pacific whiting surimi paste and their relationship to fracture gel properties. In commercial practices, the rheological properties of surimi and surimi seafood are measured using the punch or torsion test, as a means of fracture analysis. For empirical tests, such as punch, the deformation-related measurements are greatly influenced by the force measurement. Torsion, in contrast, measures the two fracture property descriptors (stress and strain) independently (Hamann and MacDonald 1992) and may provide a correlation of fracture gel quality to the dynamic rheological measurements.

Regardless of whether the punch or torsion is used, it is typically a 24 h process to obtain gel values. Therefore, when problems related to gel quality are

identified, the product may already be processed or even shipped to customers. Consequently, there is a great need to develop a rapid analysis for determining the gelation properties of surimi.

Our objectives were to investigate the effects of moisture content on the rheological properties of surimi gels based on dynamic rheological measurements and torsion testing, and to determine a relationship between small strain and large strain tests for possible early prediction of surimi gel quality.

3.3. MATERIALS AND METHODS

Frozen Pacific whiting surimi was obtained from local manufacturers. Surimi contained 4% sugar, 4% sorbitol, and 0.3% sodium phosphate as cryoprotectants, as well as 1.4% BPP (beef plasma protein) as a protease inhibitor. Immediately after the frozen surimi was transferred to the OSU Seafood Lab, the 10 kg blocks were partitioned without thawing into approximately 1 kg blocks, vacuum packed, and stored at - 30 °C until tested. Surimi used in the study was approximately 3-6 mo old.

3.3.1. Gel preparation

After determining the moisture content of the sample (AOAC 1990), surimi batch formulations with 2% salt were adjusted to obtain various moisture concentrations. Surimi gel preparation and testing were conducted according to the method of Park and others (1994) and during chopping, the paste temperature was maintained below 5 °C. A small portion (100 g) was saved for dynamic rheological testing, while the remaining sample was cooked in tubes for fracture analysis. No measurable gels for fracture were obtained when the sample moisture content was 84% or higher. Therefore, to determine a relationship between fracture gel quality and the dynamic properties, samples containing 75, 78, and 81% moisture were used for data evaluation.

3.3.2. Non-fracture gel analysis

A plastic bag of surimi paste was kept in salt-added ice water (-2 °C) to prevent pre-setting until tested. Dynamic tests were conducted using a Bohlin Rheometer, CS-50 (Bohlin Instruments Inc., East Brunswick, NJ). Dynamic rheological properties of surimi paste were monitored using a 4.0 cm dia / 4 ° angle cone and plate (CP - 4/40) geometry. Surimi paste was loaded in the gap (0.15 mm) between the cone and plate. Inside the moisture trap, a wet sponge was placed to minimize moisture loss during heating.

The region of linear viscoelastic response of surimi paste was determined by stress sweep. The lowest and highest stress values used for the stress sweep test were 0.5 and 1500 Pa, respectively, at 0.1 Hz and 20 °C. During the temperature sweep, surimi samples were heated from 10 °C to 80 °C at a 1 °C/min heating rate and 0.1 Hz frequency. The minimum and maximum frequencies used in the frequency sweep tests were 0.01 and 30 Hz, respectively. Tests were conducted at 20 °C. The stress value used for the temperature sweep and frequency sweep was selected within the range of the linear viscoelastic response.

Dynamic rheological parameters, such as G' (storage modulus), δ (phase angle), and G" (loss modulus) were obtained. G' represents the elastic characteristics of the sample while G" represents the viscous properties. Phase angle (δ), ranging from 0 ° (perfectly elastic) to 90 ° (perfectly viscous), was calculated from $\tan^{-1}$ (G"/G').

3.3.3. Fracture gel analysis

The torsion test was used to determine the fracture properties of the gels (Hamann 1983). The gels were kept at room temperature for 2 h before analysis to eliminate the effect of different gel temperatures on textural measurement. Gels were cut (2.9 cm length, 1.9 cm dia), glued on both ends to plastic disks (Duro Super Glue, Loctite Corporation, Rocky Hill, CT), and milled into an hourglass

shape, using a milling machine (Gel Consultants Inc., Raleigh, NC) with a minimum diameter of 1.0 cm in the center. Then, each gel segment was subjected to the Hamann Torsion Gelometer (Gel Consultants Inc., Raleigh, NC). By twisting the samples, shear stress was calculated based on the torque values and shear strain calculated based on the angular displacement at mechanical fracture according to the method of Hamann (1983). Shear stress indicates gel strength and shear strain denotes gel cohesiveness. At least 10 samples per treatment were measured.

3.3.4 Statistical analysis

SAS statistical package was used to analyze data (version 8, SAS Inc, Cary, NC). Least significant difference (LSD) was used to determine significant differences between mean values at $p< 0.05$.

3.3.5. Model development and verification

Linear regression analyses were used to investigate the relationship between dynamic rheology and fracture gel measurements. Temperature sweep and torsion test data from 5 different surimi batches were used to develop the equations. At first, Bayesian Information Criterion (BIC) and Cp statistics were used to

determine the best subset model using SAS/ASSIST (SAS Inc., Cary, NC). According to BIC criterion, the best models have the smallest BIC values, which means small residual mean square (σ^2) and small probability (p). The Cp criterion, on the other hand, focuses directly on the trade-off between bias due to excluding important explanatory variables and extra variance due to including too many variables (Ramsey and Schafer 1997). This process enabled us to select important explanatory variables and to eliminate insignificant ones (models with small BIC and Cp statistics). Shear stress was selected as the dependent variable (torsion test), while moisture concentration as well as G', G", and viscosity from dynamic data output (temperature sweep at 0.1 Hz) were selected as the independent (explanatory) variables. Phase angle (δ) was not used as an explanatory variable since it is a function of G' and G".

Best fitting models for each temperature sweep data point within the range of 20 - 70 °C were determined using BIC and Cp statistics. Then, a multivariate linear regression analysis was conducted using the best fitting model to get the model equation that correlates fracture gel properties to the dynamic data. This process allowed us to monitor which temperature sweep data point provided the best correlation with fracture gel properties. Plotting variables against each other indicated that there was no interaction among the variables. Therefore, the general linear regression model used was:

$$Y= \beta_0 + \beta_1 X_1 + \beta_2 X_2 \ldots. \qquad (3.1)$$

where X represents the selected independent variable, β_0 intercept term, and β_n the regression coefficient of the independent variable.

New surimi batches with different moisture concentrations were prepared to verify the linear regression models that were developed. Fracture and non-fracture gel analyses were conducted for each batch as previously described. Explanatory variables (G', G", and/or viscosity) from temperature sweep test were plugged into the corresponding model equation. For example, dynamic data output at 25 °C was used in the model developed for 25 °C. Prediction (model) shear stress values were compared to experimental (torsion) shear stress values to determine how close the predictions were.

3.4. RESULTS AND DISCUSSION

3.4.1. Fracture properties

Fracture shear stress and strain values of Pacific whiting surimi significantly decreased as moisture content increased ($p<0.05$) (Figure 3.1). The higher the moisture content, the lower the fracture shear stress and fracture shear strain. Yoon and others (1997) reported a similar trend for Pacific whiting. This observation supports the fact that the conventional method used by the surimi

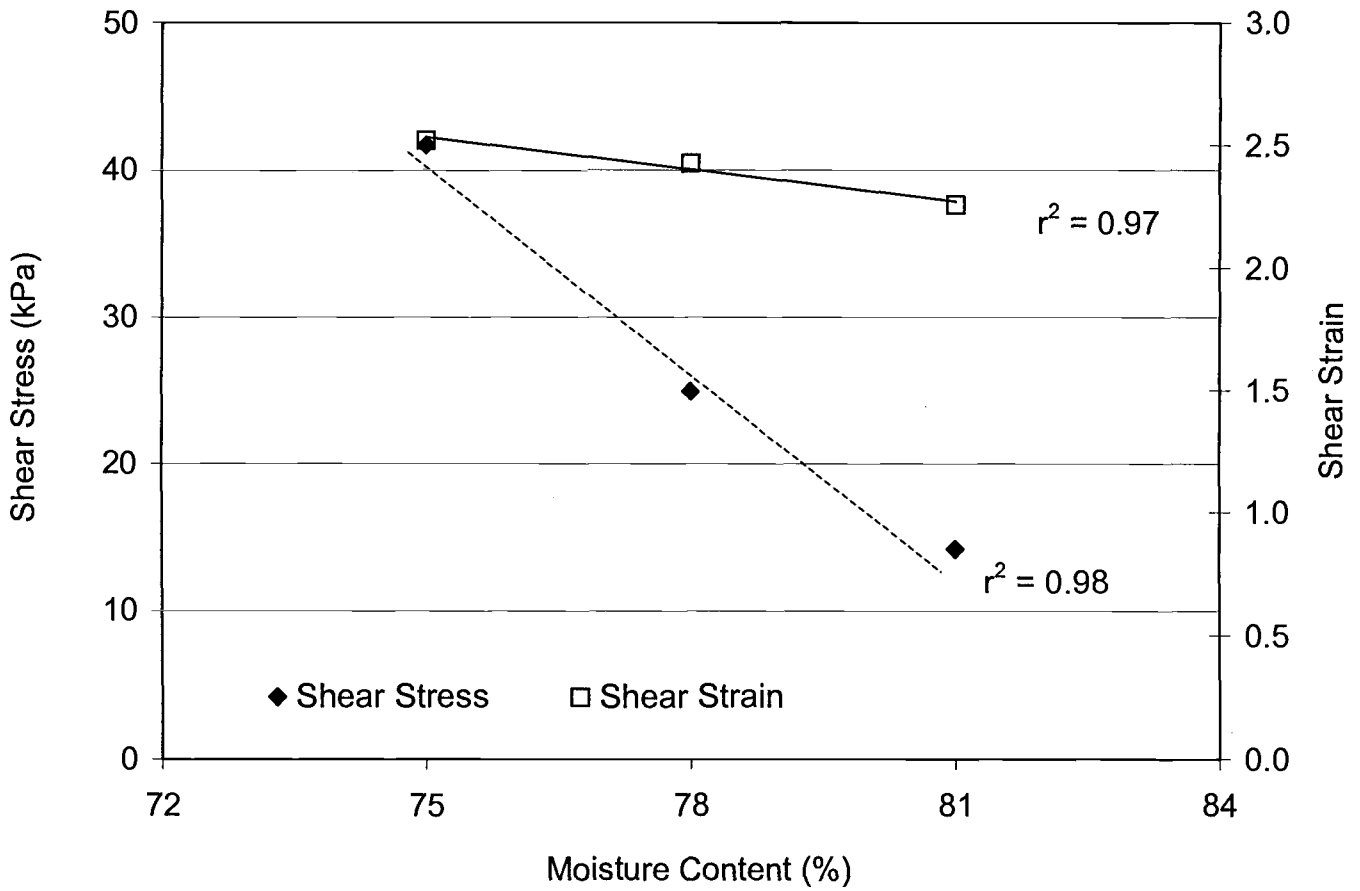

Figure 3.1. Fracture shear stress and strain values of Pacific whiting surimi as affected by moisture concentrations.

industry, where gel preparation is made without moisture adjustment, could lead to false information. Reppond and Babbitt (1997) demonstrated that water had a significant effect on fracture shear stress of various fish species. A similar trend was also reported for the fracture shear stress values of Alaska pollock surimi (Hamann and MacDonald 1992; Yoon and others 1997). However, these studies stated that moisture concentration had no effect on the fracture shear strain of Alaska pollock within the studied range (75-81%). Hamann and MacDonald (1992) suggested that, depending on species, higher shear strain values were associated with lower free moisture in the surimi gels.

Fracture shear stress showed a significantly negative linear relationship (r^2 = 0.98) with moisture content. Shear stress values dropped from 41.67 to 14.83 kPa as moisture concentration increased from 75 to 81%. Fracture shear strain, which is an indicator of protein quality (Hamann and MacDonald 1992), also showed a negative linear relationship ($r^2 = 0.97$). However, moisture content appeared to have a larger impact on the change of fracture shear stress than the change of fracture shear strain.

Consequently, fracture shear strain is not significantly influenced by protein concentration, moisture, ingredient, and processing variables. Fracture shear stress, however, is significantly affected by these factors (Hamann 1988). According to Hamann and MacDonald (1992), while fracture shear strain is proportional to the average molecular weight of the polymer chains between the nearest neighboring crosslinks (Me), fracture shear stress is proportional to the crosslinked polymer

concentration (c) at constant temperature. Increasing moisture content increases Me. However, it also reduces the number of crosslinks. The observation regarding the relationship between c and fracture shear stress were supported by our results that fracture shear stress values decrease with reduced fish protein concentration.

3.4.2. Dynamic properties

The linear viscoelastic range of a polymer is generally determined by stress sweep at a constant frequency. The effect of applied stress at the sol state (paste) on G' is given in Figure 3.2. The linear region extended significantly as moisture concentration decreased. This indicated that increasing protein concentration enforced polymer structure and increased the required stress for structural deformation.

The linear region was from 20 to 150 Pa, 20 to 75 Pa, and 20 to 46 Pa and strain values were 0.0242 at 150 Pa, 0.0217 at 75 Pa, and 0.0197 at 46 PA for 75, 78, and 81% moisture, respectively. This indicated the reduced stiffness of the polymer structure. The change of G', G", and phase angle (δ) with respect to moisture content is shown in Figure 3.3. When moisture concentration increased, G' and G" decreased, while phase angle (δ) increased (r^2 = 0.96, 0.97, and 0.97, respectively) (Figure 3.3). This linear relationship between protein concentration

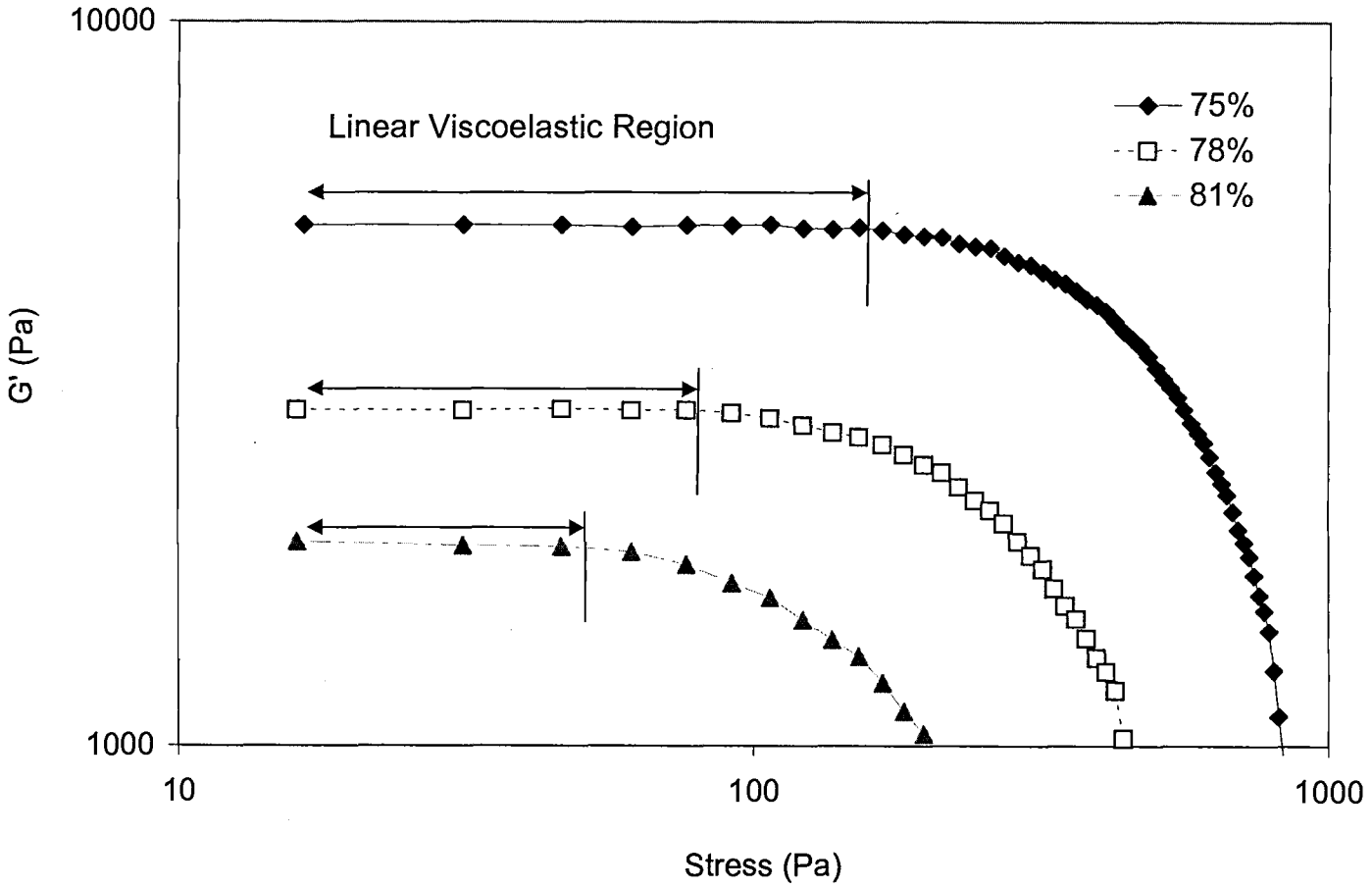

Figure 3.2. Effect of stress on G' of Pacific whiting surimi as affected by moisture concentrations at 20°C and 0.1Hz.

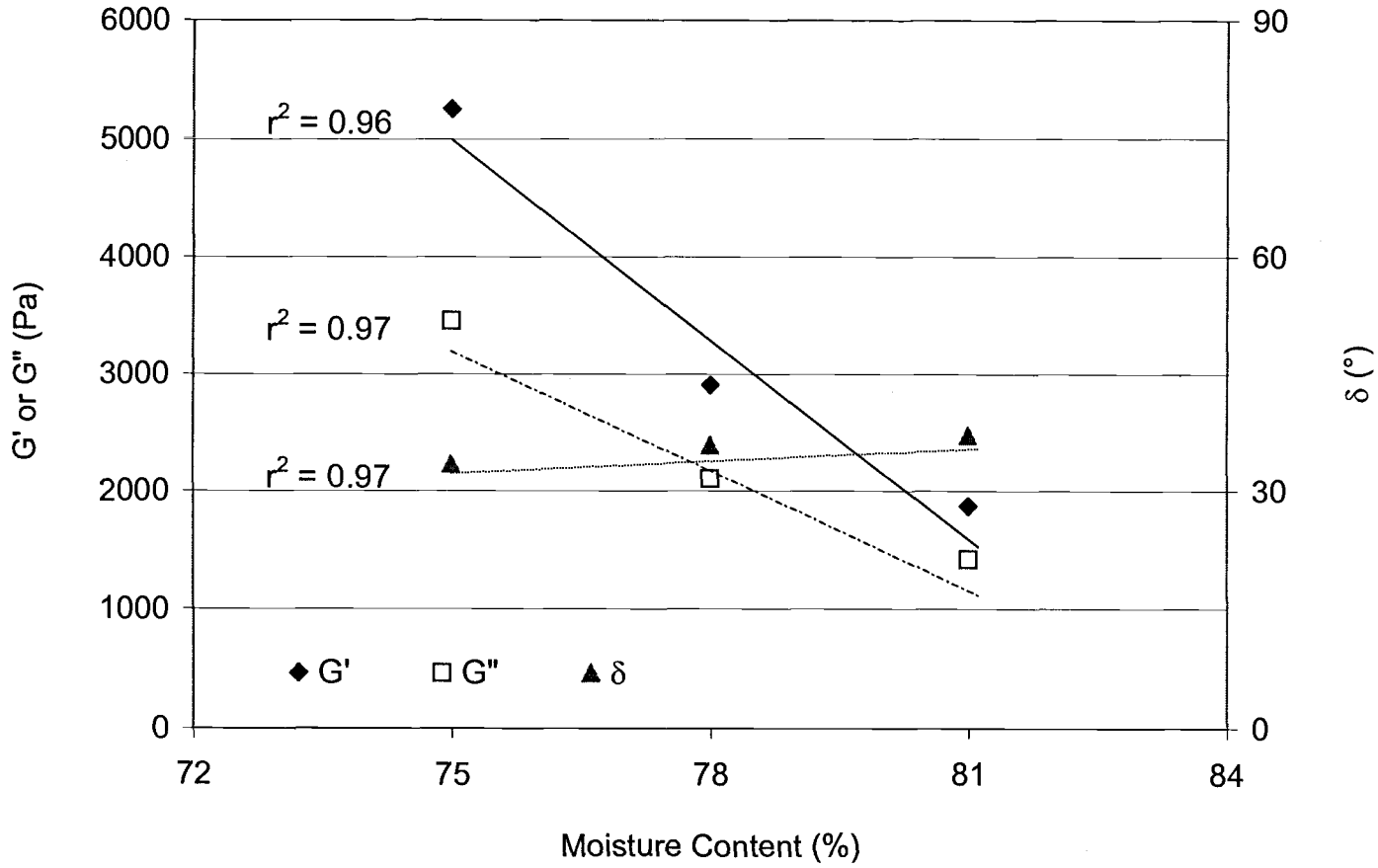

Figure 3.3. Change of G', G", and phase angle (δ) of Pacific whiting surimi as affected by moisture concentrations (0.1Hz, 46 Pa).

and dynamic rheological measurements, however, was only good within the range of the linear viscoelastic response.

Log G' against log frequency of Pacific whiting surimi at different moisture concentrations is given in Figure 3.4. As moisture concentration increased, G' decreased. Plateau modulus was obtained by plotting log G' against log frequency. According to the Rouse theory (Ferry 1980), the plateau modulus (G_N^0) is ρRT / Me, where ρ is the density, R is the gas constant, T is the temperature, and Me is the average molecular weight of the polymer between adjoining crosslinks. This relationship can also be written as $G_N^0 = \upsilon RT$, where υ is the density of the crosslinks. The larger the molecular weight, the larger the G'. When moisture content increases, Me will increase and υ will decrease. Our results confirmed the relationship given by the Rouse theory, where increasing moisture concentration reduced the G_N^0 value.

G", a measure of energy loss, started to decrease when the frequency was equal to 1.0 Hz (Figure 3.4). When frequency is significantly larger than the polymer relaxation time, the orientation cannot keep pace with the alternating shear rate and, therefore, less energy is dissipated (Ferry 1980), indicating a decrease of G". There are few polymer molecules that behave in this manner (Ferry 1980), and Figure 3.4 shows that fish myofibrillar proteins belong to this group of polymers. A crossover point (W_C) was observed at 0.02 Hz for 78 and 81% samples. At the crossover point, G' and G" are equal indicating liquid-solid phase transition.

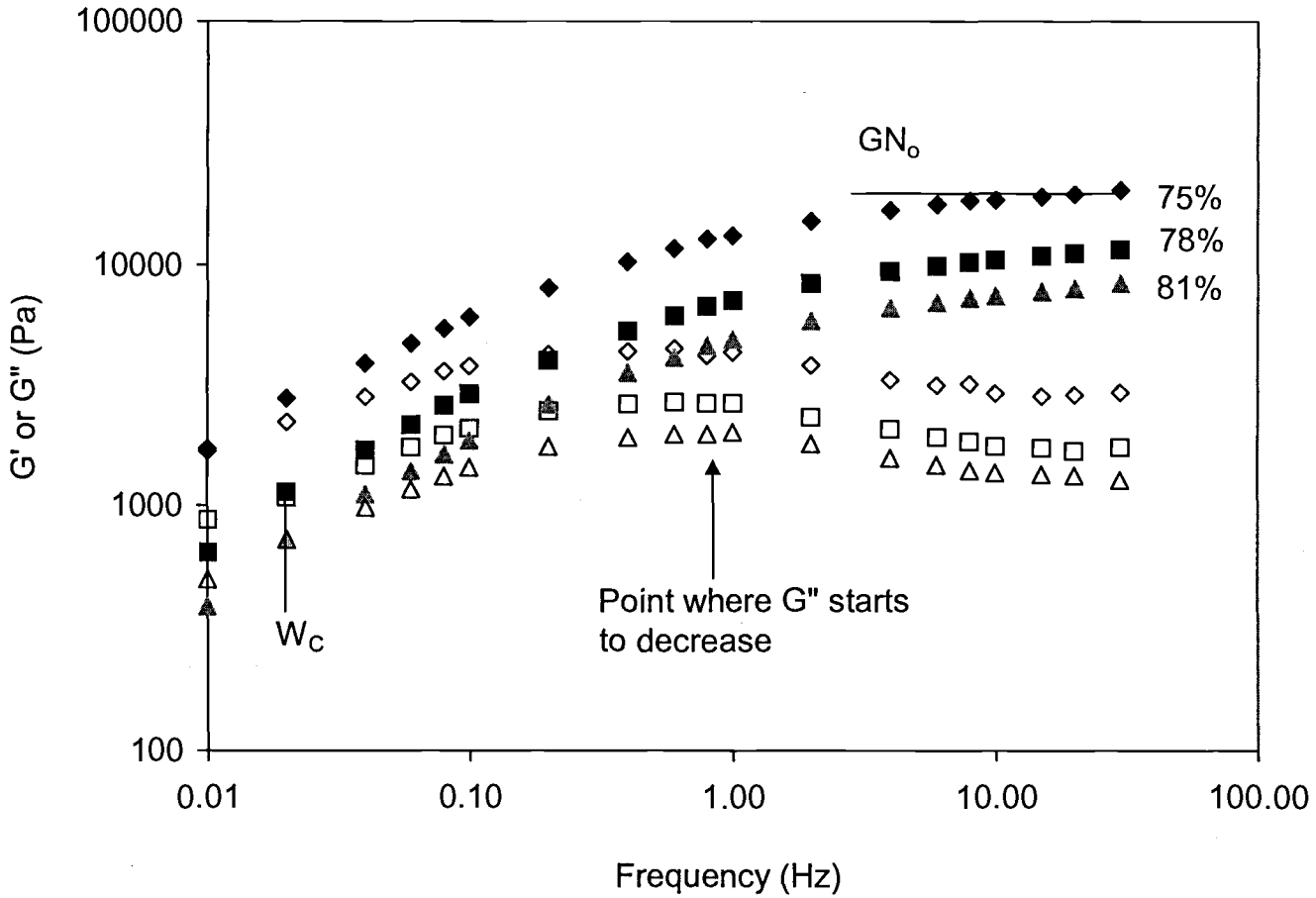

Figure 3.4. Effect of frequency on G' and G" of Pacific whiting surimi as affected by moisture concentrations. ◆ 75% G', ■ 78% G', ▲ 81% G', ◇ 75% G", □ 78% G", Δ 81% G", GN_O: plateau modulus, W_C: crossover point.

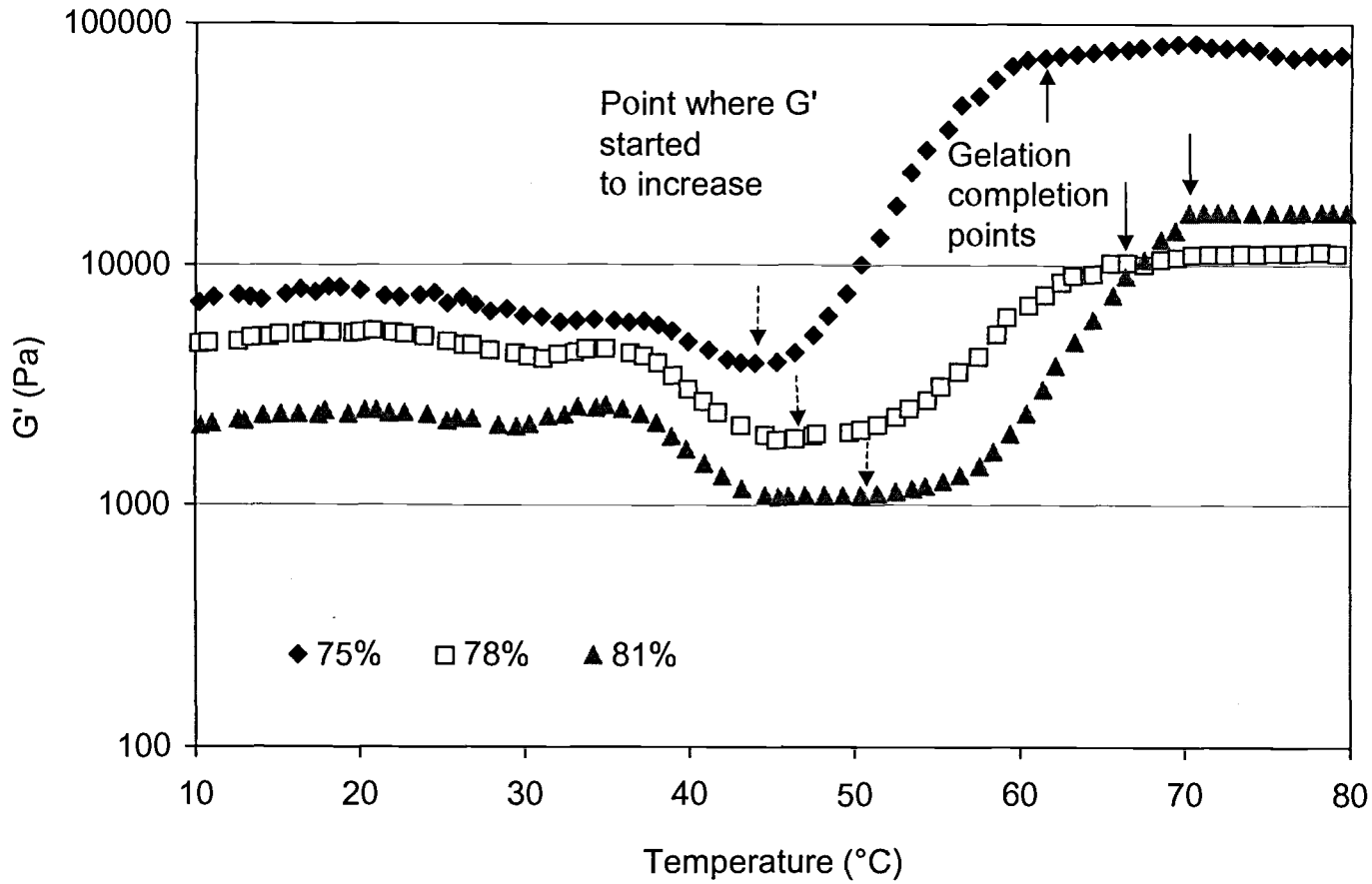

Figure 3.5. Changes of storage modulus (G') of Pacific whiting as affected by moisture concentrations.

Storage modulus (G') values of Pacific whiting surimi paste during the temperature sweep tests are shown in Figure 3.5. There was a small drop of the G' value starting at 30 °C. G' reached its lowest value at around 45 °C, then increased rapidly until reaching 60-70 °C. Thermal gelation was completed at the temperature where G' reached a maximum plateau.

Myosin gels are typically formed at two major stages during heating. First, at 30-40 °C α-helices in the tail portion of myosin molecules unfold (Ogawa and others 1995). Second, above 50 °C, the unfolded hydrophobic regions interact with each other (Sano and others 1990). Major structural changes in actomyosin occur in the 45-50 °C range, where unfolding of actomyosin is followed by aggregation. Stone and Stanley (1992) also reported a disruption of the fish muscle gel network at 45-50 °C.

The lowest G' values around 45-50 °C (Figure 3.5, 3.6) were therefore, probably due to the effect of the structural changes (unfolding) of actomyosin. An increase in G' indicates gel formation or a gel stabilized by hydrophobic interactions (Mleko and Foegeding 2000). Gelation of actomyosin continued up to 70 °C. Therefore, G' stayed relatively constant after 70 °C (Figure 3.5). As G' started to increase, moisture content also affected the aggregation rate of the unfolded protein (Figure 3.5). The higher the moisture content, the slower the aggregation rates.

The G' of surimi paste reached its lowest value regardless of moisture concentration at approximately 45°C. While G' of surimi samples containing 75%

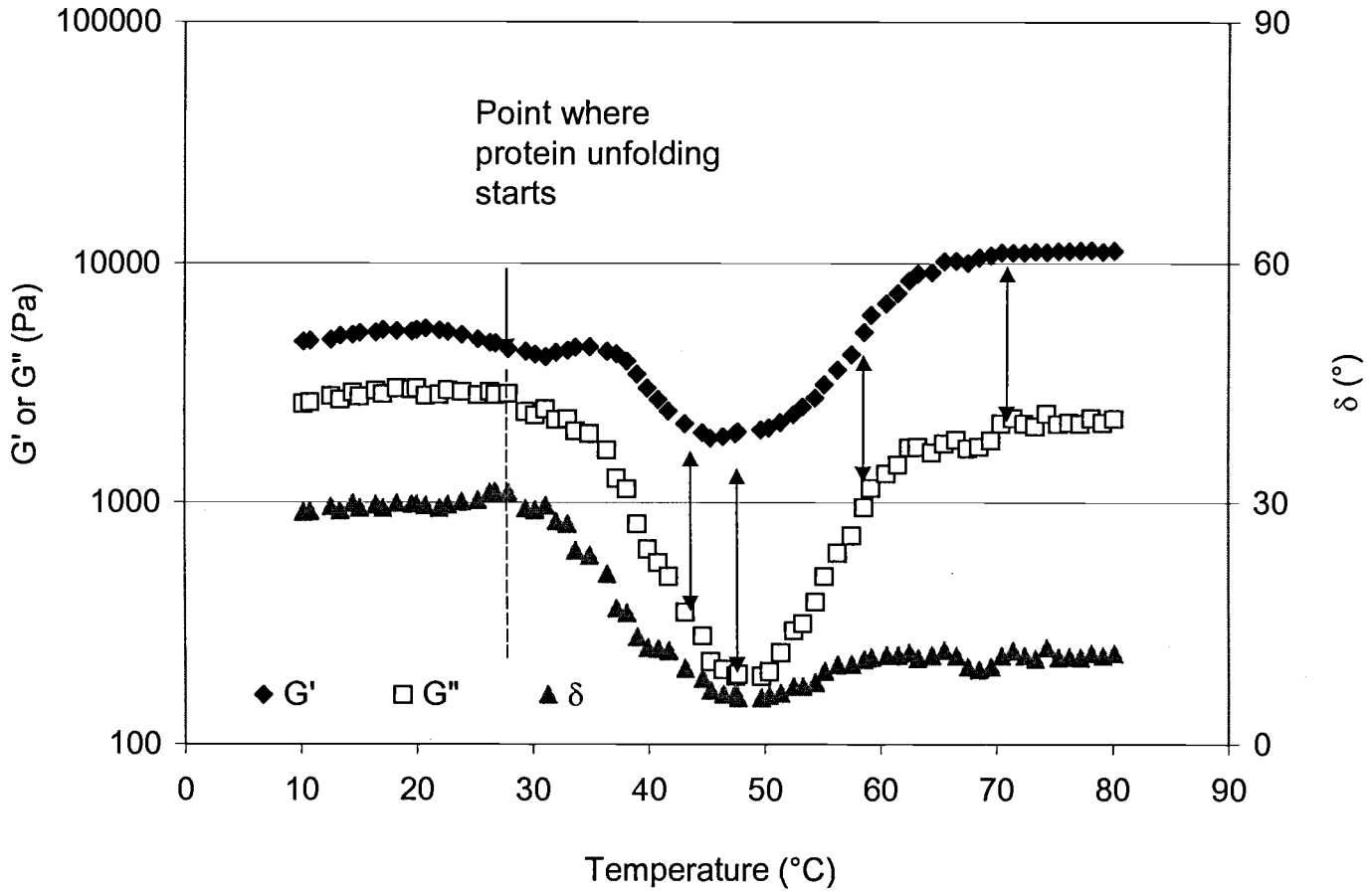

Figure 3.6. Change of G', G", and phase angle (δ) of Pacific whiting surimi containing 78% moisture.

moisture increased immediately after reaching its lowest value, G' started to increase at 52 °C for samples containing 81% moisture and measured peak area increased as moisture concentration increased. Protein denaturation (unfolding) initially started at approximately the same temperature (35 °C) regardless of moisture concentration. However, the association of unfolded proteins occurred at different rates indicating the concentration dependency of protein association.

Phase angle (δ), a measure of the ratio of energy lost to energy stored in a cyclic oscillatory deformation, was another good parameter for monitoring changes during gelation (from sol to gel state). The change of phase angle (δ) with temperature along with G' and G" for a sample containing 78% moisture is given in Figure 3.6. Phase angle (δ) started to decrease at around 30 °C, corresponding to an initial rise of G' and indicating the start of unfolding.

Another interesting observation was the difference between energy lost and stored. The difference between G' and G" was largest when fish proteins were fully unfolded at around 50 °C. It should also be noted that the phase angle (δ) was always smaller than 45° (Figure 3.6), indicating that the surimi paste behaved as a solid-like material rather than a liquid-like one. This was probably due to the high protein concentration and molecular entanglement of proteins in the surimi paste.

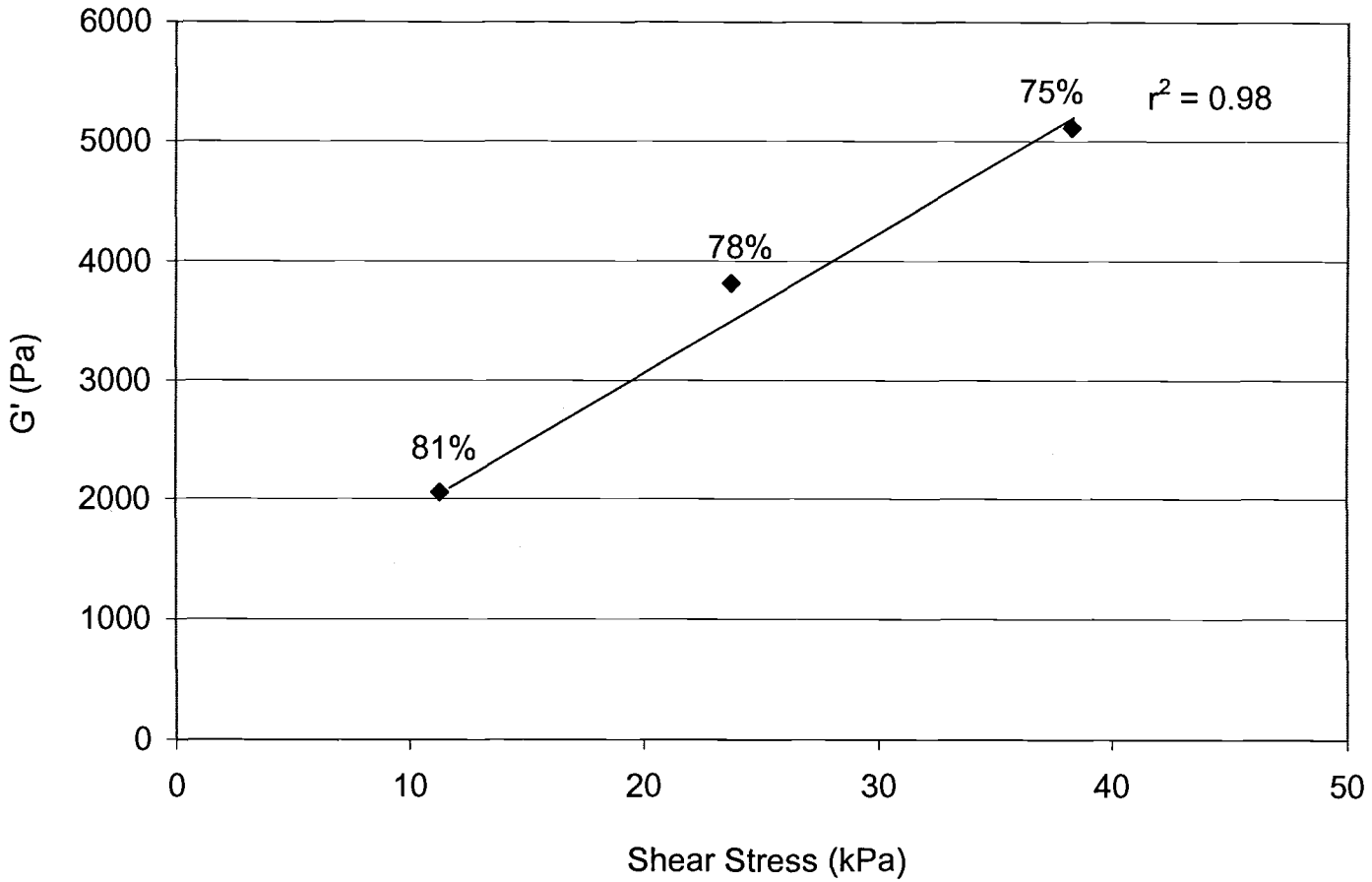

Figure 3.7. Relationship between storage modulus (G') at 30°C and fracture shear stress as affected by moisture concentrations.

3.4.3. Relationship between gel fracture and dynamic properties

The relationship between the dynamic storage modulus (G') at 30°C and fracture stress values (torsion) is given in Figure 3.7. A similar trend was observed for different surimi batches (r^2 = 0.90 - 0.99), if the G' was selected within a certain temperature range (20 - 45 °C). Temperature sweep above 45 °C did not yield a high correlation ($r^2 < 0.90$) with fracture stress. Model equations obtained from multiple linear regression analysis resulted in a similar trend. Predictions were more accurate when the dynamic data output was within the range of 25-35 °C. Table 3.1 shows the results of the regression analysis parameters used to describe the relationship between fracture shear stress or fracture shear strain and temperature sweep data at 25 °C and 30 °C, respectively. The r^2 values of the equations developed for the temperature sweep data at 25 °C and 30 °C were 0.96 and 0.97 for fracture shear stress, and 0.27 and 0.28 for fracture shear strain, respectively.

The equations of the best fitting models developed for temperature sweep data at 25 and 30 °C for fracture shear stress and fracture shear strain are:

Fracture Shear Stress = 312.612 - 4.0208*Moisture Concentration (%) - 2.1524*Log (G') + 10.14*Log (G") (3.2)

Fracture Shear Stress = 304.1571 - 3.9615*Moisture Concentration (%) - 16.0028*Log (G') + 26.3090*Log (G") (3.3)

Table 3.1. Regression analysis parameters for Eq. 3.2, 3.3, 3.4, and 3.5.

	Shear Stress		Shear Strain	
Parameters	Eq. 3.2 (25 °C)	Eq. 3.3 (30 °C)	Eq. 3.4 (25 °C)	Eq. 3.5 (30 °C)
SSE	40.26	35.90	0.73	0.72
MSE	5.04	4.49	0.07	0.07
Root MSE	2.24	2.12	0.27	0.27
r^2	0.97	0.98	0.33	0.34
adjusted r^2	0.96	0.97	0.27	0.28

SSE is the sum of squares due to error.
MSE is the mean squares of error.
adjusted r^2: [(Total mean square - Residual mean square) / Total mean square].

$$\text{Fracture Shear Strain} = -0.4068 + 0.7692*\text{Log}(G') \quad (3.4)$$

$$\text{Fracture Shear Strain} = -0.5150 + 0.8096*\text{Log}(G') \quad (3.5.)$$

As for model verification, experimental and prediction values of new surimi batches were compared using the equations developed for 25 and 30 °C. At 75% moisture, predicted shear stress values were 39.59 and 39.86 kPa (Figure 3.8) using Eq. 3.2 and Eq. 3.3, respectively. The corresponding fracture shear stress value from the experiment was 40.45 kPa. However, since a correlation between fracture

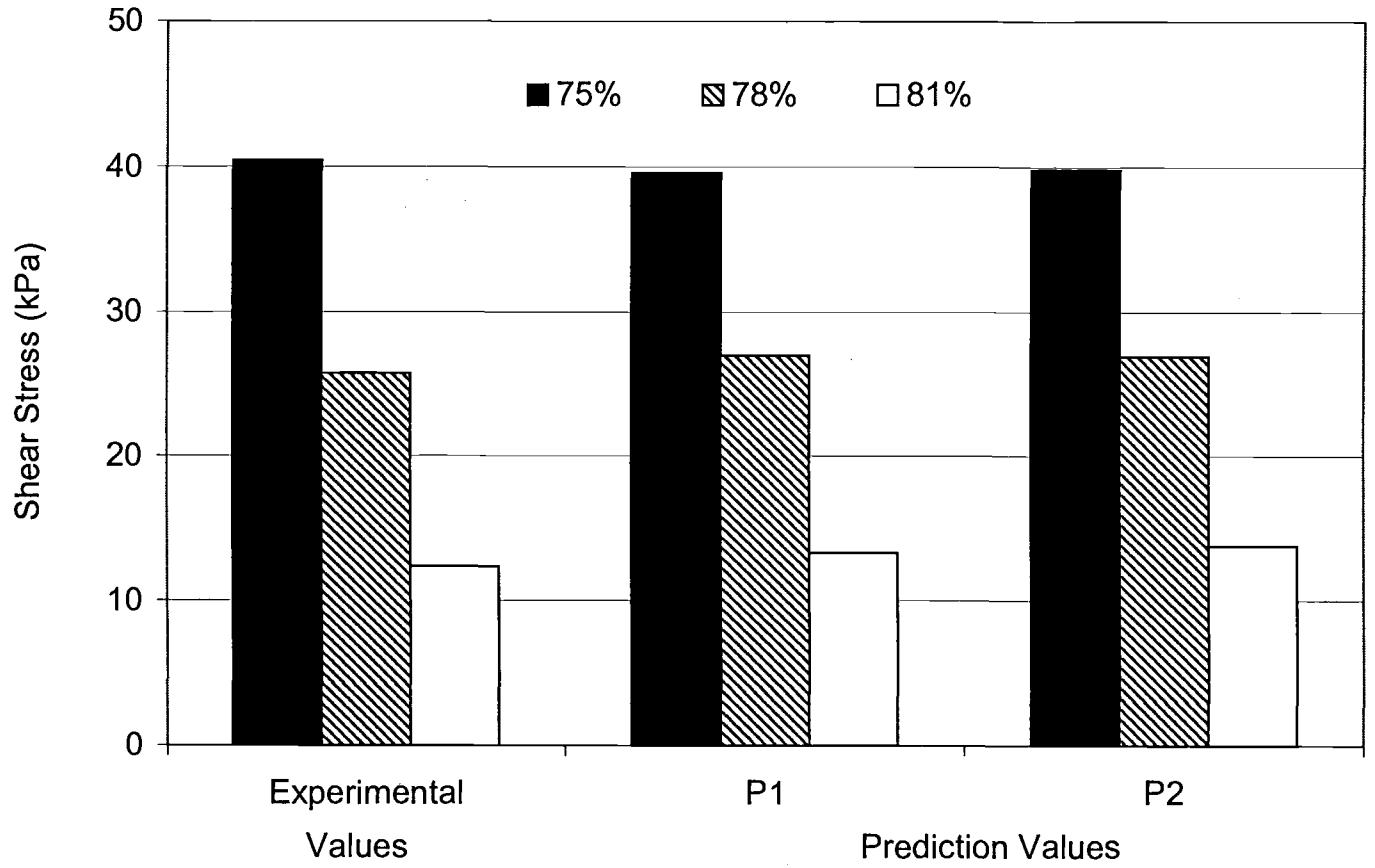

Figure 3. 8. Experimental fracture stress values from torsion test and predicted values of fracture shear stress values using the best fitting models. P1: predicted fracture shear stress using the temperature sweep data at 25 °C. P2: predicted fracture shear stress using the temperature sweep data at 30 °C.

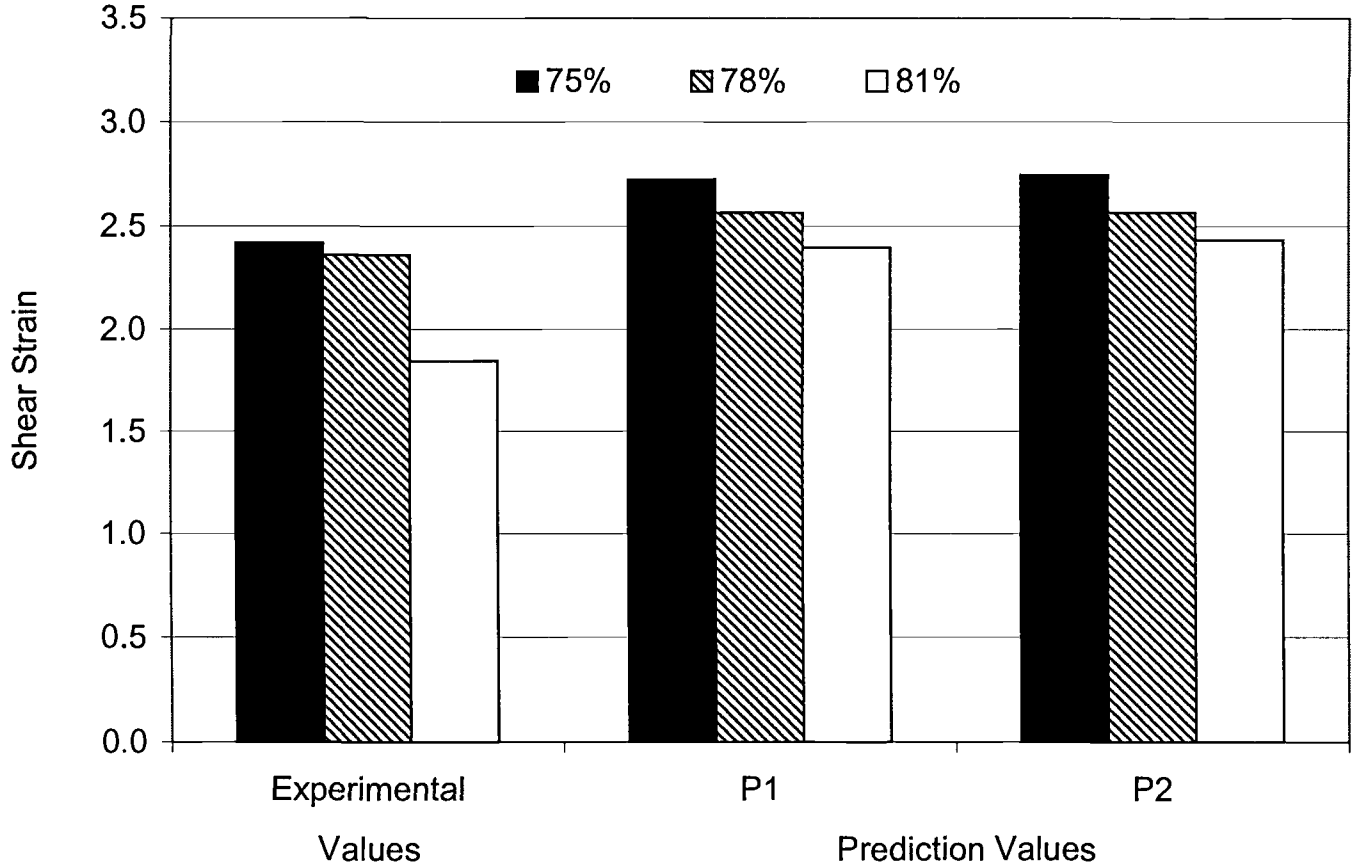

Figure 3.9. Experimental fracture strain values from torsion test and predicted values of fracture shear strain values using the best fitting models. P1: predicted fracture shear strain using the temperature sweep data at 25 °C. P2: predicted fracture shear strain using the temperature sweep data at 30 °C.

shear strain and dynamic data was low, the prediction of fracture shear strain was not as successful as for fracture shear stress (Figure 3.9). It was noted that the relationship between the dynamic rheological measurements at the sol state and the fracture measurements at the gel state could be used for surimi gel strength (shear stress) prediction, but not for deformation (shear strain) prediction.

3.5. CONCLUSIONS

Moisture content had a significant effect on the rheological properties of Pacific whiting surimi. Fracture shear stress and strain values of gels decreased as moisture concentration increased in the studied range (75 - 81%). Dynamic rheological measurements resulted in a good correlation with the fracture shear stress data. Our study showed that dynamic rheological measurements could be successfully used as a rapid test for early gel quality assessment.

3.6. ACKNOWLEDGEMENT

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CHAPTER 4

RHEOLOGICAL AND ENDOTHERMIC PROPERTIES OF PACIFIC WHITING PROTEINS AS AFFECTED BY THE PROCESSING PARAMETERS

O. ESTURK, J.W. PARK, and B.Y. KIM

4.1. ABSTRACT

Effects of pH and salt concentration on rheological and endothermic properties of Pacific whiting surimi were investigated. As pH increased, fracture shear stress and strain values increased, whereas lightness values (L*) decreased. Storage modulus (G') followed a trend similar to fracture shear stress. A linear relationship ($r^2 = 0.89$) was found between G' and fracture stress values as a function of pH. Increasing salt concentration up to 1% increased fracture shear stress and strain values, but further increase had a negative effect. The relationship between dynamic rheological data and fracture shear stress was not linear, indicating that salt concentration cannot be used as an index for estimating final gel quality. The transition temperatures obtained from temperature sweep measurements of whiting surimi at different salt concentrations showed similar peak temperatures as DSC thermograms.

Key words: whiting, rheology, pH, salt, DSC

4.2. INTRODUCTION

Heat-induced gelation is an important functional property of surimi that makes it a valuable food ingredient (Lanier 2000). Heat-induced muscle protein gels can hold a large amount of water and other food components under appropriate conditions (Kocher and Foegeding 1993). Conformational changes in myofibrillar protein structure due to partial unfolding is the reason for increased sensitivity to heat denaturation.

The pH is one of the most important factors affecting protein solubility (Turgeon and others 1992; Monahan and others 1995), which in return affects the gelation properties of fish myofibrillar proteins. Surimi has a neutral pH, and like most proteins the net charge of fish proteins is negative at pH 7 (Damodaran 1996). Increasing pH beyond the isoelectric point (pI) increases the net negative charge. These net negative charges affect the conformational state (unfolding) due to electrostatic repulsion (Biswas and Siddhartha 1995), thus providing more binding sites for water (Damodaran 1996), and increasing solubility.

Partial solubilization of myofibrillar proteins is responsible for obtaining many of the desired functional properties (Stone and Stanley 1992; Niwa 1992), such as gel formation. Partial solubilization can be achieved using relatively high salt concentrations (0.3 - 0.6 M NaCl) (Suziki 1981; Lee 1984, 1986), which makes salt an important part of surimi formulations. Solubilized myofibrillar proteins subsequently form an organized three dimensional gel network upon heating

(Lanier 1986; Niwa 1992). Addition of salt reduces the heat stability of proteins and, in return, initiates gelation at lower temperatures (Lanier 1986).

Gel strength is a major factor in determining quality and functional properties of surimi. Consequently, desired functional properties of surimi could be obtained by manipulating processing conditions based on the rheological concept. Borderias and others (1985) hypothesized that changes in the apparent viscosity of muscle homogenates were related to changes in actomyosin. Our previous study (Esturk and others 2003a) demonstrated that there was a significant relationship between surimi gel strength measured through a fracture analysis and dynamic rheological analysis. This relationship developed based on varied moisture content could be used for early determination of the fracture gel quality of surimi. Further studies, considering various factors involved in processing nature, however, are needed to find feasible methods to predict the gel quality in an early stage.

Our objectives were to investigate the effects of pH and salt concentration on both fracture and non-fracture rheological properties of Pacific whiting surimi, and to determine whether these processing parameters can be used as a guide for estimating final product quality.

4.3. MATERIALS AND METHODS

Various grades of frozen Pacific whiting surimi containing 4% sugar, 4% sorbitol, and 0.3% sodium phosphate as cryoprotectants were obtained from Pacific Surimi (Warrenton, OR). Surimi used in the study was about 3-6 mo old. Each sample (1 kg/block) was vacuum-packed and stored at –30 ° C until tested.

4.3.1. Gel preparation

Surimi gel preparation and testing were done as described by Park and others (1994). Beef plasma proteins (BPP) were added at 1.5% as a protease inhibitor. Moisture concentration was adjusted to 78% for all surimi gel formulations (AOAC 1990). Samples were prepared at various pH (6.0-7.5) and salt concentrations (0.0 - 3.0%, w/w). A small portion (100 g) was saved for dynamic rheological testing, while the remaining was cooked in stainless steel tubes.

The pH was measured using a portable pH Meter (Model HI 9025C, Whatman Labsales, Hillsboro, OR). The target pH was achieved using 1N HCl or 1N NaOH during subsequent mixing. After reaching the target pH, the paste was chopped until reaching a final temperature of 5 °C.

4.3.2. Non-fracture and fracture gel measurements

Dynamic tests were conducted by the method of Esturk and others (2003a) using a Bohlin CS-50 Rheometer (Bohlin Instruments Inc., East Brunswick, NJ). All dynamic tests (temperature sweep, frequency sweep, and stress sweep) were performed using a cone and plate (CP 4/40, 4° angle, 40mm dia).

The torsion test for fracture gel analysis was performed (Park 1994) using a Hamann Torsion Gelometer (Gel Consultants, Raleigh, NC). Fracture shear stress and fracture shear strain of the surimi gel were determined.

4.3.3. Differential scanning calorimetry (DSC)

Thermal denaturation of whiting surimi samples was studied in a Setaram micro DSCIII (Lyon, France). Samples were accurately weighed in a standard stainless steel vessel (approximately 500 mg), and distilled water was used as the reference material. The scanning temperature range was 20-90 °C at a heating rate of 1 °C/min.

4.3.4. Color analysis

A Minolta Chroma Meter CR-300 (Minolta Camera Co., Ltd., Ramsey, NJ) was used to measure the color of surimi gels. CIE L*, a*, and b* values were obtained to measure the degree of lightness, red-green hue, and yellow-blue hue.

4.3.5. Statistical analysis

SAS statistical package was used to analyze data (version 8, SAS Inc, Cary, NC). Least significant difference (LSD) was used to determine significant differences between mean values at $p < 0.05$.

Linear regression analyses were used to investigate the relationship between the dynamic rheology properties of the surimi paste and fracture properties of the surimi gel at various pH levels and salt concentrations. The general linear regression model used was:

$$Y = \beta_o + \beta_1 X_1 \ldots \qquad (4.1)$$

where Y represents the selected dependent variable, X independent variable, β_o intercept term, and β_i the regression coefficient of the independent variable.

4.4. RESULTS AND DISCUSSION

4.4.1. Effect of pH on fracture, non-fracture properties, and color

The effect of pH on fracture shear stress and strain was significant ($p < 0.05$). Fracture shear stress values of Pacific whiting surimi gels increased linearly as pH increased within the experimental range (pH 6.0 - 7.5) (Figure 4.1). Fracture shear stress values were 15.40, 23.45, 28.19, and 31.32 kPa for pH 6.0, 6.5, 7.0, and 7.5, respectively.

The effect of pH on protein solubility was likely to have a direct relationship with the increased gel strength. As the pH increased beyond the pI, the net charge increased negatively, resulting in increased solubility thorough electrostatic repulsion (Biswas and Siddhartha 1995) and providing more binding sites for water (Damodaran 1996).

Fracture shear strain values increased up to pH 7.0. At pH 7.5, fracture shear strain values decreased slightly, but remained insignificant ($p>0.05$) (Figure 4.1). The linearity of shear strain as a function of pH, however, was not high enough to be used as a quality index.

Chung and others (1993) reported that shear stress and strain values of Pacific whiting surimi gel increased steadily when the pH increased from 5 to 10. Punch force and depth also increased significantly in Alaska pollock surimi when

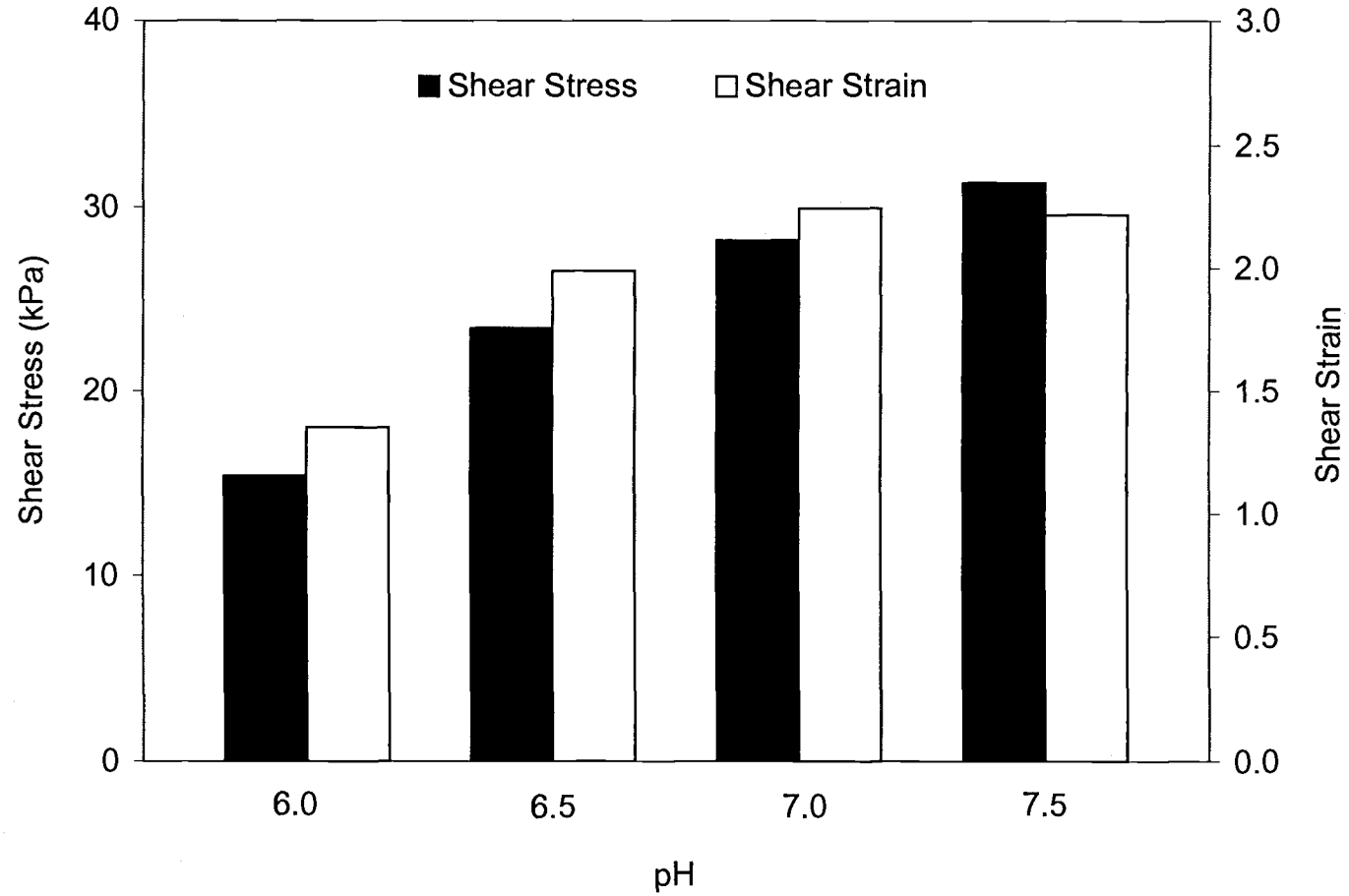

Figure 4.1. Fracture shear stress and fracture shear strain values of Pacific whiting surimi as affected by pH.

the pH increased from 6 to 7 (Akahane and Shimizu 1989). Funatsu and Arai (1991) reported that Alaska pollock prepared with 2.5% salt showed maximum breaking strength and breaking strain values at pH 7.35 and between pH 6.5 - 7.6, respectively. There was a dramatic decrease in gel strength of Alaska pollock surimi when the pH was lowered from 6.75 to 3.77 (Nishino and others 1991). Low pH had a strong effect on the proteolytic activity of Pacific whiting, resulting in reduced gel strength (Morrissey and others 1993, 1995).

The profiles of the storage modulus during temperature sweep were similar regardless of pH, except the pH 6.0 sample. While G' of the pH 6.0 sample had the lowest values at 29 and 41 °C, similar peaks were observed at 31 and 46 °C for pH 7, and 7.5 samples, respectively (Figure 4.2). Esturk and others (2003a) suggested that decreases in G' around 30 and 45 °C probably related to protein denaturation (unfolding).

At the beginning of the temperature sweep, the highest G' values were observed at pH 7 and 7.5, while the lowest value was observed at pH 6.0 (Figure 4.2). However, at temperatures above 50 °C, G' of all surimi paste increased rapidly until 75 °C, and showed the same pattern at all pH values.

Proteins are more stable against denaturation at the isoelectric point (pI) than at any other pH (Damodaran 1996). When the pH gets closer to the pI, a condition that favors aggregation, the net charge of a protein molecule decreases and in return protein-protein interactions increase through ionic linkages (Kinsella

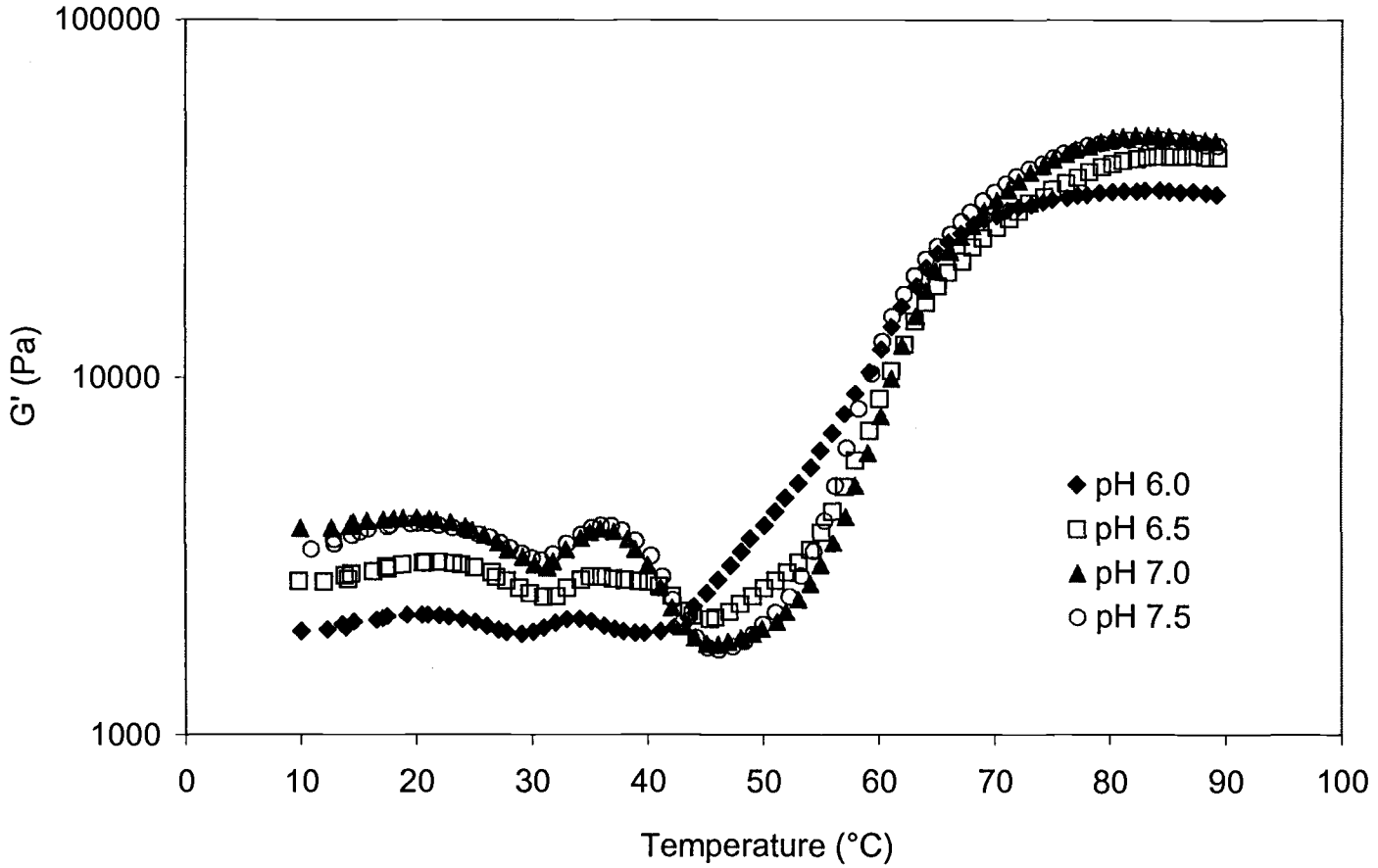

Figure 4.2. Change of storage modulus (G') of Pacific whiting surimi as affected by pH.

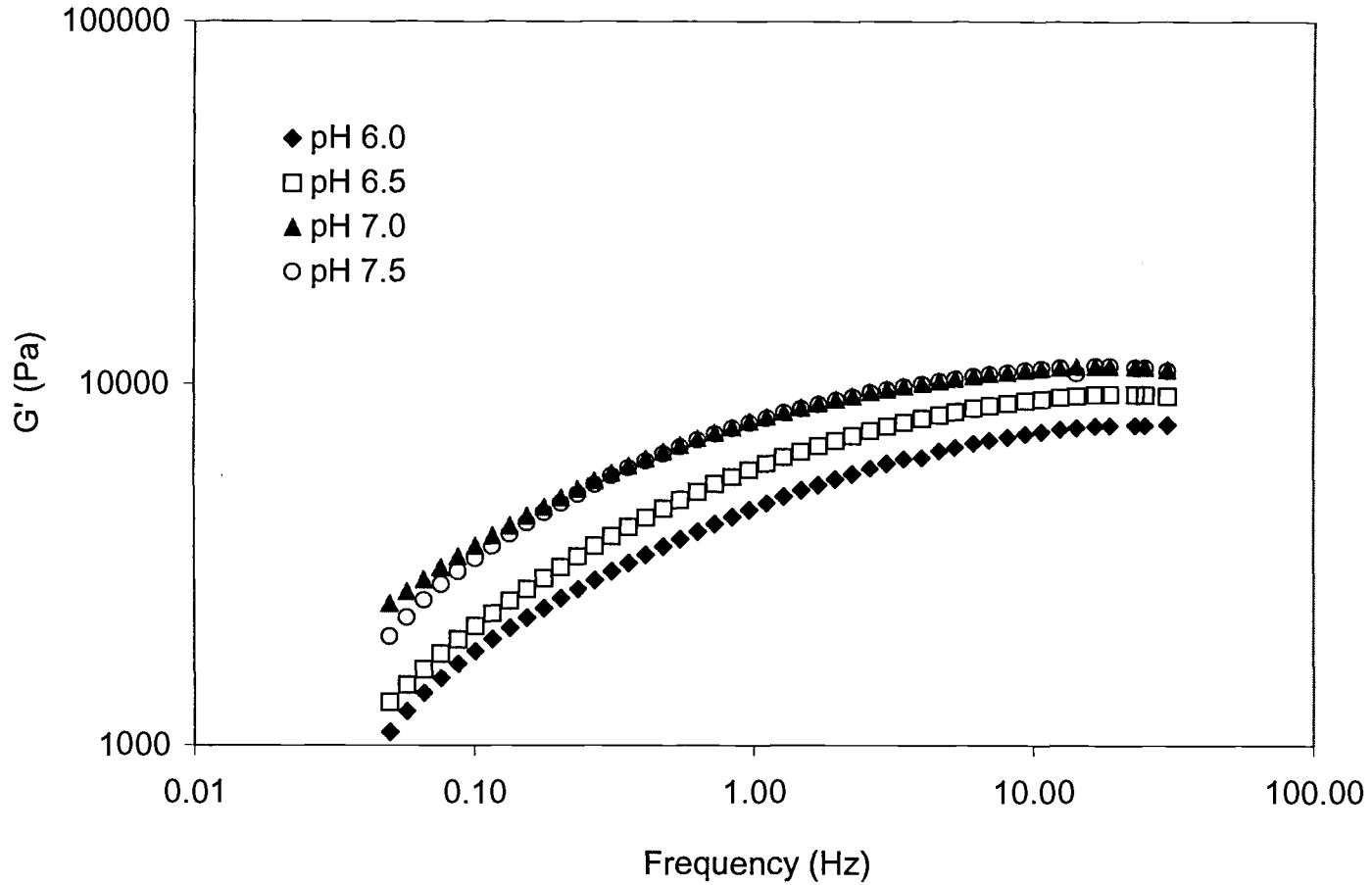

Figure 4.3. Effect of frequency on G' of Pacific whiting surimi as affected by pH.

1984). As a result of increased protein-protein association, protein solubility decreases and protein stability increases. This could be the reason for the different patterns of G' at pH 6.0 since it is closer to the pI than the other studied pH levels.

A similar pattern was observed for G' from the frequency sweep data (Figure 4.3). The pH 6.0 sample had the lowest G' value followed by pH 6.5. Storage modulus (G') at pH 7.0 and 7.5 was highest and almost identical to each other.

There was a significant effect of pH on the lightness (L*) of surimi gel samples ($p < 0.05$). Lightness decreased from 86.22 to 78.86 as pH increased from 6.0 to 7.5. The increased lightness and reduced shear stress of surimi gels at lower pH may have resulted from a coagulum type of aggregation, which yields more opaque and brittle gels (Wicker and others 1986). However, at higher pH values, the gels were more translucent and elastic, indicating that denaturation led to a more orderly gel formation.

4.4.2. Effect of salt concentration on fracture properties

The lowest fracture shear stress (hardness) and fracture shear strain (cohesiveness) values were obtained when salt was not incorporated during comminution (Figure 4.4). As salt concentration increased up to 1.0%, the fracture

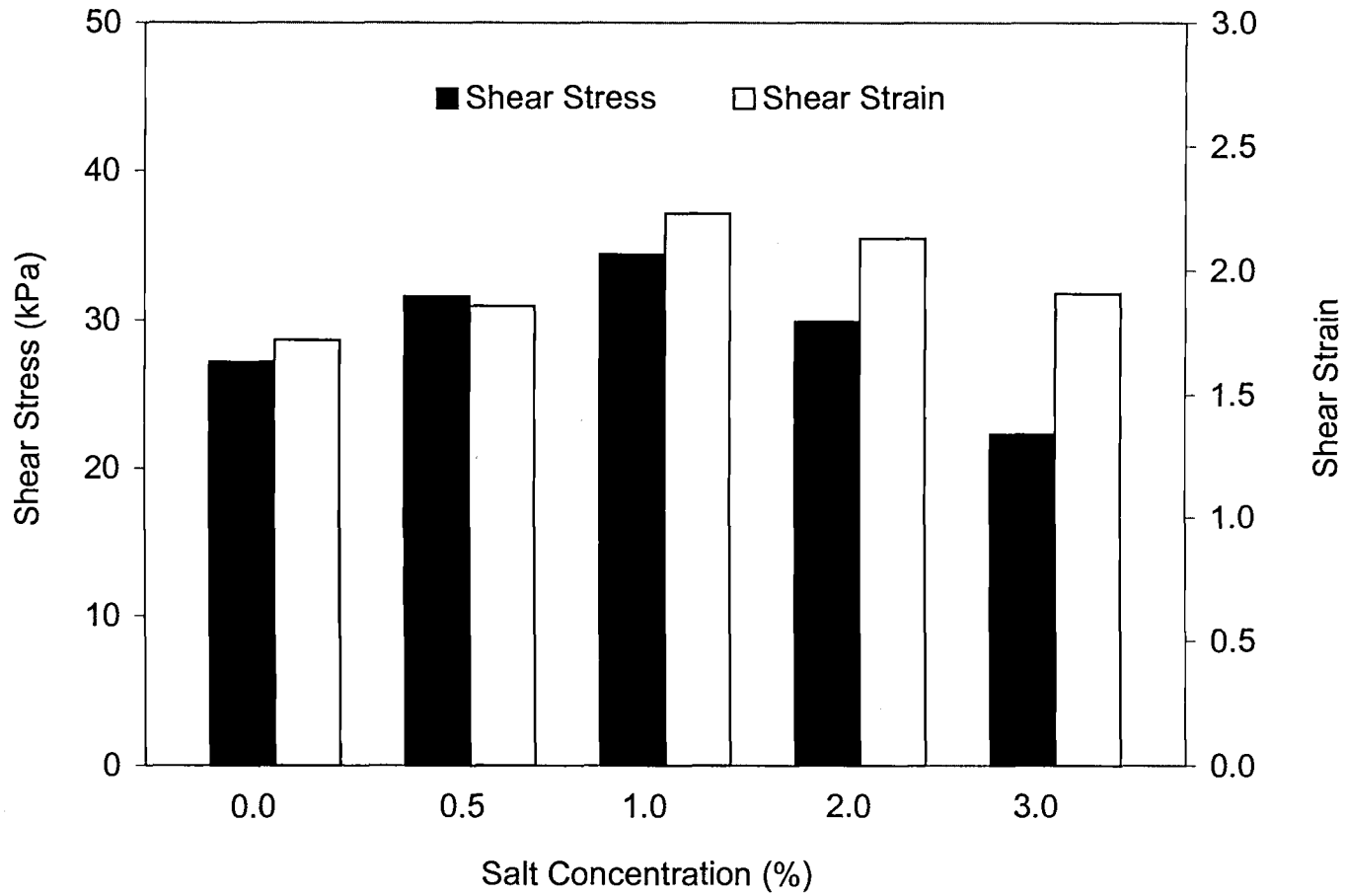

Figure 4.4. Fracture shear stress and fracture shear strain values of Pacific whiting surimi as affected by salt concentration.

shear stress value increased. However, shear stress values decreased at 2 and 3% salt. The decreased gel strength and cohesiveness of surimi gel at higher salt concentrations were probably due to partial denaturation of myofibrillar proteins during frozen storage. The fish myofibrillar proteins might have partially denatured prior to the addition of salt. When higher concentration (3%) of salt added , salt might cause salting-out effect resulting in some protein aggregation. Fracture shear strain followed a similar trend to fracture shear stress.

Partial solubilization of myofibrillar proteins is necessary to obtain the desired functional properties (Niwa 1992). This can be achieved using relatively high salt concentrations (0.3 - 0.6 M NaCl) (Suziki 1981). The main function of salt is to help solubilize the myofibrillar proteins, thus improving gel formation.

Stefansson and Hultin (1994) reported that almost all of the proteins from cod muscle were soluble when the ionic strength was near zero. Solubility reached a minimum level when the ionic strength was 0.025M. A salting-in effect was seen at higher ionic strengths, which had a 50% solubility at 0.75 M within the range of 0.15-1M. At higher salt concentrations (>1 M), however, salts have ion specific effects that affects the structural stability of proteins (Damodaran 1996).

Addition of excessive salt induces protein-protein interactions, resulting in precipitation, a phenomenon known as salting-out. As the protein unfolds, anionic groups in several amino acids are exposed. These groups can interact with water or other compounds such as Na^{+}. This cation-anion interaction between the carboxyl

group of an amino acid and Na^+, reduces the electrostatic repulsion, thus reducing unfolding.

Cl^- ions also play an important role in protein solubility, where Cl^- ions selectively neutralize positively charged sites on the protein molecule, shifting the pI to a lower value, resulting in increased solubility at the existing pH (Hamm 1986). Suzuki (1981) reported that high salt concentration (> 1 M) reduces the gel forming ability of myofibrillar proteins, probably due to the decreased solubility of myosin caused by the salting out effect.

Park (1995a) reported that Alaska pollock gels prepared with 0.5% salt had the lowest shear stress and strain values. While stress values of higher salt content samples were similar, strain values of 2% salt gels were highest and reducing salt concentration resulted in decreased strain values. Chung and others (1993) stated that increasing pH increased whiting surimi gel strength regardless of salt concentration, and the stress values of the lower salt surimi samples (0.0 and 0.9%) were significantly greater than that of high salt surimi samples (1.7 and 2.5%) when the pH was 7 or higher. Gomez-Guillen and others (1997) studied the influence of salt on surimi gels with different protein additives and found that the highest gel strength was obtained with gels containing soy protein and 2.5% salt.

4.4.3. Effect of salt concentration on dynamic properties and DSC thermogram patterns

As salt concentration increased, storage modulus (G') consistently decreased at all temperatures (Figure 4.5). Fish proteins prepared with 3% salt, in particular, had a significantly lower G' value at all temperatures and showed the biggest depth at 40 °C. This was probably due to increased solubility of whiting proteins. Increasing salt concentration improved protein solubilization resulting in reduced the viscosity of surimi paste, which was evidenced by higher phase angle values. As shown in our previous study (Esturk and others 2003a) and pH studies, a linear relationship was observed between the storage modulus (G') and fracture shear stress values. However, samples with various salt concentrations did not follow this trend. Increasing salt concentration constantly reduced G' values. Therefore, salt concentration may not be used as a quality index for whiting surimi.

The transition temperatures obtained from the storage modulus (G') of whiting surimi with different salt concentrations (Figure 4.5) were similar to the peak transition temperatures (T_d) obtained from the DSC thermograms (Figure 4.6, and Table 4.1). This indicated that peaks obtained from the dynamic tests were related to protein unfolding. There were 4 endothermic peaks clearly distinguishable at low salt concentrations (0.0, 0.5%). However, the peaks observed around 35 and 40 °C were less noticeable and shifted to the lower temperatures at increased salt concentrations (2.0 or 3.0%) (Figure 4.6).

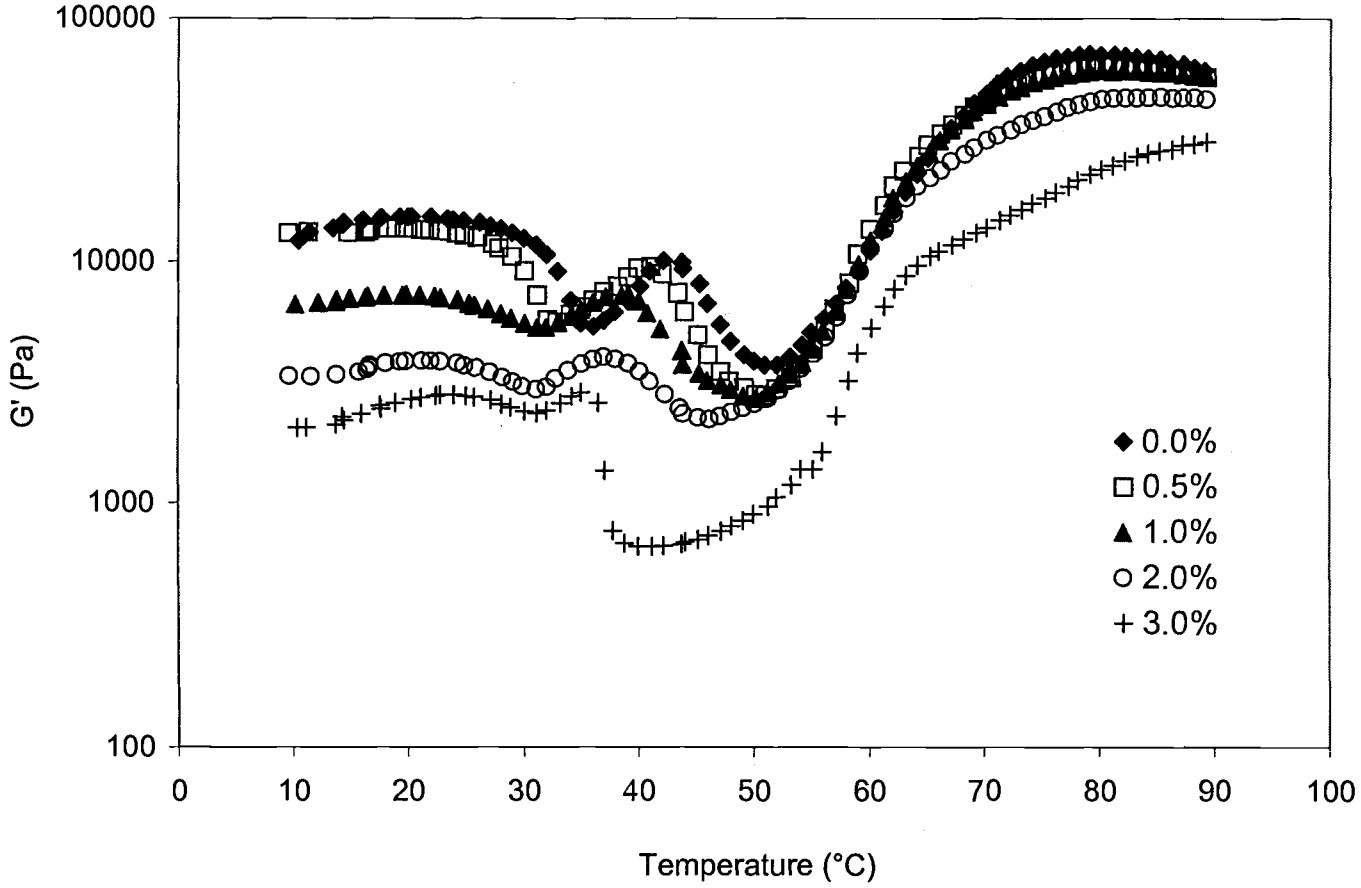

Figure 4.5. Change of storage modulus (G') of Pacific whiting surimi as affected by salt concentration.

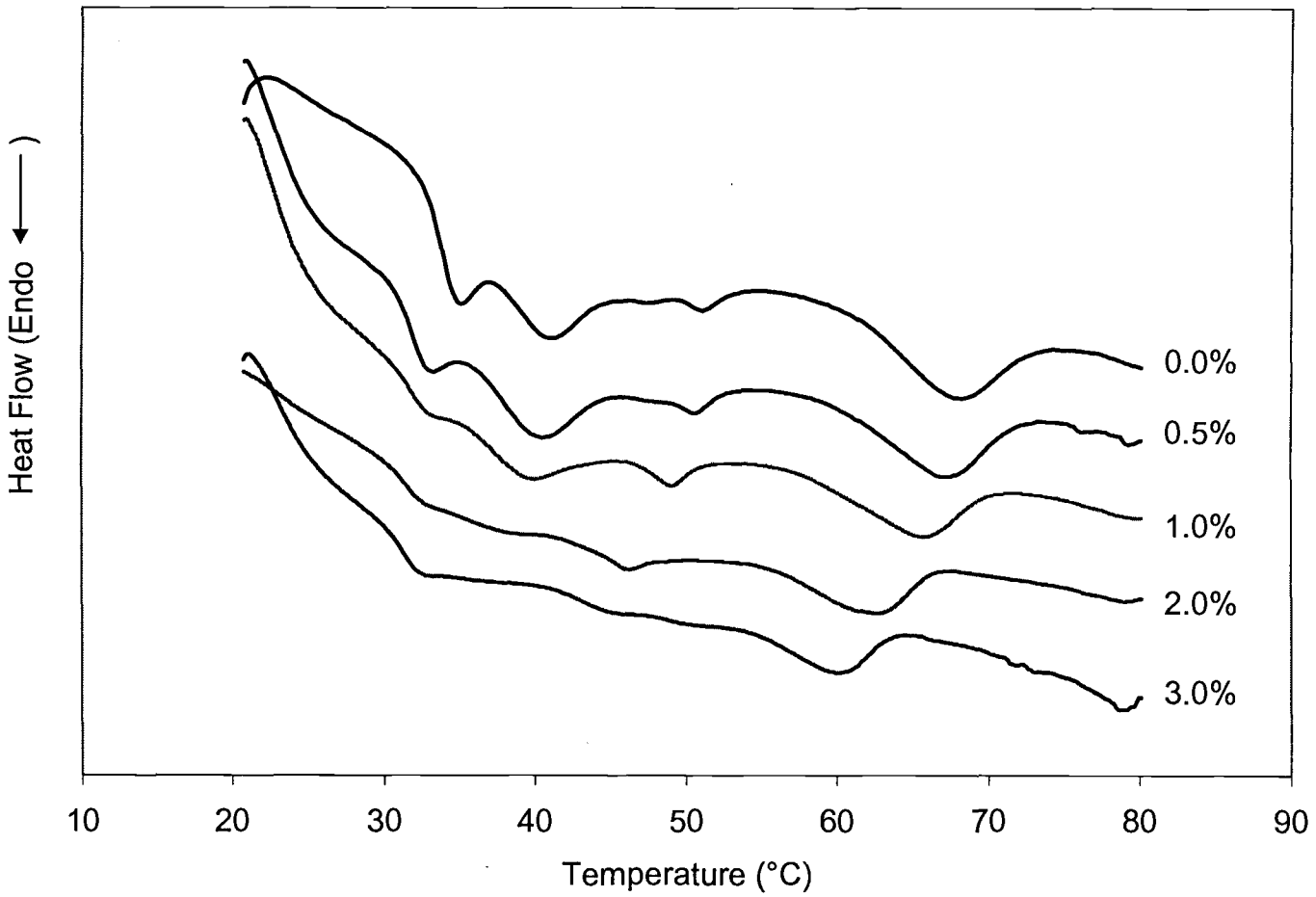

Figure 4.6. Changes in DSC thermogram of Pacific whiting surimi samples as affected by salt concentration.

Observed peak transition (denaturation) temperatures for the first peak, referring to the denaturation of myosin (Hasting and others 1985; Fernandez-Martin and others 1998), at 0.0, 0.5, and 1.0% salt concentrations were 35.4, 33.5 and 32.2 °C from DSC and 35.8, 33.2, and 31.9 °C from dynamic rheology measurements (temperature sweep), respectively (Table 4.1). The dynamic rheometer was not able to detect peaks observed at temperatures higher than 60 °C. DSC, however, seems to be more sensitive to thermal denaturation of various subunits of fish proteins.

Angsupanich and others (1999) and Fernandez-Martin and others (1998) reported four endothermic transition temperatures at 34.2 °, 43.8 °, 50.6 °, and 60.9 °C and at 33.5 °, 44.5 °, 51 ° and 74.7 °C for cod and blue whiting, respectively. They attributed the first three peaks for myosin and the last peak for actin. Therefore, the first three peaks in our study presumably attribute to myosin transition temperatures, and the fourth peak to actin transition temperature.

Dynamic and DSC data indicated that an increase in salt concentration shifted the transition (protein denaturation) temperatures to lower values (Figure 4.5 and 4.6). Salt concentration also affected enthalpies of heat. They were 0.3078, 0.2711, 0.2505, 0.1959, and 0.1411 J/g for 0.0, 0.5, 1.0, 2.0, and 3.0% salt concentrations, respectively. Similar results were reported for cod (Howell and others 1991), herring (Hasting and others 1985), shark (Chen 1995), and tilapia (Park and Lanier 1989).

Table 4.1. Temperature sweep (TS) and differential scanning calorimetry (DSC) peak temperatures of Pacific whiting surimi at various salt concentrations.

Salt Concentration (%, w/w)		Peak 1 (°C)	Peak 2 (°C)	Peak 3 (°C)	Peak 4 (°C)
0.0	DSC	35.4	41.2	51.1	68.8
	TS	35.8		51.0	
0.5	DSC	33.5	40.6	50.6	67.3
	TS	33.2		50.7	
1.0	DSC	32.2	40.1	49.1	65.8
	TS	31.9		50.2	
2.0	DSC			46.3	62.7
	TS	31.8		46.2	
3.0	DSC				60.3
	TS	31.2		41.2	

DSC: Differential Scanning Calorimetry (20-90 °C, 1.0 °C/min heating rate)
TS: Temperature Sweep (at 0.1Hz, 10-90 °C, 1.0 °C/min heating rate)

4.4.4. Linear regression model

There was a linear relationship between the storage modulus (G') and fracture shear stress values, and between pH and lightness (L*). These relationships were used to predict surimi gel strength and L*. However, samples with various salt concentrations did not have this linear relationship. Therefore, salt concentration data was not used in linear regression analyses.

Table 4.2 shows the results of the regression analysis parameters used to describe the relationship between fracture shear stress and temperature sweep data at 25 °C. Best fitting models for each temperature sweep data point within the range of 20-45 °C were determined using BIC and Cp statistics as described by Esturk and others (2003a). Multivariate linear regression analysis indicated that predictions were more accurate when the dynamic data output was within the range of 20-35 °C. The equations developed for temperature sweep data at 25 °C are:

$$\text{Fracture Shear Stress} = -29.5729 + 6.2893\ pH + 0.0063G' \qquad (4.2)$$

$$L^* = 112.862 - 4.6060\ pH \qquad (4.3)$$

where, pH is the pH of the sample, G' is the measured storage modulus value at 25 °C, and L* is the lightness value of the cooked gel. The relationship between fracture shear stress and pH was significant when a 95% confidence level was used for Eq. 4.2. However, for Eq. 4.3, a 90% confidence level was used to obtain a significant relationship between fracture shear stress and G', and L* and pH respectively.

As for model verification, experimental and prediction values of new surimi batches were compared using the equations developed using G' values at 25 °C. At pH 7.0, predicted shear stress and L* values were 29.04 kPa and 81.39 (Figure 4.7) using Eq. 4.2 and Eq. 4.3, respectively. The corresponding fracture shear stress and L* values from the experiment were 28.19 kPa and 80.54, respectively.

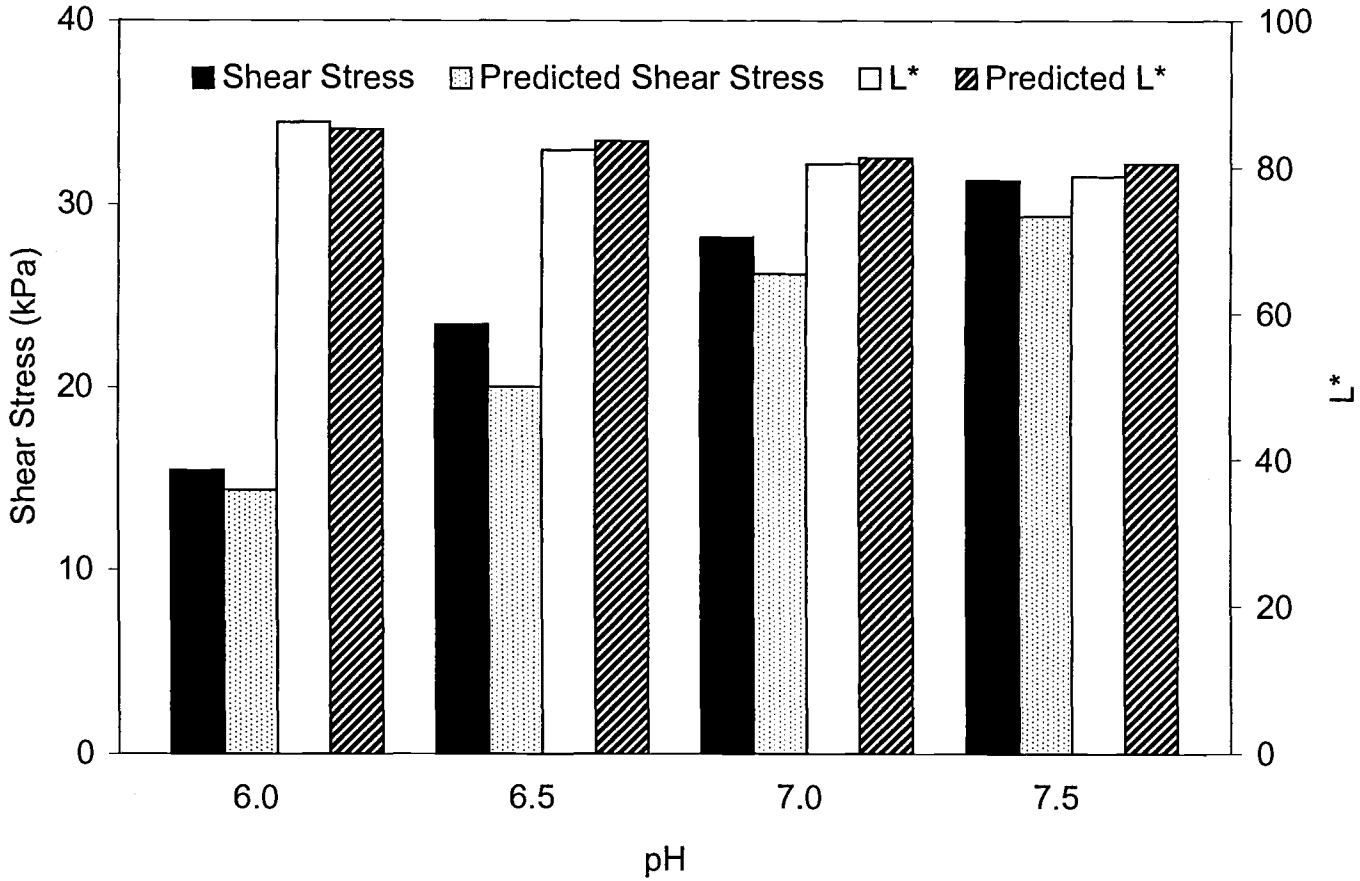

Figure 4.7. Experimental and predicted fracture shear stress and lightness (L*) values of Pacific whiting surimi as affected by pH.

Table 4.2. Regression analysis parameters for Eq. 4.2 and 4.3.

Parameters	**Eq. 4.2**	**Eq. 4.3**
SSE	118.38	34.9307
MSE	23.68	0.9192
Root MSE	1.79	0.9588
r^2	0.94	0.88
Adjusted r^2	0.93	0.88

SSE is the sum of squares due to error.
MSE is the mean squares of error.
Adjusted r^2: [(Total mean square - Residual mean square) / Total mean square]

However, the correlation between fracture shear strain and dynamic data was low. Therefore, prediction of fracture shear strain was not successful (data not shown). It was noted that the relationship between the dynamic rheological measurements at the sol state and the fracture measurements at the gel state could be used to predict surimi gel strength (shear stress) and L* (lightness).

4.5. CONCLUSION

A linear relationship was found between fracture shear stress and dynamic test data as a function of pH, and this relationship was used for the prediction of surimi gel strength. However, a similar relationship was not observed when salt concentration was used as a variable. Peak temperatures obtained from the temperature sweep test were very close to those obtained from DSC, indicating that peaks obtained from the dynamic tests were related to protein unfolding.

CHAPTER 5

THERMAL STABILITY OF FISH PROTEINS FROM VARIOUS SPECIES

O. ESTURK and J.W. PARK

5.1. ABSTRACT

Thermal stability of myofibrillar proteins from various fish species was compared at various setting conditions and chopping temperatures. The respective Ca-ATPase activity was also measured. Significant species effects were observed with regards to the optimum temperature for gel setting and chopping. While cold water fish species like Alaska pollock had the highest shear stress values when chopping temperatures were at 5 °C or lower, warm water fish species had higher fracture shear stress values when chopping temperatures were between 20 ° to 30 °C. SDS-PAGE demonstrated that the decreased intensity of myosin heavy chain (MHC) resulted in the formation of large molecular weight proteins due to polymerization, except for Pacific whiting. A first order reaction kinetics was observed for Ca-ATPase activity for all fish species.

Key words: Thermal stability, rheology, setting, SDS-PAGE

5.2. INTRODUCTION

Thermal stability of fish myosin has been studied by the thermodynamic analysis of myofibrillar ATPase activity and a relationship between thermal stability and the environmental habitat temperature has been reported (Johnston and others 1973, 1975; Hashimoto and others 1982; Tsuchimoto and others 1988). In addition, fish myosin from cold water species was determined to be more labile to thermal denaturation than warm water species.

Maintaining protein functionality is one of the most important goals in the production of surimi and surimi seafood. It is, therefore, of primary importance to understand the effect of processing parameters, such as chopping temperature, on protein stability. Based on the study conducted by Arai and others (1973), regarding the relationship between species and thermostability of actomyosin ATPase, Lee (1986) suggested that warm water fish species could tolerate higher washing water temperatures than cold water fish species without a significant reduction in protein functionality.

Unlike land animal protein, fish myofibrillar proteins form an elastic and translucent gel below 40 °C if sufficient time is given after properly comminuting with salt (Lanier and others 1982). This textural strengthening of salted surimi paste at low temperatures is known as setting (Lee and others 1997a). Polymerization of myosin through the formation of non-disulfide covalent crosslinks is reportedly responsible for the textural enhancement during setting

(Numakura and others 1987; Nishimoto and others 1987, 1988). This setting phenomena is catalyzed by a calcium-dependent endogenous transglutaminase (TGase) (Seki and others 1990; Kamath and others 1992).

Currently, the surimi and surimi seafood manufacturing industry has used only one principle concerning the effect of chopping temperature of surimi paste on gel strength: it is the colder the chopping temperature, the better the gel strength. Regardless of the species origin, the manufacturers have controlled their chopping temperature between 7-15 °C. However, if thermal stability of the fish species was taken into consideration during chopping, we hypothesized different gel texture values could be obtained.

Our objective was to investigate the thermal stability of fish myofibrillar proteins from various fish species as affected by various comminution and setting temperatures.

5.3. MATERIALS AND METHODS

Frozen Alaska pollock (AP), Pacific whiting (PW), big eye (BE), lizardfish (LF), and threadfin bream (TB) surimi were obtained from various manufacturers: AP from Trident Seafoods (Seattle, WA), PW from Pacific Surimi (Warrenton, OR), BE, LF, and TB from Andaman Surimi Industries (Bangkok, Thailand). The grade of surimi ranged from medium (A) to high (FA). All surimi were stored

frozen (- 18 °C) for 6 ± 3 mo. AP represented cold water species, while BE, LF, and TB represented warm water species. PW was selected as a species living between these two extreme temperatures. Each surimi was partitioned into 1 kg blocks, vacuum-packed, and stored at –30 ° C until tested.

5.3.1. Gel preparation

Surimi gel preparation and testing were done as described by Park and others (1994). Moisture concentration was adjusted to 78% (AOAC 1990), and 2% salt was added for all surimi gel formulations. Surimi paste temperature was maintained below 5 °C during chopping for the samples tested at various setting conditions (control, 5 °C/18 h, 25 °C/3 h, 40 °C/1 h, and 60°C/30 min) followed by cooking at 90 °C for 15 min.

As for the thermal stability of the surimi from various species, six different chopping temperatures (0, 5, 10, 20, 25, and 30 °C) were tested. 1.5% (w/w) beef plasma proteins (BPP) was added to PW samples as a protease inhibitor during chopping. At least ten samples per treatment were measured.

5.3.2. Non-fracture and fracture gel measurements

Dynamic tests were conducted by the method of Esturk and others (2003a) using a Bohlin Rheometer, CS-50 (Bohlin Instruments Inc., East Brunswick, NJ). All dynamic tests (temperature sweep, frequency sweep, and stress sweep) were performed using cone and plate (CP - 4/40).

The torsion test was performed (Esturk and others 2003a) using a Hamann Torsion Gelometer (Gel Consultants, Raleigh, NC) to determine the fracture shear stress (gel strength) and fracture shear strain (cohesiveness) of the gel.

5.3.3. Sodium dodecylsulfate polyacrylamide electrophoresis (SDS-PAGE)

Twenty seven mL of 5% (w/v) sodium dodecylsulfate (SDS) was added to 3 g of gel sample. The mixture was homogenized for 3 min at a speed of 2 using a Polytron (Brinkmann Instruments, Westburry, NY). The homogenates were incubated in a water bath (80 °C) for 30 min and centrifuged at 5,000 x g (Sorval, DuPont Co., Newton, CT) for 15 min at room temperature to remove insoluble debris. The protein concentration of the supernatants was determined by the Lowry method (Lowry and others 1951). Electrophoresis was performed according to the procedure of Laemmli (1970). Stacking gels and separating gels were prepared using 4% and 10 % (w/v) polyacrylamide, respectively. Separated proteins were

stained with 0.125% Coomassie brilliant blue R-250 (Bio-Rad, Richmond, CA) and destained in a solution containing 10% methanol and 10% acetic acid.

5.3.4. Ca-ATPase activities

Ca-ATPase activity was determined using the method of MacDonald and Lanier (1994) with slight modifications. Surimi was homogenized with 20 mM phosphate buffer (pH 7.0) at a 1:9 ratio and centrifuged at 5,000 x g for 30 min to remove cryoprotectants. Three grams of precipitate were homogenized with 50 mL of 0.6 M NaCl containing 20 mM Tris-HCl (pH 7.0) for 2 min. The homogenate was extracted for 3 h in a cold room (4 °C) and centrifuged at 10,000 x g for 30 min. The protein concentration was adjusted to 2.0 mg/mL for the measurement of Ca-ATPase activities. Samples were incubated at 5 °, 20 °, 25 °, 30 °, 40 °, and 50 °C for 0-24 h. Ca-ATPase activities were assayed in 5mM $CaCl_2$, 100mM KCl, 20 mM Tris-maleate (pH 7.0) and 1mM ATP, respectively. The reaction was stopped by adding 5 mL chilled 15% trichloroacetic acid.

The concentration of inorganic phosphate (C) released during incubation was determined according to the method of Arai (1974). The specific activity of Ca-ATPase was defined as micromoles of inorganic phosphate liberated per milligram of protein (μM Pi/mg protein/min) at the incubation temperature.

The first order reaction rate constant (K_D) for Ca-ATPase activity was calculated using the plot of *ln* C against time.

$$ln\left(\frac{C}{C_o}\right) = K_D t$$

where C_O and C are the ATPase activities before and after incubation, and the slope is equal to $-K_D$.

5.3.5. Statistical analysis

SAS statistical package was used to analyze data (version 8, SAS Inc, Cary, NC). Least significant difference (LSD) was used to determine significant differences between mean values at $p<0.05$.

5.4. RESULTS AND DISCUSSION

5.4.1. Effect of setting on gel fracture properties

Setting had a significant effect on the fracture shear stress values (Figure 5.1) of surimi samples ($p<0.05$). Setting temperatures up to 40 °C increased

fracture shear stress values significantly compared to the control group. Fracture shear stress reached a maximum at the optimum setting temperature, which varied depending on the fish species, and reached a minimum at 60 °C for all species tested. Optimum setting temperatures were approximately 5, 25, 40, 40, and 25 °C for AP, PW, BE, LF, and TB, respectively. Setting at 60 °C induced gel weakening for all fish species indicating all tested surimi contained proteolytic enzymes. Especially PW, which resulted in no measurable gels, appeared to have the highest concentration of proteolytic enzymes.

Similar to our findings, Lee and Park (1998) also reported that optimum setting temperatures were 5 °C for cold water species, such as pollock, and 25 °C for temperate water species, like whiting. Warm water fish species such as Atlantic croaker (Kamath and others 1992), tilapia (Yongsawatdigul and others 2000), and other surimi achieved maximum gel strength at 40 °C.

All of these results confirm the variability in optimum setting conditions among species. Calcium dependent endogenous TGase has been reported to be responsible for the unique setting ability of surimi (Seki and others 1990; Kamath and others 1992; Lee and others 1997b). Endogenous TGase initiates the crosslinking between the glutamyl and lysine [ε-(γ-glutamyl) lysine dipeptide crosslinks] residues in the protein molecules (Park 2000a). Imai and others (1996) also suggested that the content of nondisulfite crosslinks generally correlate well with gel strength. In addition, Araki and Seki (1993) investigated the reactivity of carp TGase in various fish species in terms of polymerization velocity of myosin

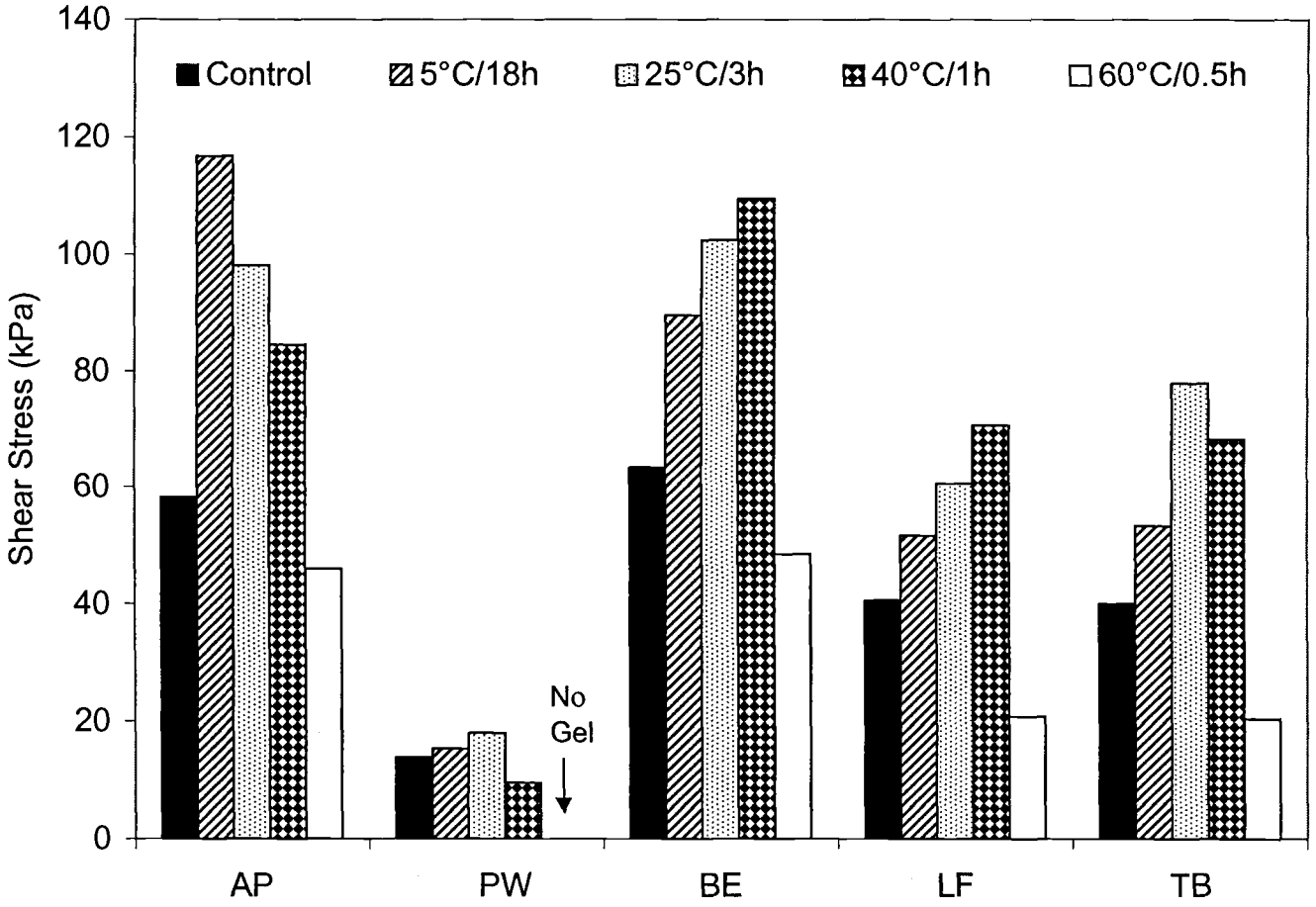

Figure 5.1. Fracture shear stress values of AP, PW, BE, LF and TB at various setting conditions. AP: Alaska pollock, PW: Pacific whiting, BE: big eye, LF: lizard fish, TB: threadfin bream.

heavy chain and reported that polymerization velocity varied significantly depending upon the fish species.

Setting temperature had less effect on fracture shear strain values of surimi gels except 60 °C/0.5h treatment (Figure 5.2). While addition of microbial transglutaminase increased gel strength with appropriate setting time and temperature (Seguro and others 1995; Lee and others 1997a, 1997b, 1997c), it had little effect on fracture shear strain (Lee and others 1997a).

Fracture shear stress values of Pacific whiting surimi, containing 1.5% BPP, were evaluated using surimi produced at different dates of processing and/or at different plants (Figure 5.3). The magnitude of shear stress values was different for different PW samples, but followed a similar trend. Setting increased fracture shear stress values and setting at 25 °C was more effective than at 5 °C or control (no setting) treatments.

Endogenous TGase is water soluble (Nowsad and others 1994) and TGase content in surimi can vary depending on the washing method used during surimi manufacturing. Natural TGase content also shows variation among different fish species and within individuals of the same fish species (Lanier 2000). Therefore, among other factors such as protein quality and Ca^{+2} content, variation in endogenous TGase activity in surimi could be a factor contributing to the differences within the same setting treatment.

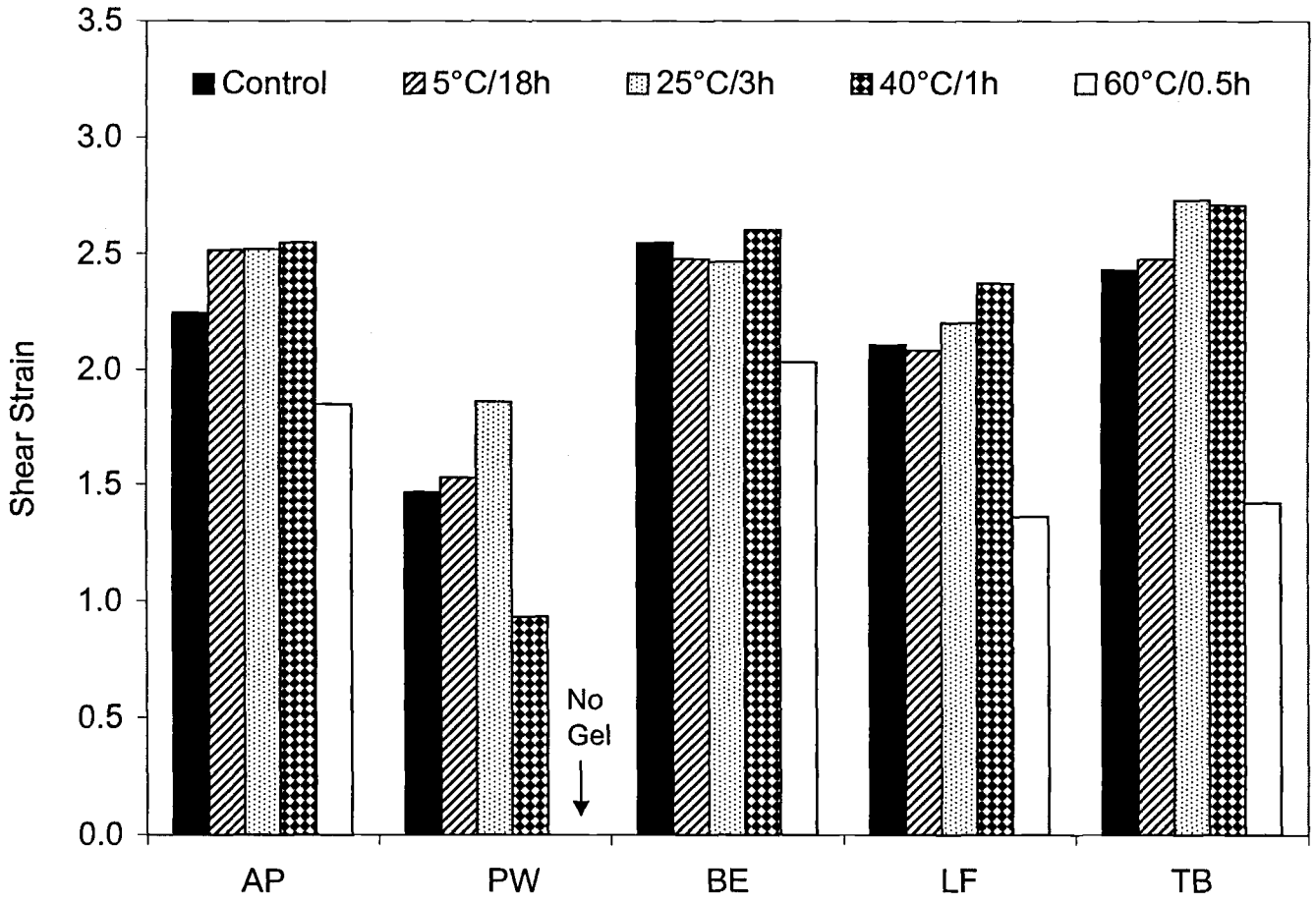

Figure 5.2. Fracture shear strain values of AP, PW, BE, LF and TB at various setting conditions. AP: Alaska pollock, PW: Pacific whiting, BE: big eye, LF: lizard fish, TB: threadfin bream.

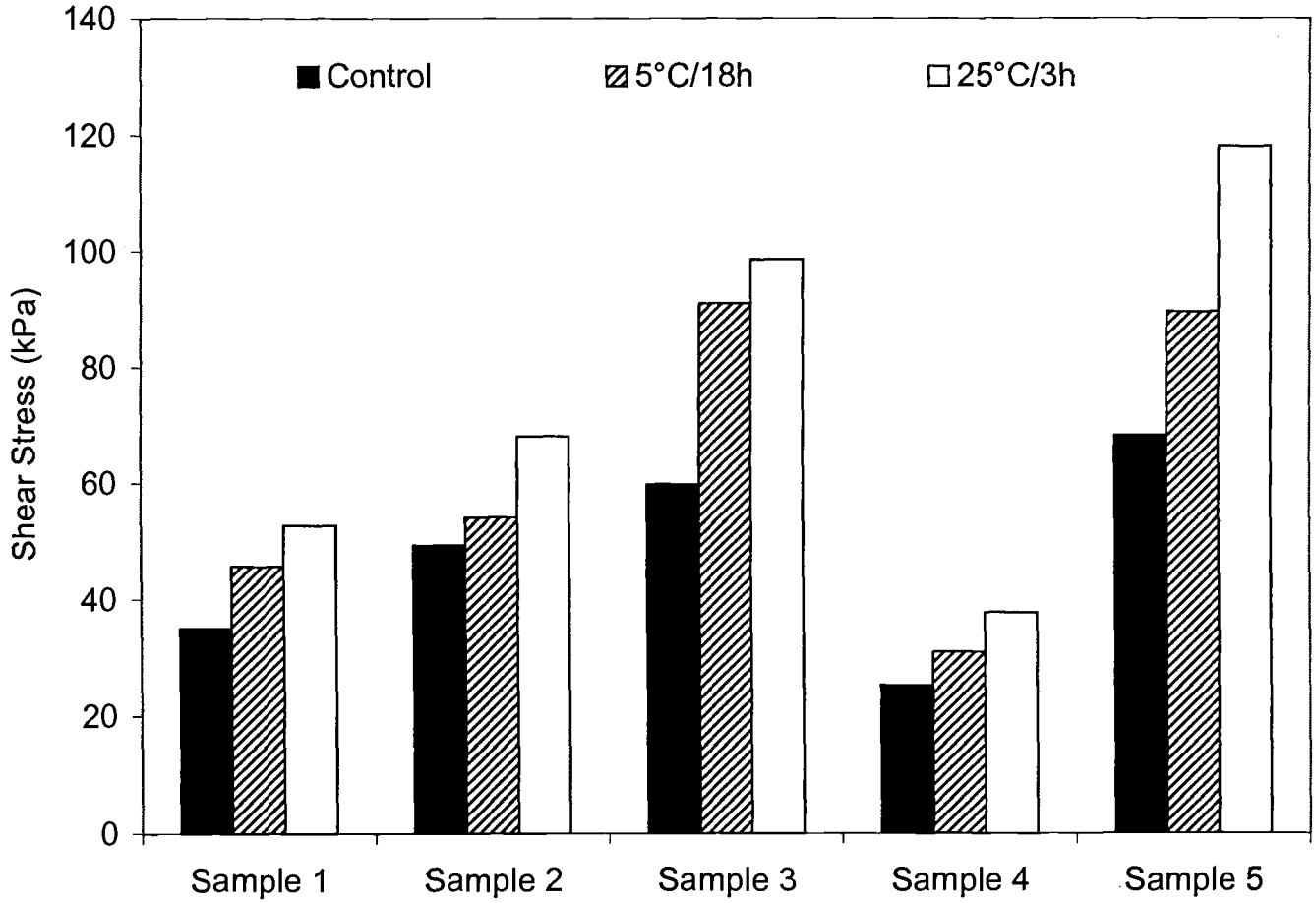

Figure 5.3. Fracture shear stress values of Pacific whiting surimi (containing 1.5% beef plasma proteins) produced at different plants and/or dates at various setting conditions.

5.4.2. SDS-PAGE

Protein patterns of AP, PW, BE, LF, and TB surimi at various setting temperatures are shown in 10% (w/v) polyacrylamide gel (Figure 5.4). Degradation of myosin heavy chain (MHC) was noted at 25 °C/3 h treatment. The maximum degradation of MHC occurred while setting at 25 ° and 40 °C for BE, LF, and TB. This was probably due to MHC polymerization since medium molecular weight (MMW) (66 < Mw <205 kDa) and low molecular weight (LMW) protein bands (<45 kDa), as a result of proteolytic degradation, did not appear.

At 40 °C setting for PW, the intensity of the MHC band decreased and resulted in MMW proteins. At a higher setting temperature (60 °C), MHC degradation occurred for all fish species. In addition to MHC degradation (appearance of MMW bands), however, TGase induced MHC polymerization was also observed (appearance of a thickening band at the top of SDS-PAGE gel) for AP, BE, LF and TB. TGase activity at higher setting temperatures (60 °C) was probably due to the presence of cryoprotectants in surimi. Sugar and sorbitol might have stabilized TGase against denaturation at higher temperatures. The intensity of the PW actin band (45 kDa) was reduced, and LMW protein bands appeared at 60 °C setting. These LMW protein bands, however, were not observed for the other fish species (Figure 5.4).. An and others (1994b) reported that cathepsin L, a cysteine proteinase, was responsible for the proteolytic degradation in whiting surimi, which exhibited maximum activity at 55 °C and pH5.5.

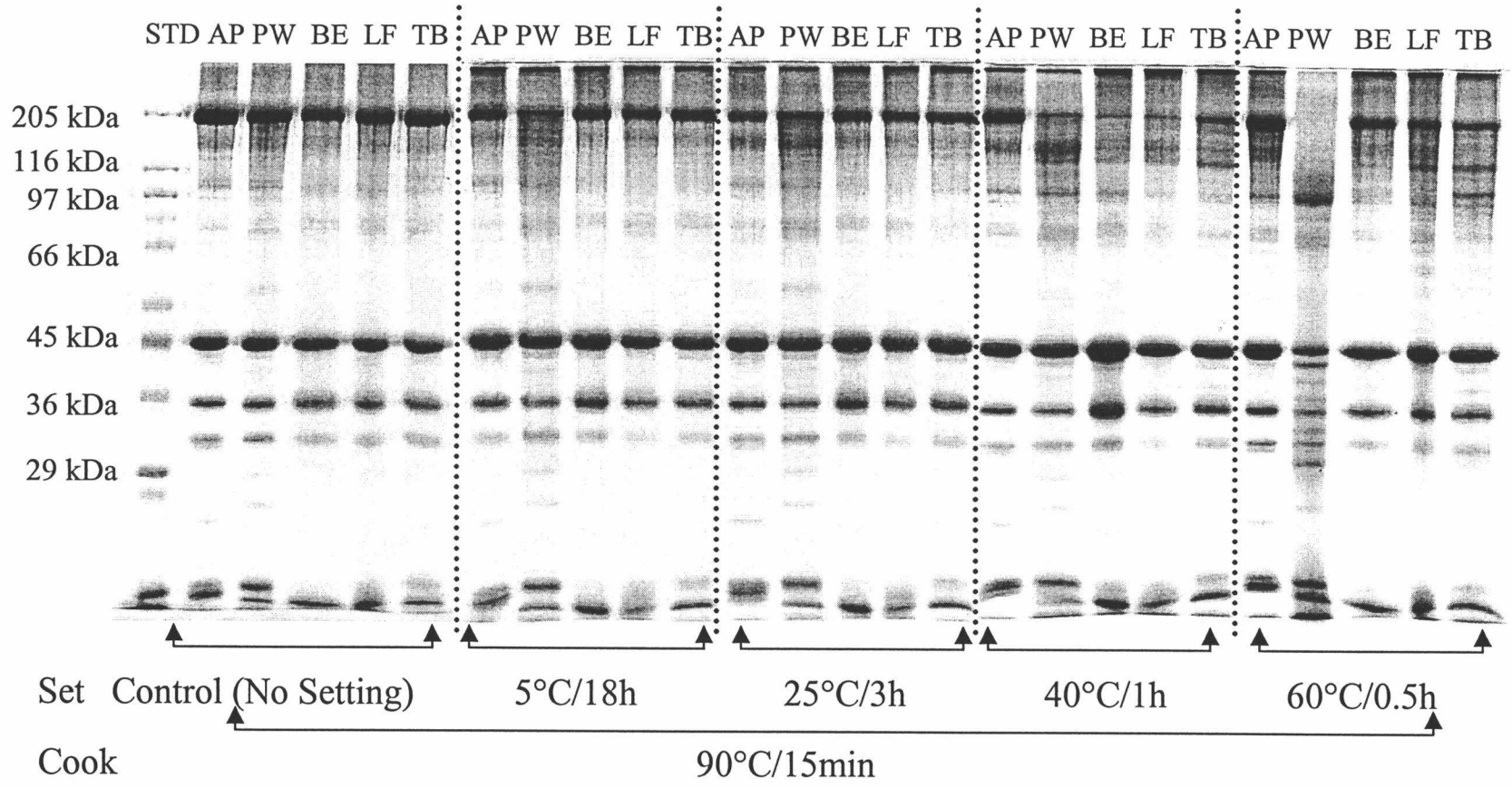

Figure 5.4. SDS-PAGE patterns of AP, PW, BE, LF and TB at various setting conditions in 10 % polyacrylamide gel. STD: wide range molecular weight standard, AP: Alaska pollock, PW: Pacific whiting, BE: big eye, LF: lizard fish, TB: threadfin bream.

While the major changes resulting from setting were seen in the density of MHC band, the actin bands appeared to remain unchanged. Numakura and others (1985) showed that a slight reduction occurred in troponin-T content in AP surimi during 30 °C setting. The reduction in MHC and no significant decrease in other myofibrillar protein intensities suggested that protein polymers were predominantly derived from the polymerization of MHC. At 40 °C (optimum setting temperature for BE and LF), MMW proteins were also observed in addition to HMW proteins, indicating that proteolysis and polymerization were occurring at the same time.

5.4.3. Effect of chopping temperature on fracture properties

The effect of chopping temperature on fracture shear stress and fracture shear strain was very significant with regard to the thermal stability of various fish proteins. AP had the highest gel strength near 0 °C, but gel strength steadily decreased as chopping temperature increased. PW, containing 1.5% BPP, showed maximum gel strength near 5 °C. Although increasing chopping temperature reduced the gel strength of PW surimi gels, the temperature effect was not as pronounced as for AP. BE and LF exhibited an identical trend, where gel strength increased when the final chopping temperature reached 20 °C, and further increases in chopping temperature decreased gel strength. TB reached its maximum gel strength at around 25 °C (Figure 5.5). Temperature rise beyond the optimum

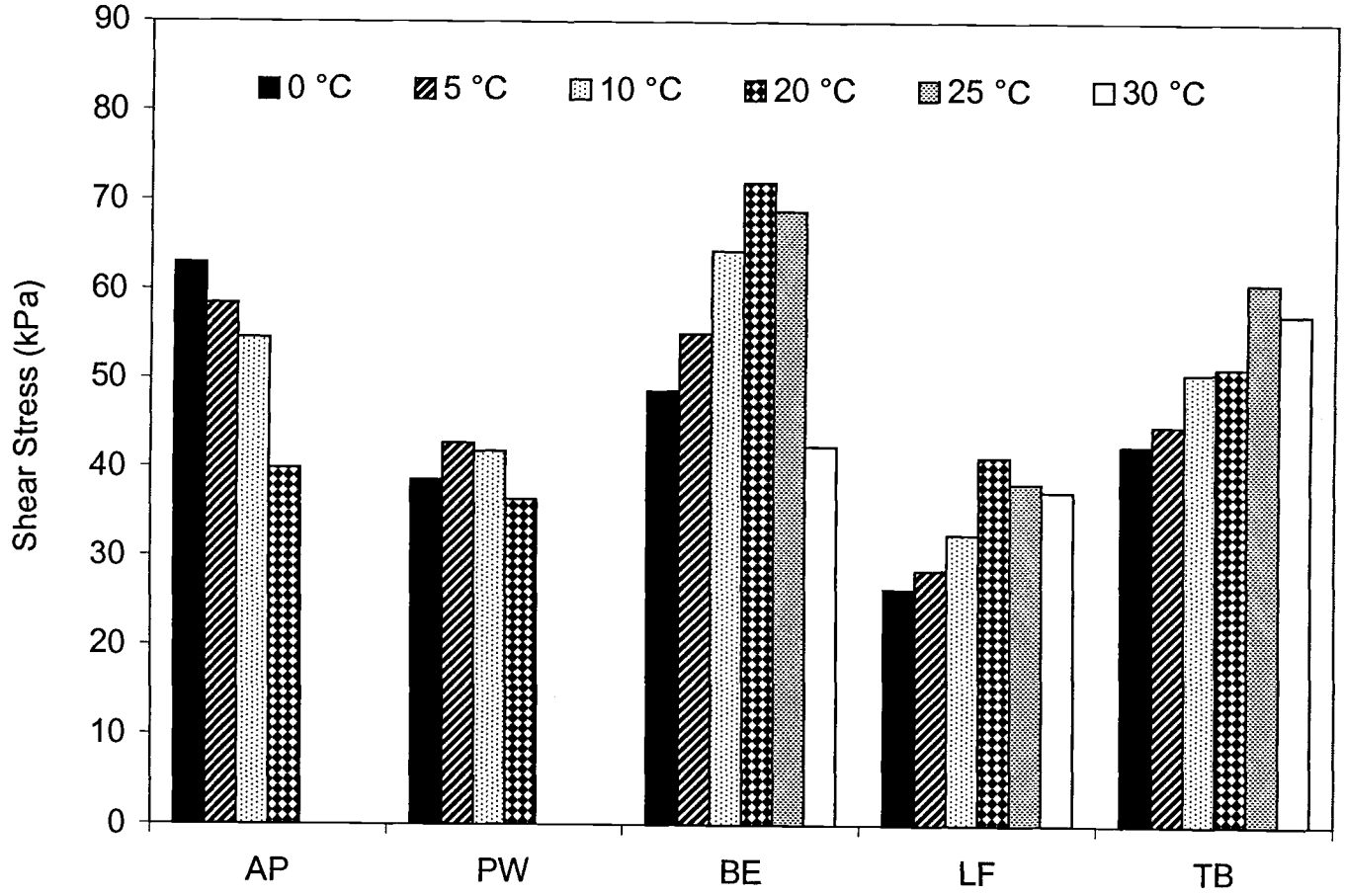

Figure 5.5. Fracture shear stress values of AP, PW (containing 1.5% beef plasma proteins), BE, LF and TB at various chopping temperatures. AP: Alaska pollock, PW: Pacific whiting, BE: big eye, LF: lizard fish, TB: threadfin bream.

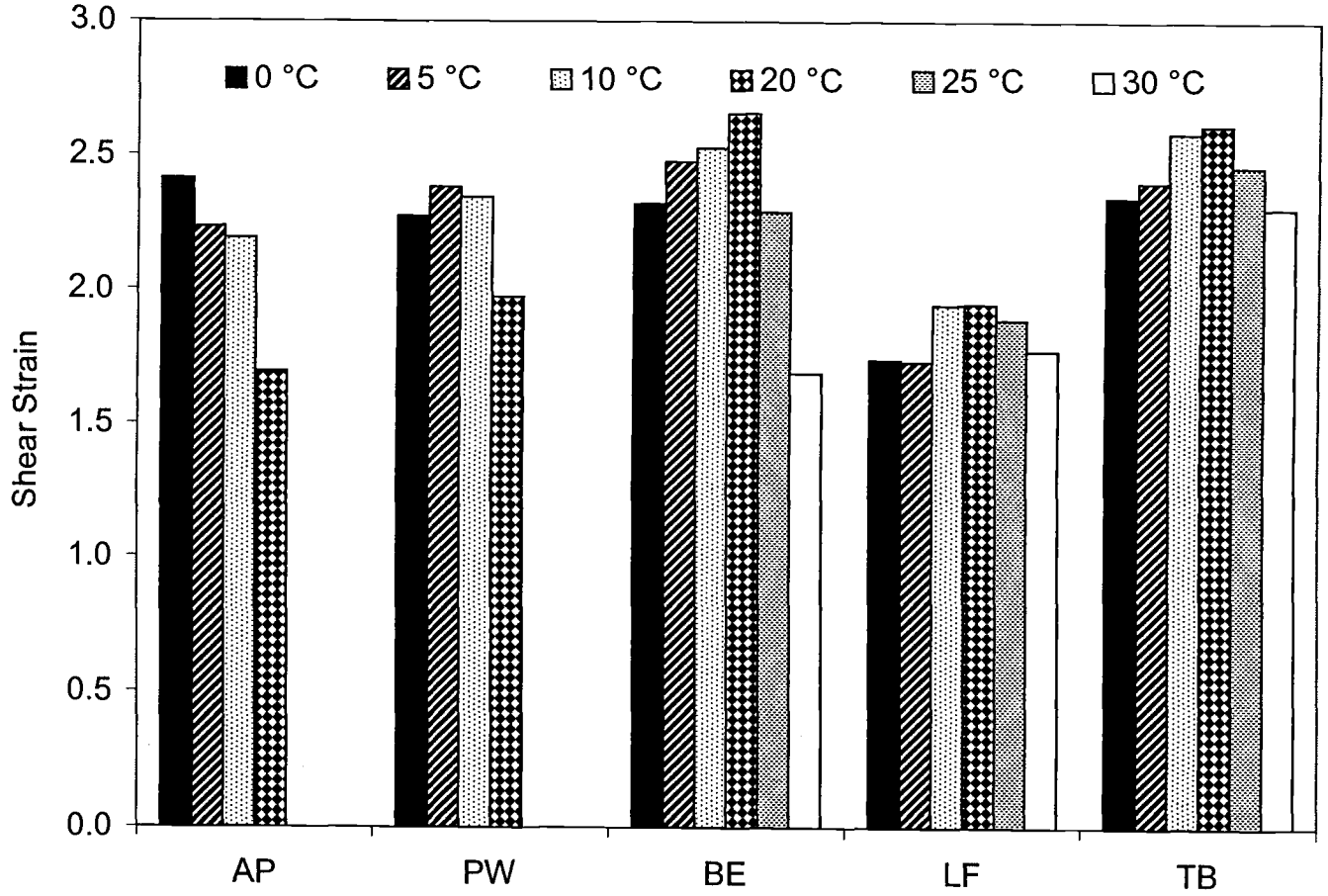

Figure 5.6. Fracture shear strain values of AP, PW (containing 1.5% beef plasma proteins), BE, LF and TB at various chopping temperatures. AP: Alaska pollock, PW: Pacific whiting, BE: big eye, LF: lizard fish, TB: threadfin bream.

chopping temperature might have caused excessive protein denaturation and, therefore, reduced the gel forming ability.

Shear strain values followed a similar trend to shear stress values (Figure 5.6). For all fish species maximum shear stain was observed when the maximum shear stress was obtained except for TB. TB had its highest shear strain value at 20 °C compared to 25°C for maximum shear stress.

Our observations indicated that a strong relationship exists among fish habitat temperature, optimum chopping temperature, and protein functionality. While cold water fish species, such as AP, had their maximum gel strength and cohesiveness at lower temperatures (0 °C), warm water fish species (BE, LF, and TB), exhibited maximum gel strength and cohesiveness at higher temperatures (20-25 °C). However PW performed the best between 5 to 10 °C, which is relevant to its habitat temperature.

Douglas-Shwartz and Lee (1988) reported a similar trend for Alaska pollock and red hake, which found the optimum chopping temperatures were 4° and 12 °C, respectively. Park (1997) compared the effects of temperature during chopping on shear strain and shear stress values of Alaska pollock surimi gels. According to this study, chopping temperatures between 0-5 °C provided maximum gel strength, but gel strength decreased at temperatures above 5 °C

5.4.4. Effect of chopping temperature on non-fracture properties

BE surimi prepared at various chopping temperatures were subjected to temperature sweep and the storage modulus (G') was obtained (Figure 5.7). The magnitude of G' among the pastes prepared using the various chopping temperatures was different within the range of 10 ° - 60 °C. G' values of BE surimi chopped at 20 °C or higher were not significantly different from each other and were greater than samples prepared at lower temperatures. Dynamic rheological measurements agreed with the gel strength from the torsion test. This suggested that the G' of surimi reflected the gel structure network as affected by chopping temperature. While warm water fish species (BE, LF and TB) exhibited the highest storage modulus values when samples were chopped at 20 °C or higher, AP and PW exhibited a higher storage modulus value when the samples were chopped at temperatures lower than 20 °C (data not shown).

5.4.5. Ca-ATPase

Ca-ATPase activity was time dependent and its activity decreased linearly with time within the first 30 min of incubation at different incubation temperatures. Ca-ATPase activity of all fish species followed first order reaction kinetics within this time period (Table 5.1).

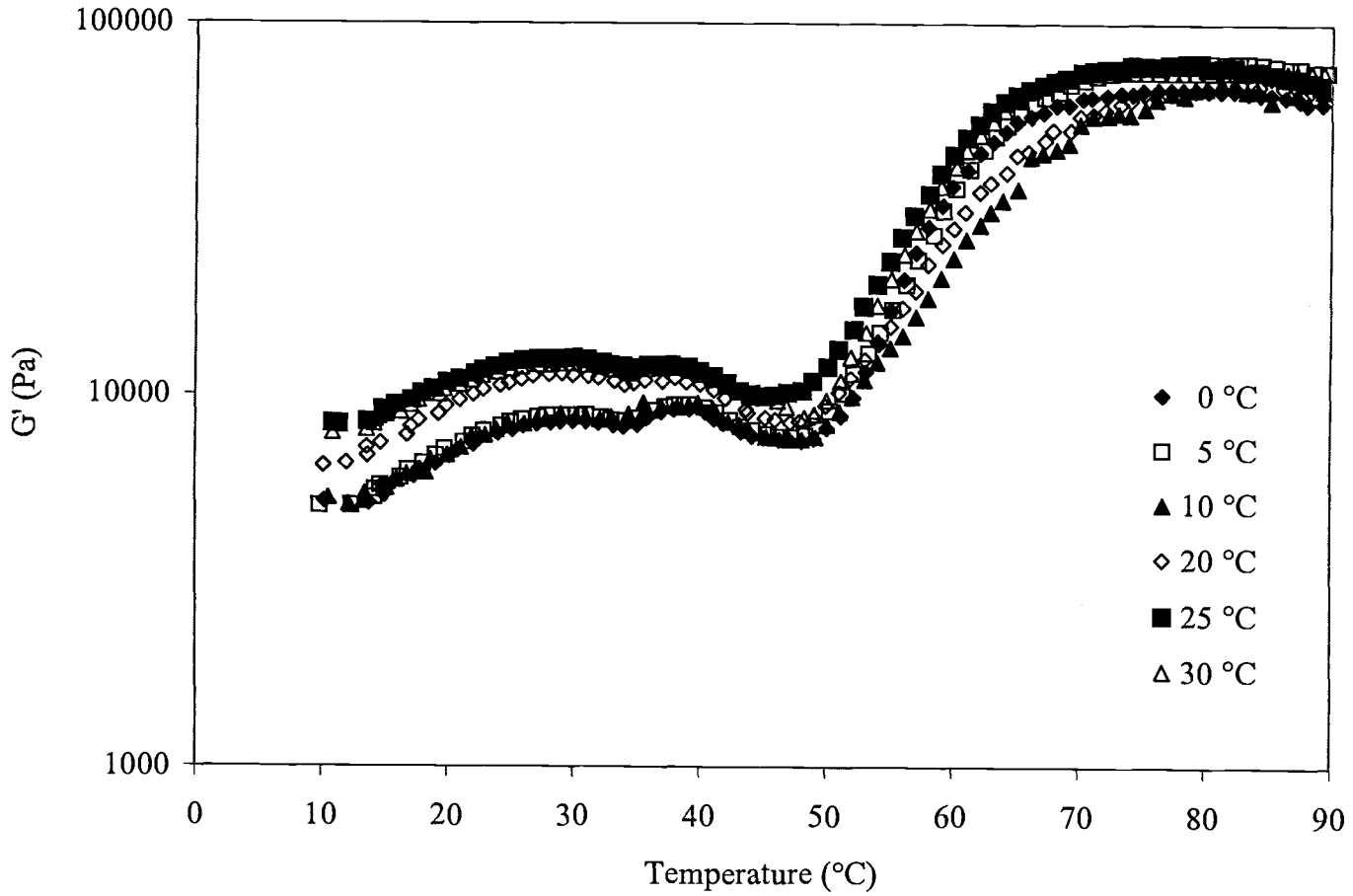

Figure 5.7. Effect of various chopping temperatures on storage modulus (G') of big eye surimi.

Habitat temperature had a marked influence on K_D values (Table 5.1). Cold water species (AP) and temperate water fish species (PW) had higher K_D values at the tested incubation temperatures than warm water fish species (BE, LF, and TB). Similar results were reported for the effect of habitat temperature (Johnston and others 1973; Arai and others 1973; Hashimoto and others 1982, Tsuchimoto and others 1988), suggesting that there was a strong relationship between the thermal stability of fish myofibrillar proteins and the environmental temperature at which the fish lives. Slopes of K_D against temperature were smaller for AP and PW than for LF, BE, and TB suggesting that the Ca-ATPase activity of cold or temperate water fish species was less affected by sample incubation temperature than warm water fish species.

Tsuchimoto and others (1988) studied the thermal stability of various fish species at 25 °C and found that K_D values ($x10^{-6}/s$) of AP, shortfin LF, and cutlassfish were 551.63, 161.3, and 155.9, respectively. Saeki and others (1986) studied the effect of $CaCl_2$ on the thermal stability of AP and reported that addition of $CaCl_2$ increased T_D several times. Type of cryoprotectant used in surimi also affected CA-ATPase activity. Besides fish habitat temperature, sample composition should also be taken into consideration for ATPase activity measurements (MacDonald and Lanier 1994).

There was a species effect with extended incubation time. AP, PW, LF, BE, and TB showed maximum activity at 20 °, 25 °, 30 °, 30 °, and 30 °C,

Table 5.1. Reaction rate constants (K_D) and r^2 values of Alaska pollock, Pacific whiting, big eye, lizard fish, and threadfin bream at various incubation temperatures.

Fish Species	Incubation Temperature (°C)	K_D ($x10^{-6}$/s)	r^2
Alaska pollock	5	542.40	0.9473
	20	637.72	0.9840
	25	863.99	0.9460
	30	918.11	0.9518
	40	1067.50	0.9370
Pacific whiting	5	412.21	0.9778
	20	628.64	0.9622
	25	734.87	0.9287
	30	824.57	0.8947
	40	931.15	0.9341
Big eye	5	204.63	0.9852
	20	428.11	0.9628
	25	569.86	0.9965
	30	617.67	0.954
	40	717.04	0.9697
Lizardfish	5	253.31	0.9796
	20	470.60	0.9846
	25	539.51	0.9908
	30	753.90	0.9889
	40	797.31	0.9627
Threadfin bream	5	175.81	0.9898
	20	353.19	0.9835
	25	457.12	0.9888
	30	577.69	0.9762
	40	685.05	0.9764

CHAPTER 6

DEGRADATION AND AGGREGATION BEHAVIOURS OF FISH PROTEINS WITH RESPECT TO THE THERMAL STABILITY OF VARIOUS SPECIES

O. ESTURK and J.W. PARK

6.1. ABSTRACT

Proteolytic degradation and thermal aggregation behaviors of various fish species were compared. Proteolytic enzyme activity increased linearly with incubation time when the test was conducted at the respective optimum temperature up to 240 min. SDS-PAGE analysis showed that myosin heavy chain was the major protein targeted by proteolytic enzymes. For all tested fish species, a 0.5 °C/min heating rate induced more aggregation followed by 1 °C/min, and then 2 °C/min. There was a species effect with respect to the temperature where proteins started to aggregate, as evidenced by turbidity. Peak temperatures obtained from temperature sweep tests were very similar to those obtained from DSC, indicating that peaks obtained from the dynamic tests were related to protein unfolding.

Key words: degradation, aggregation, rheology, DSC, SDS-PAGE

6.2. INTRODUCTION

Maintaining functionality of myofibrillar proteins is of prime importance for muscle foods. Better understanding of the thermal stability of fish proteins with respect to proteolytic enzymes and temperature sensitivity would help to optimize surimi and surimi seafood manufacturing, as well as provide a manufacturing short cut to maximize fish protein functionality.

Alaska pollock is the major fishery resource used for surimi production. In recent years, however, other species such as, Pacific whiting, big eye, lizardfish, and threadfin bream, have also been introduced into surimi production. Esturk and Park (2003) reported that the functionality of fish myofibrillar proteins was species dependent and processing guidelines for utilization should be specifically determined for each particular species.

Serine proteases are responsible for textural breakdown of threadfin bream (Toyohara and Shimizu 1988), and lizardfish (Suwansakornkul and others 1993). Cathepsin L, a cysteine protease, hydrolyzes muscle proteins causing severe textural degradation of Pacific whiting surimi (An and others 1994b, Morrissey and others 1993). Proteolytic degradation of myofibrillar components, consequently, presents a technical problem for the better utilization of those fish species.

Thermal denaturation is a prerequisite for the heat-induced gelation of food proteins. Denaturation and aggregation are the two major steps in heat-induced gelation. When aggregation is slow, with respect to denaturation, heat-denatured

proteins align in an orderly fashion to form a fine gel network resulting in more elastic gels (Hermansson 1979). Although the effect of heating rate on the gelation properties of myofibrillar proteins has been well established (Foegeding and others 1986; Camou and others 1989; Yongsawatdigul and Park 1999), the effect of heating rate with respect to the thermal stability of fish protein has not been documented.

Our objectives were 1) to investigate the degradation behaviors of fish myofibrillar proteins from various fish species and 2) to determine the influence of heating rates on the denaturation and aggregation behaviors of fish myofibrillar proteins.

6.3. MATERIALS AND METHODS

6.3.1. Sample preparation

Frozen Alaska pollock (AP), Pacific whiting (PW), big eye (BE), lizardfish (LF), and threadfin bream (TB) surimi were obtained from various manufacturers: AP from Trident Seafoods (Seattle, WA), PW from Pacific Surimi (Warrenton, OR), BE, LF, and TB from Andaman Surimi Industries (Bangkok, Thailand). All surimi were stored frozen (- 18 °C) for 6 ± 3 mo. The grade of surimi ranged from medium (A) to high (FA). AP represented cold water species, while BE, LF, and

TB represented warm water species. PW was selected as a species living between these two extreme temperatures. Each surimi was partitioned into 1 kg blocks, vacuum packed, and stored at –30 ° C until tested.

Surimi was homogenized with 20 mM phosphate buffer (pH 7.0) at a 1:9 ratio and centrifuged at 5,000 x g for 30 min to remove cryoprotectants and other soluble materials. All tests were conducted using the recovered crude actomyosin precipitate.

6.3.2. Autolysis

The optimum temperature for proteolysis was determined by conducting autolysis at 25 °, 30 °, 40 °, 50 °, 55 °, 60 °, 65 °, 70 °, and 75 °C for 90 min. Three grams of fish myofibrillar proteins were incubated at the optimum autolysis temperature for 0, 30, 60, 120, 180, and 240 min. Autolysis was stopped by adding 27 mL of 5% cold trichloroacetic acid (TCA) solution. The mixture was incubated at 4 °C for 15 min and centrifuged at 6,000 x g for 15 min. The TCA soluble proteins were analyzed for the oligopeptide content using Lowry's assay (Lowry and others 1951) and expressed as μmol/mL of tyrosine released (An and others 1994b). Sample blanks were kept on ice and analyzed to correct for oligopeptide present in the fish muscle.

6.3.3. Sodium dodecylsulfate polyacrylamide electrophoresis (SDS-PAGE)

SDS-PAGE samples were prepared as described by Esturk and Park (2003). Retention of crude actomyosin, which was incubated at the optimum autolysis temperature and sampled at various time intervals, was investigated using SDS-PAGE as described by Laemmli (1970). Stacking gels and separating gels were prepared at 4% and 10 % (w/v) polyacrylamide, respectively. The amount of protein loaded on the polyacrylamide gel was 50μg/lane. The separated proteins were stained with 0.125% Coomassie brilliant blue R-250 (Bio-Rad, Richmond, CA) and destained in a solution containing 10% methanol and 10% acetic acid.

6.3.4. Turbidity

Three grams of washed surimi were homogenized with 50 mL of 0.6 M KCl containing 20 mM Tris-HCl (pH 7.0) for 2 min. The homogenates were extracted for 3 h in a cold room (4 °C) and centrifuged at 10,000 x g for 30 min. Supernatant was diluted with 20 mM $NaHCO_3$ solution (1:9) and centrifuged at 10,000 x g for 30 min. Crude actomyosin (precipitate) was diluted to 0.5 mg/mL using 0.6 M KCl, 20 mM This-HCl buffer, pH 7.0, and heated from 10 ° to 80 °C at various heating rates (0.5 °, 1 ° or 2 °C/min). A programmable water bath (Neslab, Model RTE 100LP, Portsmouth, NH) was connected to a UV-VIS spectrophotometer (UV-2401 PC, Shimadzu Scientific Instruments Inc., Baltimore, MD) to heat the

samples. Protein solution was placed into a quartz cell (light path length 10 mm). Protein aggregation was determined by measuring absorbance at 320 nm (Xiong 1992).

6.3.5. Dynamic and thermal analysis

Dynamic tests were conducted by the method of Esturk and others (2003a) using a Bohlin Rheometer, CS-50 (Bohlin Instruments Inc., East Brunswick, NJ). All dynamic tests (temperature sweep, frequency sweep, and stress sweep) were performed using a cone and plate (CP - 4/40) probe.

Thermal denaturation of surimi samples using differential scanning calorimetry (DSC) was studied in a Setaram micro DSC III system (Lyon, FR) as described by Esturk and others (2003b). The scanning temperature ranged between 20 and 90 °C at a 0.5 ° or 1.0 °C/min heating rate.

6.4. RESULTS AND DISCUSSION

6.4.1. Autolysis

LF and PW did not show signs of proteolytic degradation until the temperature reached >25 °C. BE and TB required higher temperatures for proteolysis, and there was no significant rise in proteolytic activity until the temperature reached >40 °C (Figure 6.1). However proteolytic activity of AP remained very low, regardless of temperature. The autolytic activity for all species gradually increased and reached a maximum at 55 °, 65 °, 60 °, 55 °, and 65 °C for AP, BE, LF, PW, and TB, respectively.

Proteolytic activity increased linearly with incubation time at the optimum temperature for proteolysis throughout the time-course experiment (Figure 6.2). PW had the highest proteolytic activity, followed by LF, BE, TB, and AP, in descending order. Similar trends were reported for tilapia surimi (Yongsawatdigul and others 2000) and Pacific whiting (An and others 1994b).

Fish myofibrillar proteins undergo textural softening upon slow heating due to endogenous heat stable protease enzymes, which primarily hydrolyze myosin (An and others 1994a). An and others (1994b) reported that cathepsin L, a cysteine protease, was responsible for the textural degradation of whiting surimi. The enzyme, which has a molecular weight of 28.8 kDa (Seymour and others 1994), exhibited maximum activity at 55 °C and pH 5.5.

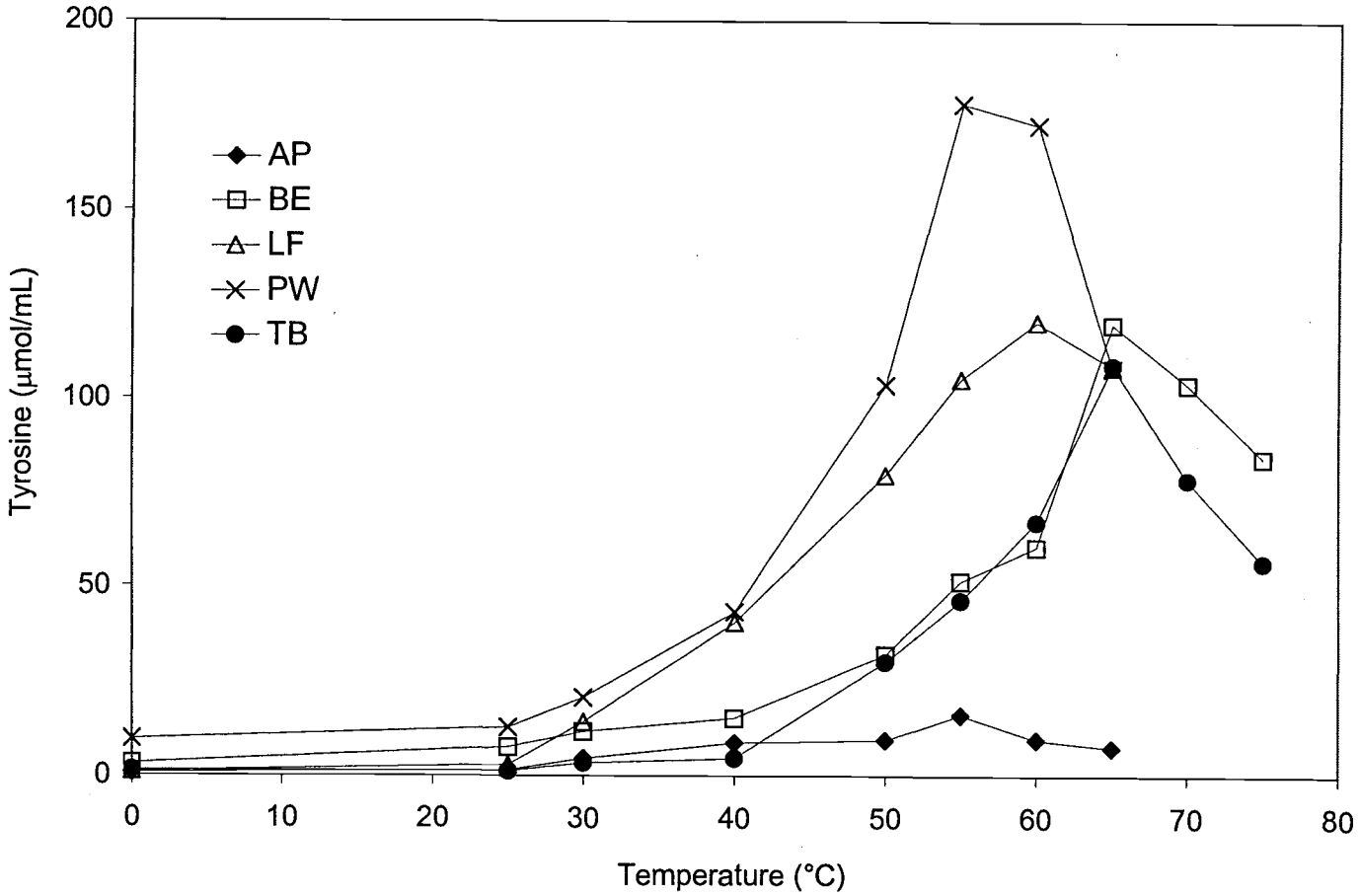

Figure 6.1. Effect of temperature on autolytic activity of AP, BE, LF, PW, and TB. AP: Alaska pollock, PW: Pacific whiting, BE: big eye, LF: lizardfish, TB: threadfin bream.

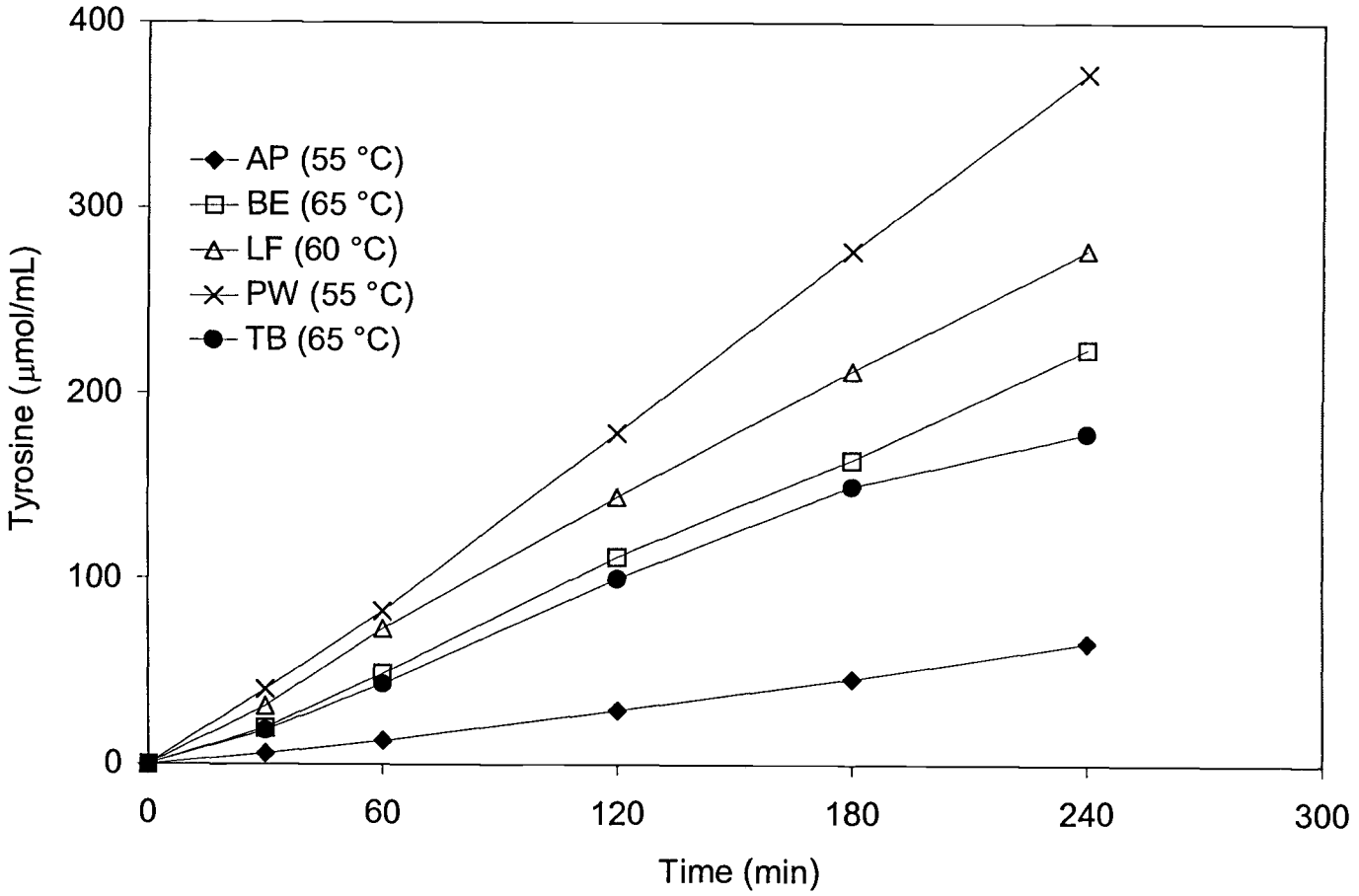

Figure 6.2. Autolytic activity of AP, BE, LF, PW, and TB incubated at their optimum proteolysis temperature. AP: Alaska pollock, PW: Pacific whiting, BE: big eye, LF: lizardfish, TB: threadfin bream.

Toyohara and Shimizu (1988) reported that the gel weakening phenomenon of TB surimi was caused by a serine protease exhibiting maximum activity at 65 °C. Similarly, serine proteases were found to be responsible for the textural breakdown of lizardfish (Suwansakornkul and others 1993), and oval-filefish (Toyohara and others 1992). Yongsawatdigul and others (2000) also suggested that a serine protease, exhibiting its optimum proteolytic activity at 65 °C, was responsible for the textural degradation of tilapia surimi.

Proteolytic degradation of fish muscle proteins could be minimized using either food grade enzyme inhibitors (Morrissey and others 1993) or rapid heating (Yongsawatdigul and Park 1996). Beef plasma protein, egg white, whey proteins, and potato extract have been reported to inhibit proteolytic activity and improve the gel forming ability of whiting myofibrillar proteins (Morrissey and others 1993; Porter and others 1993; Weerasingh and others 1996).

Protein patterns of AP, BE, LF, PW, LF, and TB for various incubation times at their optimum proteolysis temperature are given in Figure 6.3, 6.4, 6.5, 6.6, and 6.7, respectively. AP incubated at 55 °C did not exhibit significant proteolytic activity and the myofibrillar proteins remained intact with prolonged incubation time (Figure 6.3). A different protein band (116<Mw<205 kDa), however, was observed and its intensity increased as incubation was prolonged, indicating there is a minimum level of proteolytic degradation for Alaska pollock.

Protein patterns of BE myofibrillar proteins incubated at 65 °C are shown in Figure 6.4. There was a gradual decrease in the BE myosin heavy chain (MHC)

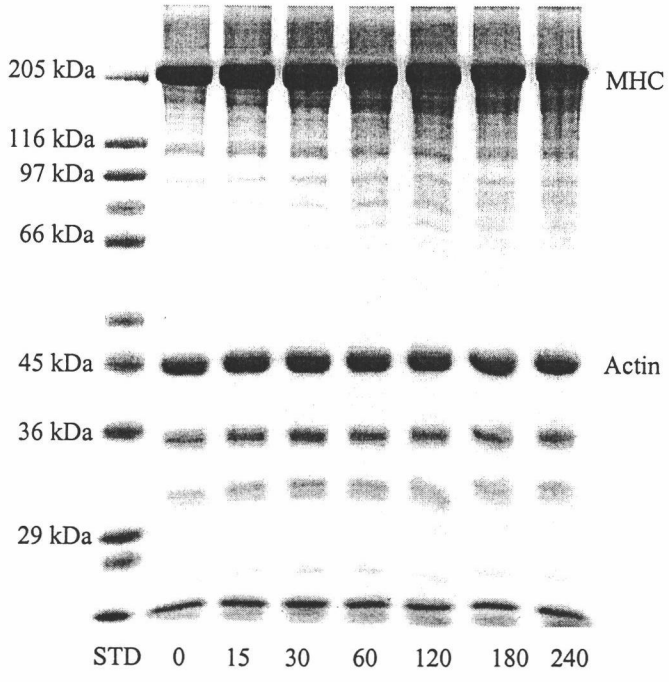

Figure 6.3. SDS-PAGE pattern of Alaska pollock incubated at 55 °C
STD: wide molecular weight standard, 0-240 incubation times in min.

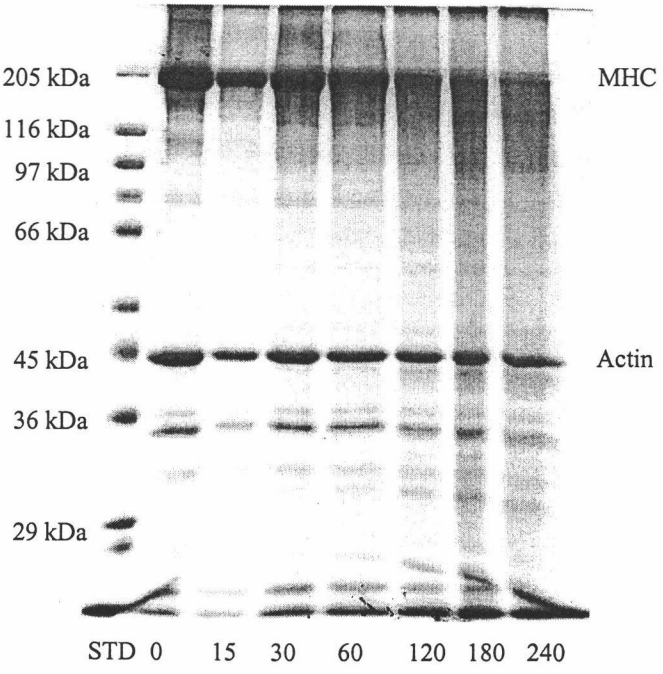

Figure 6.4. SDS-PAGE pattern of big eye incubated at 65 °C.
STD: wide molecular weight standard, 0-240 incubation times in min.

band intensity on SDS-PAGE gel as incubation time increased. Disappearance of MHC was evident at 3 h incubation. Medium molecular weight (MMW) proteins (66 < Mw <205) and low molecular weight (LMW) proteins (<45) appeared as a result of MHC degradation with prolonged incubation time.

LF, incubated at 60 °C, showed significant proteolytic activity. Degradation of LF MHC band was gradual. After 2 h of incubation, only trace amounts of the MHC band remained (Figure 6.5). In addition to MHC, intensity of actin, troponin, and tropomyosin bands also decreased with extended incubation time. In return, new MMW and LMW proteins appeared. Intensity of a protein band with a molecular weight of 97 kDa increased with incubation time. An additional protein band was observed right below actin (36<Mw<45) and its intensity also increased as incubation time increased.

PW, incubated at 55 °C, exhibited the most severe MHC degradation, which was notable after 30 min of incubation (Figure 6.6). However, degradation of actin, troponin, and tropomyosin, was slow and gradual. A decrease in the intensity of MHC, actin, and troponin resulted in the subsequent appearance of MMW and LMW proteins. An and others (1994b) studied the autolytic pattern of PW surimi, and reported that MHC band intensity was substantially reduced within 5 min incubation and completely disappeared within 20 min. In addition, MHC and actin were extensively hydrolyzed when PW surimi was slowly heated, a rate of 1 °C/min, from 10 to 90 at (Yongsawatdigul and Park 1996).

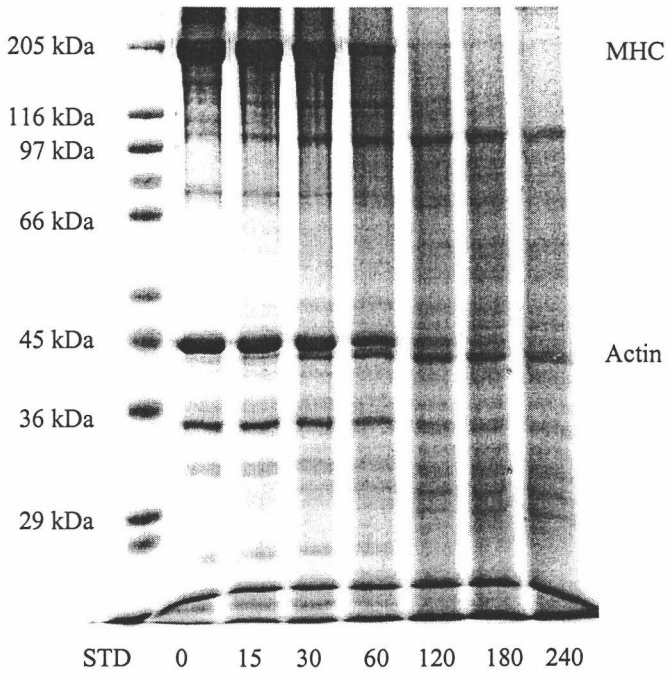

Figure 6. 5 SDS-PAGE pattern of lizardfish incubated at 60 °C.
STD: wide molecular weight standard, 0-240 incubation times in min.

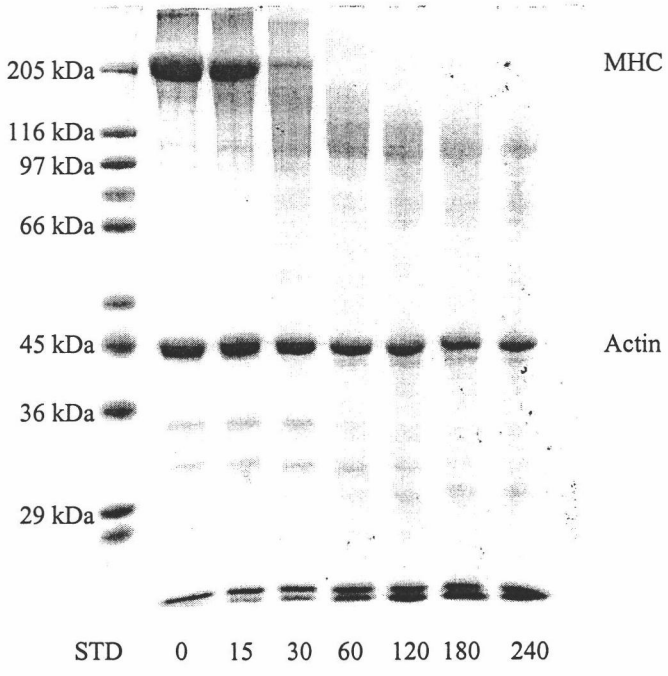

Figure 6.6. SDS-PAGE pattern of Pacific whiting incubated at 55 °C.
STD: wide molecular weight standard, 0-240 incubation times in min.

There was a gradual decrease in TB MHC, actin, troponin, and tropomyosin intensity (Figure 6.7). Disappearance of MHC was evident after 3h incubation. MMW and LMW proteins appeared as a result of degradation with prolonged incubation time.

Optimum proteolysis temperature and extent of proteolysis varied with fish species. MHC was the primary target for proteolytic degradation. PW MHC exhibited the fastest hydrolysis compared to the other fish species, which completely disappeared after 1 h of incubation. LF had the fastest actin hydrolysis followed by PW and TB. Differences in degradation patterns indicated that the type of proteases (serine or cysteine) may play a role in the hydrolysis rates of MHC and actin. Similarly, An and others (1994a) reported that PW surimi exhibited maximum proteolytic activity at 55 °C, corresponding to cathepsin L, a cysteine proteinase (Seymour and others 1994). Wasson (1992) suggested that a cysteine proteinase from arrowtooth flounder completely degraded MHC in 20 min at 55 °C, but actin was unaffected after 1 h of incubation.

6.4.2. Turbidity

All fish species followed a similar trend. Initially, absorbance values remained fairly constant until reaching the onset temperature, where absorbance started to increase. At this initial stage, the heating rate did not have a significant

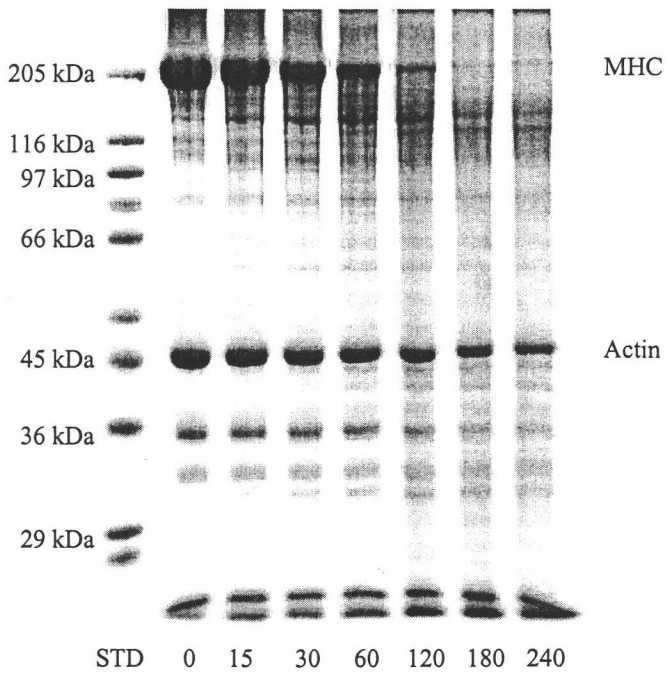

Figure 6.7. SDS-PAGE pattern of threadfin bream incubated at 65 °C.
STD: wide molecular weight standard, 0-240 incubation times in min.

effect ($p>0.05$) on the absorbance values of any fish species (Figure 6.8, 6.9, 6.10, 6.11, and 6.12). Absorbance of (320 nm) fish myofibrillar protein solutions increased with temperature due to the formation of protein aggregates.

The onset temperature appeared to be heating rate dependent. Slower heating rates were related to lower onset temperatures. The onset temperatures of the samples heated at 0.5 °, 1.0 °, and 2 °C/min heating rates were 32.5 °, 35.5°, and 37.5 °C for AP (Figure 6.8), 38 °, 40.2°, and 46,7 °C for BE (Figure 6.9), 26.2 °, 26.2, and 32.8 °C for LF (Figure 6.10), 27.2 °, 29.2 °, and 30.6 °C for PW (Figure 6.11), and 39.1 °, 42.3 °, and 45.5 °C for TB (Figure 6.12), respectively.

After reaching the onset temperature, absorbance readings increased rapidly with temperature. Similar results were reported for herring (Chan and others 1993), cod (Chan and others 1993; Yongsawatdigul and Park 1999), chicken (Xiong and Blanchard 1994), and a mixture of cod and herring (Chan and Gill 1994) myofibrillar proteins. Gill and others (1992) demonstrated that an increase in absorbance of heated fish myosin correlated with the formation of myosin aggregates.

For all fish species, there was a shift in absorbance readings around 60 °C. This could be due to the formation of larger aggregates as a result of actin and myosin aggregation. Heating rate also affected the magnitude of absorbance values for all fish species. A 0.5 °C/min heating rate resulted in the highest turbidity values followed by 1 °C/min, and then 2 °C/min. Higher absorbance readings at slower heating rates were probably due to the formation of larger aggregates in respectively longer time.

The patterns of turbidity for LF were quite different, where turbidity started to increase at 38.8 °, 41.7 °, and 46.5 °C for 0.5 °, 1.0 °, and 2 °C/min heating rates, respectively. LF and PW had high proteolytic activity, however, this did not seem to affect turbidity measurements. After reaching the onset temperature, turbidity increased constantly with temperature. Yongsawatdigul and Park (1999), however, reported that absorbance of PW myosin heated at 0.5 °, 1.0 °, and 2 °C/min started to decrease at 42 °, 48 °, and 50 °C, respectively.

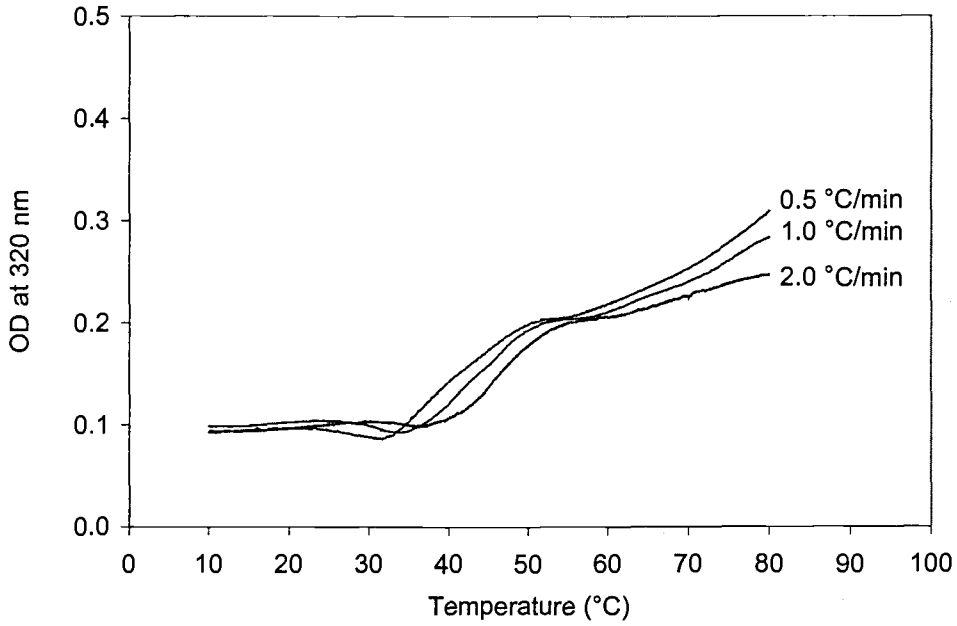

Figure 6.8 Changes in turbidity of Alaska pollock crude actomyosin in 0.6 M KCl, 20 mM Tris-HCl, pH7 heated linearly at 0.5 °, 1.0 °, 2.0 °C/min.

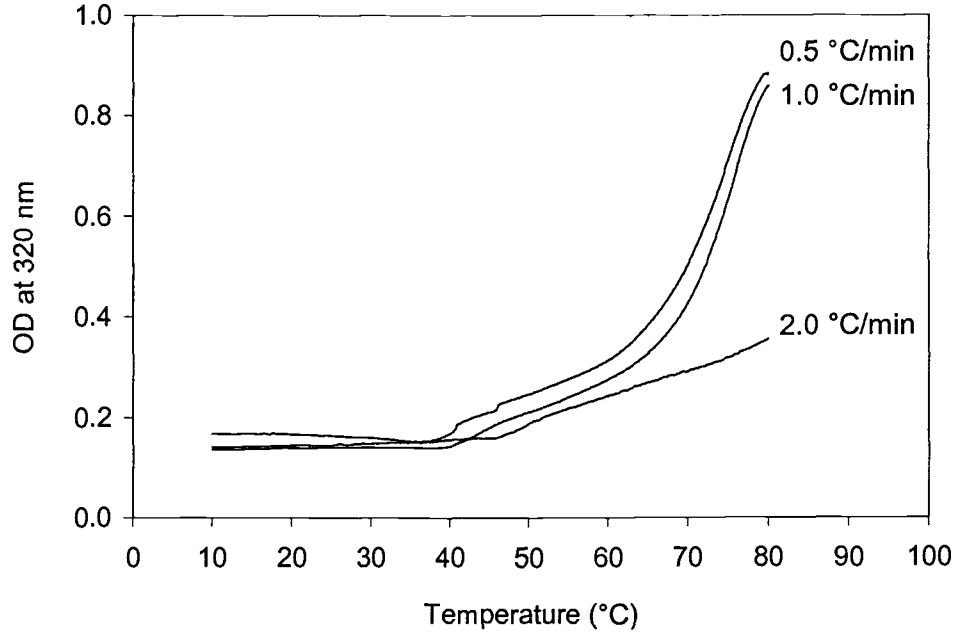

Figure 6.9. Changes in turbidity of big eye crude actomyosin in 0.6 M KCl, 20 mM Tris-HCl, pH7 heated linearly at 0.5 °, 1.0 °, 2.0 °C/min.

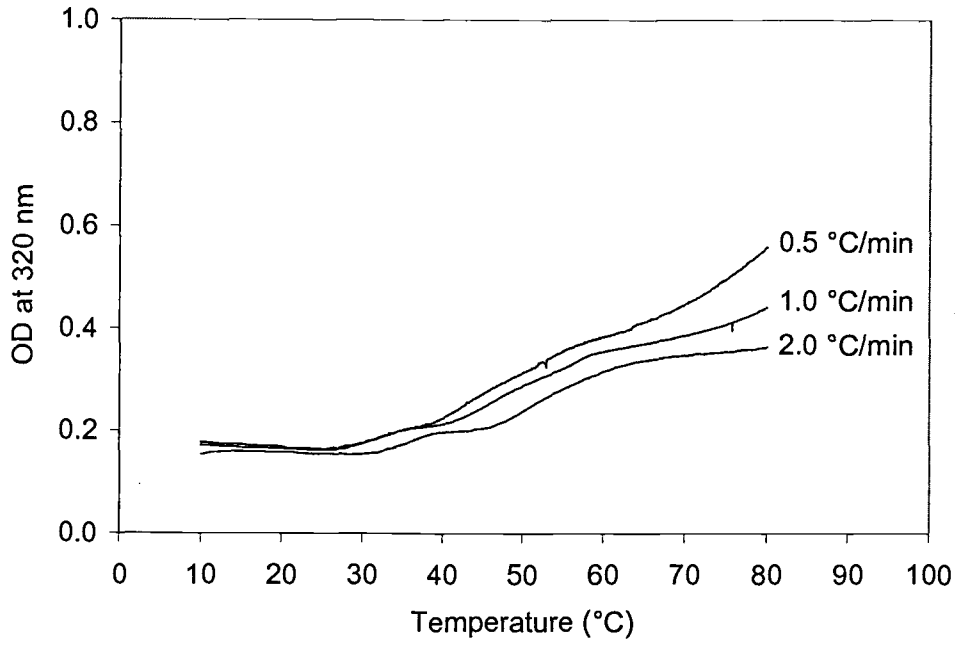

Figure 6.10. Changes in turbidity of lizardfish crude actomyosin in 0.6 M KCl, 20 mM Tris-HCl, pH7 heated linearly at 0.5 °, 1.0 °, 2.0 °C/min.

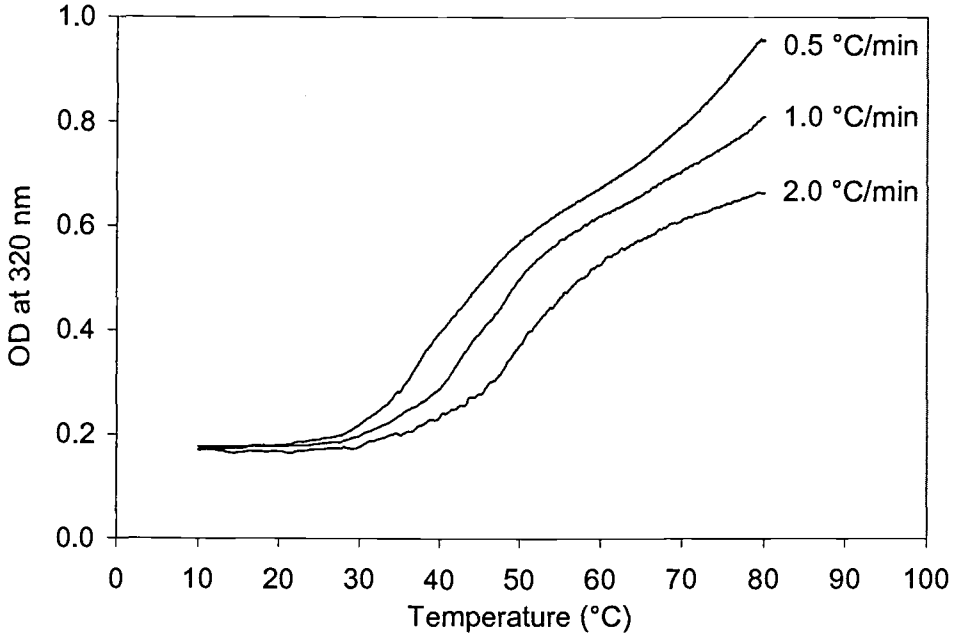

Figure 6.11. Changes in turbidity of Pacific whiting actomyosin in 0.6 M KCl, 20 mM Tris-HCl, pH7 heated linearly at 0.5 °, 1.0 °, 2.0 °C/min.

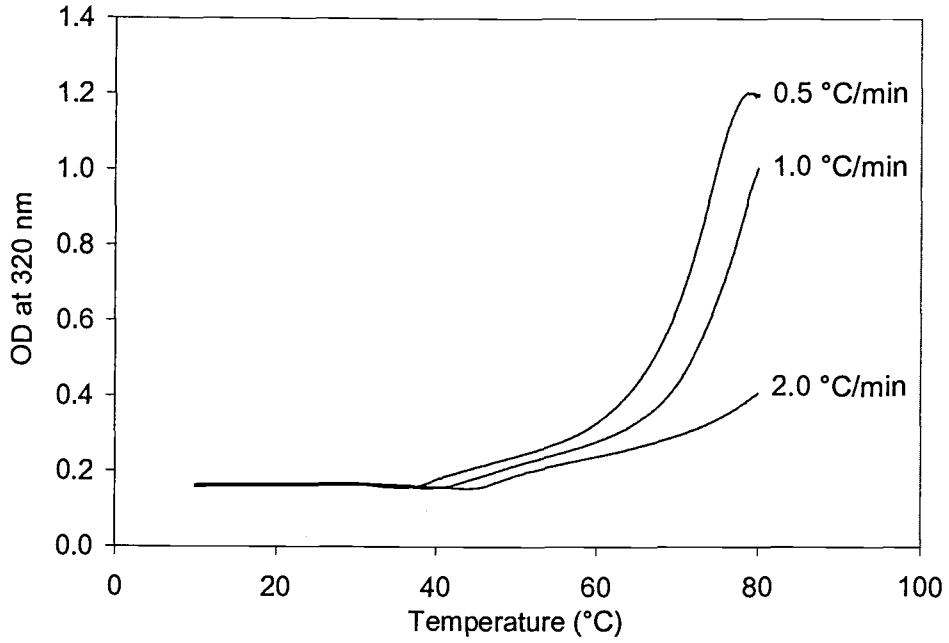

Figure 6.12. Changes in turbidity of threadfin bream actomyosin in 0.6 M KCl, 20 mM Tris-HCl, pH7 heated linearly at 0.5 °, 1.0 °, 2.0 °C/min.

6.4.3. Dynamic and thermal analysis

Storage modulus (G') transition temperatures obtained from temperature sweep (TS) measurements and peak temperatures obtained from the DSC thermograms at 0.5 ° and 1.0 °C/min are given in Table 6.1 and Table 6.2, respectively. Similarity of G' and the DSC thermal transition temperatures (T_d) for the first peak (myosin) indicated that peaks obtained from the dynamic tests were related to the protein unfolding temperatures. There were 3 endothermic peaks clearly distinguishable for all fish species from DSC measurements and 2 transition peaks from TS.

Myosin thermal denaturation is known to occur on several structural domains capable of undergoing independent transitions (Privalov 1982) depending on the biological source and the experimental environment (pH, ionic strength). Fernandez-Martin and others (1998) reported that thermal transition temperatures of blue whiting were 33.5 °, 44.5 °, and 74.7 °C. These peaks reflected the thermal response of myosin (subfragment S1), myosin rod, and actin, respectively.

Thermal transition temperature (T_d) of myosin and actin for various fish species are reported in literature (Hasting and others 1985; Poulter and others 1985; Davies and others 1988; Kim and others 1986; Park and Lanier 1989; Howell and others 1991). Myosin T_d values varied within the range of 29 to 59 °C. A narrower temperature range, however, was reported for actin T_d (65-75 °C).

Fish habitat temperature clearly affected the thermal transition temperature. For the first peak (myosin), AP had the lowest thermal denaturation temperature, followed by PW, BE, LF, and finally TB. These results suggested that the stability of myosins from different fish species is related to the environmental temperature at which fish live and supported the general statement that proteins of warm water species were more thermostable than those of cold or temperate water species. Hasting and others (1985) and Howell and others (1991) reported similar findings supporting our results. For the third peak (actin), however, there was a reverse relationship where AP had the highest thermal transition temperature and then PW, BE, LF, and TB.

Table 6.1. Differential scanning calorimetry (DSC) and temperature sweep (TS) peak temperatures of Alaska pollock, big eye, lizardfish, Pacific whiting and threadfin bream at 0.5 °C/min heating rate

		Peak 1 (°C)	**Peak 2 (°C)**	**Peak 3 (°C)**
Alaska pollock	DSC	32.91	38.48	64.47
	TS	32.80	47.60	
Pacific whiting	DSC	34.15	39.32	62.07
	TS	34.30	44.20	
Big Eye	DSC	37.85	45.52	
	TS	38.60	46.60	
Lizardfish	DSC	36.98	46.08	60.30
	TS	37.80	50.20	
Threadfin bream	DSC	39.26	48.31	59.75
	TS	39.40	47.60	

Table 6.2. Differential scanning calorimetry (DSC) and temperature sweep (TS) peak temperatures of Alaska pollock, big eye, lizardfish, Pacific whiting and threadfin bream at 1 °C/min heating rate.

		Peak 1 (°C)	**Peak 2 (°C)**	**Peak 3 (°C)**
Alaska pollock	DSC	33.63	41.78	67.26
	TS	33.30	48.20	
Pacific whiting	DSC	35.16	41.59	64.60
	TS	35.40	48.20	
Big Eye	DSC	38.8	47.76	62.85
	TS	38.70	48.30	
Lizardfish	DSC	37.73	47.87	61.60
	TS	38.80	51.60	
Threadfin bream	DSC	39.26	48.31	60.30
	TS	39.90	48.60	

TS and DSC measurements suggested that slower heating rates required lower transition temperatures. This was probably due to the fact that a slow heating rate allowed more time to unfold.

6.5. CONCLUSIONS

While LF and PW showed the highest proteolytic activity, AP exhibited limited hydrolysis. Optimum autolysis temperature was species dependent and habitat temperature seemed to have an effect as well. Slow heating rate induced aggregation of fish myofibrillar proteins at lower temperatures due to longer protein unfolding time. Thermal aggregation and degradation properties of fish species should, therefore, be considered for determining the optimum heating regime.

CHAPTER 7

GENERAL CONCLUSION

Dynamic rheological measurements resulted in a good correlation with fracture shear stress data as affected by various processing parameters. A linear relationship was found between fracture shear stress and dynamic test data as a function of moisture and pH. However, this relationship was not observed for all processing parameters, such as salt concentration. Our study indicated that dynamic rheological measurements could be successfully used in early gel quality prediction.

It has been suggested that dynamic rheological measurement results are sensitive to chemical composition and physical structure, and therefore, could be used to relate dynamic rheological parameters to molecular changes. Comparison of peak temperatures obtained from the temperature sweep tests and DSC confirmed the assumption that peaks obtained from dynamic tests were related to protein unfolding. However, the rheometer was not sensitive enough to detect thermal transition temperatures above 55 °C.

Understanding the thermal stability of fish proteins is very important for proper utilization of surimi. Correct processing temperature could prevent the thermal damage of myofibrillar proteins, which in return, may result in better product quality. Our study indicated that the stability of fish myofibrillar proteins

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