AN ABSTRACT OF THE THESIS OF

Jirawat Yongsawatdigul for the degree of Doctor of Philosophy in Food Science and Technology presented on January 29, 1996

Title: Textural and Electrical Properties of Pacific Whiting Surimi under Ohmic Heating

Abstract approved: _____
Jae W. Park

Feasibility of ohmic heating to overcome gel-weakening in Pacific whiting surimi was investigated. An ohmic heating apparatus was developed using two rhodium-coated stainless steel electrodes inside a polyvinyl chloride (PVC) tube, a variable transformer, and voltage and current transducers. Rapid heating associated with the ohmic process quickly inactivated endogenous proteinase(s), resulting in significantly high shear stress and shear strain of surimi gels (78% moisture content, 2% NaCl). Degradation of myosin heavy chain (MHC) and actin examined by SDS-PAGE were significantly reduced and continuous gel structure were shown by scanning electron microscopy. Whiting surimi heated in a 90°C water bath for 15 min exhibited poor gel quality and disordered microstructure due to proteolysis of MHC.

Electrical conductivity, a critical parameter influencing rate of heat generation during ohmic heating, was elucidated. Electrical conductivities of whiting surimi pastes with four moisture contents (75, 78, 81, and 84% wet basis) and added NaCl (1, 2, 3, and

4%) were measured from 10 to 90°C using ohmic heating at voltage gradient of 3.3, 6.7, and 13.3 V/cm. Electrical conductivity significantly increased with temperature and salt content and slightly increased with moisture content. The effect of the voltage gradient was evident at combinations of high moisture (81, 84%) and NaCl content (3, 4%), due to electrochemical reactions at the electrodes. The empirical model of electrical conductivity as a function of temperature and compositional characteristics predicted values with an error range of 0-15.6%.

Finally, kinetic models of textural degradation of whiting surimi were developed using two different approaches: isothermal and nonisothermal procedure over a range of temperature (40-85°C) and time (0.5-35 min). The effect of thermal lag was accounted for using the models derived from the Arrhenius equation. Textural degradation obtained from both methods followed first order kinetic. Degradation of MHC derived from nonisothermal procedure was best described by apparent reaction order of 1.4. Degradation rate of gel texture and MHC increased with temperature and reached the highest rate at 55 and 57°C, respectively. Then they decreased to minimum rate at 70 and 75°C, respectively. The kinetic model for the loss of MHC satisfactorily estimated MHC content of the controls with an averaged error of 10.8%. Relationship between degradation of MHC and gel texture are discussed.

Textural and Electrical Properties of Pacific Whiting Surimi under Ohmic Heating

by

Jirawat Yongsawatdigul

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirement for the
degree of

Doctor of Philosophy

Presented January 29, 1996
Commencement June 1996

Doctor of Philosophy thesis of Jirawat Yongsawatdigul presented on January 29, 1996

APPROVED:

Major Professor, representing Food Science and Technology

Head of the Department of Food Science and Technology

Dean of Graduate School

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

Jirawat Yongsawatdigul, Author

ACKNOWLEDGEMENT

I would like to thank Dr. Jae W. Park, my major advisor, for his academic guidance and support throughout the entire course of my graduate study. I would also like to express my gratitude to Dr. Edward Kolbe for his valuable advice and constructive criticism on my thesis work. My appreciation is also expressed to Dr. David Thomas for his assistance on statistical analysis. Special thanks are also extended to Dr. Michael T. Morrissey for his support and advice throughout my stay at the OSU Seafood Lab. I also would like to thank Dr. Michael H. Penner and Dr. Robert Jarvis for serving as my committee members and providing invaluable help.

It has been a great experience to work in the OSU Seafood Laboratory where I have met several wonderful friends and colleagues. I am very thankful to Nancy Chamberlain for her friendship and constant moral support. I also appreciate the help from Lynne Johnson who worked tirelessly on producing the prints used in my thesis. I will always remember my fellow graduate students whom I have known in this past three years.

I also wish to thank my loving parents who have always supported and encouraged me in my pursuit of a higher education. This thesis work is totally dedicated to them.

CONTRIBUTION OF AUTHORS

Dr. Jae Park and Dr. Edward Kolbe were involved in the experimental design and data interpretation of each manuscript. Dr. Michael Morrissey participated in the first phase of this study. Mr. Yaser AbuDagga assisted in the hardware assembly for the ohmic heating device.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF TABLES

LIST OF APPENDIX FIGURES

TEXTURAL AND ELECTRICAL PROPERTIES OF PACIFIC WHITING SURIMI UNDER OHMIC HEATING

Chapter 1: INTRODUCTION

Annual harvest of Pacific whiting (*Merluccius productus*) has been approximately 200,000 metric tons in the United States over the last decade (OCZMA, 1994). Most of the harvested fish is processed into surimi. Surimi is a minced fish which has been washed, dewatered, and mixed with cryoprotectants to stabilize the proteins during frozen storage (Okada, 1992). Washing and dewatering steps are primarily to remove sarcoplasmic proteins, fat, blood, as well as pigments and also to concentrate myofibrillar proteins. The most important functional property of surimi is its ability to form a strong elastic gel, which mostly depends on quality of myofibrillar proteins. The production of whiting surimi was not practical until 1991 due to severe textural degradation accompanied by substantial loss of myosin heavy chain upon slow heating (Chang-Lee et al., 1989; Morrissey et al., 1993). This is caused by proteolytic activity of the endogenous heat stable proteinase(s) which primarily hydrolyze myosin, a myofibrillar component required to form three-dimensional gel networks.

Since 1991, the addition of food grade enzyme inhibitors has made whiting surimi commercially acceptable as a raw material for surimi-based seafoods. Beef plasma protein, egg white, potato extract, and whey protein concentrate are found to inhibit proteolytic activity and enhance gel forming ability of surimi (Change-Lee et al., 1989; Change-Lee et al., 1990; Porter et al., 1990; Morrissey et al., 1993). Hardness and elasticity of surimi gel measured by an Instron Universal Testing Machine significantly

improve when either 3% egg white or a combination of 3% egg white and 5% potato starch are added (Chang-Lee et al., 1989; Chang-Lee et al., 1990). Morrissey et al. (1993) reported that beef plasma protein shows the highest inhibitory activity as compared to egg white and potato extract. Proteolytic activity in whiting surimi based on autolytic assay is inhibited about 85% by addition of 1% beef plasma protein. Moreover, surimi gels with beef plasma protein exhibit desirable textural characteristics as indicated by failure shear stress and shear strain (Morrissey et al., 1993). Proteolysis can also be inhibited by whey protein concentrate with a concentration of 2-3%, depending on its protein content (Piyachomkwan, 1993).

Although the food grade enzyme inhibitors have been proven to be effective in inhibiting proteolysis, the use of these compounds provides some negative side effects to the surimi-based products. Whiteness is one of the most important characteristics of surimi and is used to evaluate its quality (Lanier, 1992). Addition of 1-1.5% beef plasma protein decreases whiteness of surimi and increases yellowness, which is an undesirable color (Park, 1995). Labeling beef plasma protein as food additive in surimi-based seafoods sometimes draws adverse perceptions from consumers. Furthermore, sensory properties of surimi containing beef plasma protein at concentration greater than 1% are seldom accepted by Japanese consumers (Akazawa et al., 1993). Egg white is expensive and provides an undesirable sulfur-like odor at the levels needed for effective inhibition (Porter et al., 1993). Although potato extract and whey protein concentrate have less adverse effect on sensory properties, they must be added at higher concentration as compared to beef plasma protein (Chang-Lee et al., 1989; Chang-Lee et al., 1990; Akazawa et al., 1993; Morrissey, et al. 1993). In addition, textural properties of surimi

with potato extract or whey protein concentrate are lower than those of surimi with beef plasma protein (Akazawa et al., 1993; Morrissey et al., 1993). Thus, higher usage level of these two inhibitors would increase the cost. It is obvious that alternative approaches to overcome the textural deterioration of Pacific whiting surimi are needed.

The endogenous proteinase in Pacific whiting flesh is purified and identified as cathepsin L (Seymour et al., 1994). The purified enzyme (pH 5.5) exhibits maximal activity at 55°C and decreases to minimal activity at 70°C. The pattern of myosin degradation caused by the purified proteinase is consistent with the autolytic pattern of surimi, indicating that cathepsin L is responsible for myosin and texture degradation in whiting surimi (An et al., 1994).

Since the endogenous proteinase is heat stable, slow heating rate associated with conventional heating methods allows the proteinase to hydrolyze myosin before it is thermally inactivated. When surimi packed in a stainless steel tube (i.d. = 1.9 cm) is heated in a 90°C water bath, temperature at the geometric center gradually increases and remains in the enzyme activation range, 45-60°C, for about 85 sec (AbuDagga and Kolbe, 1996). This could be ample time for hydrolytic reaction of myofibrillar proteins, especially myosin. In contrast, proteolysis of whiting fillets is minimized when they are deep-fried at 196°C or heated within 1.5 min using microwaves (Patashnik et al., 1982). Textural degradation in arrowtooth flounder, another kind of fish exhibiting the endogenous proteinase problem, also markedly decreases when cooked by microwaves (Greene and Babbitt, 1990). Thus, rapid heating methods could be a potential means to cope with textural breakdown induced by proteolysis.

Ohmic heating is a heating process in which alternating electric current passes through electrically conducting food products (de Alwis and Fryer, 1990). Heat is internally generated due to electrical resistance of the food materials, and conducts within the samples. For this reason, heat is readily transferred within the sample, resulting in rapid heating rate and uniform heat distribution. This is a striking contrast to conventional heating methods in which temperature of the product increases relatively slow because heat penetrates from external heating medium.

Ohmic heating has been successfully applied to the sterilization process of particulate foods (Skudder and Biss, 1987; Biss et al., 1989). The advantage of ohmic heating over conventional sterilization is that both solid and liquid phases with identical electrical conductivity are simultaneously heated, therefore, temperature uniformly distributes in both phases (Halden et al., 1990). The problems associated with thermal lag in solid phase or overheating in liquid phase are eliminated. As compared with microwave heating, ohmic heating provides more uniform temperature distribution because heat penetrates throughout the bulk of the sample (Biss et al., 1989). Moreover, energy utilization of ohmic heating process is more efficient than that of microwave heating (Biss et al., 1989). Although the advantages of ohmic heating are obvious, its application in seafood products is very limited in the United States. In Japan, ohmic heating has recently been used in manufacturing surimi-based products (Shiba, 1993). Textural properties of the ohmically heated products are superior to those heated in a 90°C water bath (Shiba, 1992; Shiba and Numakura, 1992). Since ohmic heating provides a rapid heating rate with uniform temperature distribution, it could be a promising process to overcome gel weakening in whiting surimi.

One of the most important properties of food needed to be considered in ohmic heating is electrical conductivity. Rate of heat generation in ohmic heating is controlled by electrical conductivity of food materials (Palaniappan and Sastry, 1991a,b; de Alwis and Fryer, 1992). Several studies have indicated that electrical conductivity of food materials is dependent on various factors, such as temperature, applied voltage gradient, moisture, and salt content (Palaniappan and Sastry, 1991a,b; Halden et al., 1990). Therefore, knowledge of electrical conductivity of food materials under ohmic heating is critical for process design.

Time and temperature are also important parameters influencing textural properties of surimi gel. Surimi gelation occurs upon heating, and it involves denaturation and aggregation processes (Ferry, 1948). Denaturation is a process in which native protein undergoes structural changes (Ziegler and Acton, 1984). Denatured proteins associate with one another and form gel network during aggregation period (Ziegler and Acton, 1984). According to the gelation kinetics, stronger gel network tends to form when slow heating rate is applied because the aggregation process of denatured proteins is favored (Ferry, 1948; Foegeding et al., 1986a; Camou et al., 1989; Arntfield and Murray, 1992). However, gelation kinetics alone cannot explain or predict textural changes in whiting surimi gel. This is because the hydrolytic reaction of myofibrillar proteins, especially myosin, catalyzed by the endogenous proteinase occurs concurrently with gelation during heating. Therefore, it is essential to develop the kinetic models that can adequately explain the changes of gel texture in whiting surimi so that optimum time and temperature required to obtain a desired surimi gel can be quantitatively determined.

Research Objectives

The overall objective of this research was to apply a rapid heating process, ohmic heating, as a means to overcome gel-weakening phenomenon in Pacific whiting surimi. This research demonstrated an alternative approach to minimize proteolysis in fish muscle proteins, besides the use of food grade enzyme inhibitors. The importance of various parameters, namely electrical conductivity, time, and temperature, on ohmic heating process and textual properties of whiting surimi gel was illustrated. The outcome of this research could also be applicable to surimi made from other fish species that inherently exhibit endogenous proteinase problems. Specific objectives of this research were:

1. To investigate the feasibility of ohmic heating as a method to reduce textural degradation caused by endogenous proteinase(s) in whiting surimi without enzyme inhibitors.
2. To study the effect of temperature and compositional characteristics (moisture and salt content) on electrical conductivity of surimi during ohmic heating as well as to develop an empirical model describing electrical conductivity of whiting surimi as a function of such variables.
3. To determine degradation kinetics of gel texture and myosin heavy chain of whiting surimi during heating.

Literature Review

Proteolysis of fish muscle proteins

Textural degradation of Pacific whiting at 50-65°C has been extensively studied. The extent of proteolytic activity is associated with degree of infection of myxosporidian parasite, *Kudoa paniformis* (Patashnik et al., 1982; Erickson et al., 1983; Toyohara et al., 1993). It is still unclear whether the proteinase is produced from the parasite or from immunological responses of the fish. Nevertheless, most researchers have reported the role of cathepsins in proteolysis of Pacific whiting. Cathepsins are lysosomal enzymes and are involved in cellular immunity induced by pathological conditions (Asghar and Bhatti, 1987). Hydrolysis of muscle proteins in post-mortem is caused by the release of cathepsins from lysosomes (Zeece et al., 1992). Erickson et al. (1983) studied proteolytic activity in the sarcoplasmic fluid of Pacific whiting and concluded that the proteinases involved in textural degradation were cathepsin B and C. Based on the studies of specific substrates and inhibitors, the heat stable proteinase exhibits cysteine proteinase characteristics and is identified as cathepsin L (Toyohara et al., 1993; Seymour et al., 1994). Seymour et al. (1994) reported that cathepsin L purified from whiting exhibited two forms, namely P-I and P-II with pH optima at 5.5 and 6.0, and temperature optima of 55 and 60°C, respectively. It was later suggested that P-I is complex-formed with an endogenous inhibitor; whereas, P-II is the free enzyme (An et al., 1995). The purified cathepsin L hydrolyzes myofibrillar proteins, myosin, and native as well as heat-denatured collagen (Toyohara et al., 1993; An et al., 1994). The degradation pattern of myofibrillar proteins induced by the purified cathepsin L at its pH optimum (5.5) is consistent to that

of proteolysis of surimi at 55°C, at pH 6.8-7.0, suggesting that textural degradation of whiting surimi at elevated temperatures is attributed to proteolytic activity of cathepsin L (An et al., 1994). The presence of cathepsin B and H in the whiting flesh is also noted (An et al., 1994). However, it is unlikely that both enzymes are causative agents of proteolysis at high temperature because the optimum temperatures of cathepsin B and H as assayed using synthetic substrates are at 37 and 20°C, respectively. It also should be mentioned that activity of both enzymes is negligible in whiting surimi, but activity of cathepsin L is predominant. This indicates that cathepsin B and H are removed by extensive washing during surimi processing, while cathepsin L is highly associated with myofibrillar proteins.

Cathepsin L and B are also found in white muscle of chum salmon (*Oncorhynchus keta*) during the spawning season (Yamashita and Konagaya, 1990a,b). Yamashita and Konagaya (1990c, 1991) indicated that cathepsin L hydrolyzed myofibrillar proteins to a greater extent than cathepsin B at 20°C. Therefore, cathepsin L was responsible for textural degradation of muscle proteins in chum salmon. However, proteolytic activity of cathepsin L at high temperatures has not been reported. A recent study by Saeki et al. (1995) demonstrated that surimi made from chum salmon during the spawning season displayed poor gel-forming characteristics and substantial loss of myosin heavy chain upon heating. Gel quality was improved when cysteine proteinase inhibitor, L-trans-epoxysuccinylleucylamino(4-guanidino)butane (E-64), was added, indicating involvement of heat stable cysteine proteinase(s) in the textural degradation of chum salmon surimi.

Arrowtooth flounder (*Atheresthes stomias*), a large biomass in the Gulf of Alaska, also suffers from tissue-softening due to the endogenous proteinase. Although infection

of parasites was observed in arrowtooth, there was no correlation between parasite density and degree of proteolysis (Greene and Babbitt, 1990). The partially-purified proteinase is cysteine proteinase, exhibiting highest activity at 55°C at pH 6.0-7.0 against casein (Wasson et al., 1992a). Proteolysis of arrowtooth surimi is inhibited by beef plasma protein, egg white, and α_2 macroglobulin at concentrations of 1%, 1-1.5%, and 0.2%, respectively (Wasson et al., 1992b). On the other hand, whey protein concentrate up to 1% does not have any inhibitory effect on proteolytic activity in arrowtooth surimi.

Serine proteinases are responsible for textural breakdown of threadfin bream (*Nemipterus bathybius*) (Toyohara and Shimizu, 1988), oval-filefish (*Navodon modestus*) (Toyohara et al., 1992), and lizardfish species (*Saurida sp*) (Suwansakornkul et al., 1993). Proteolysis of surimi made from these species can be inhibited by soybean trypsin inhibitor. Distribution of heat-stable serine proteinase in muscle proteins varies among species. The proteinase is only found in the sarcoplasmic portion of threadfin bream (Kinoshita et al., 1991), but it is strongly bound to myofibrillar proteins of oval-filefish (Toyohara et al., 1992). The heat-stable proteinase(s) effectively hydrolyzes myosin heavy chain at pH 7.0 and 60°C.

In early studies of gel weakening phenomenon, the endogenous proteinase responsible for textural degradation was normally categorized as heat stable alkaline proteinase (Iwata et al., 1974; Boye and Lanier, 1982; Makinoden et al., 1985). A distinct characteristic of alkaline proteinase is its high activity in alkaline condition (pH ~ 8.5-9.1) at 60-65°C. Makinodan et al. (1987) purified alkaline proteinase from white croaker (*Sciaena schlegeli*) and identified it as cysteine proteinase consisting of four subunits with molecular weight of 430,000 daltons. The enzyme showed maximum activity toward

myosin heavy chain, actin, and tropomyosin at 60°C. However, Busconi et al. (1984) reported two purified alkaline proteinases, proteinase I and proteinase II, in white croaker (*Micropogon opercularis*) muscle. Proteinase I showed serine proteinase characteristics, while proteinase II exhibited cysteine proteinase properties. Folco et al. (1984) further investigated hydrolytic activity of these proteinases against myofibrillar proteins at 60°C and found that both proteinase I and II hydrolyzed purified myosin, but only proteinase I was able to degrade myosin heavy chain from actomyosin. Makinodan et al. (1985) reported that alkaline proteinase was the principle enzyme responsible for textural degradation of white croaker surimi although the presence of other proteinases, such as cathepsin D and calpain were also found. This was because alkaline proteinase was the only enzyme that retained hydrolytic activity at the pH of surimi paste (6.8) and at 60°C. Textural degradation of surimi made from Atlantic menhaden (*Brevoorti tyrannus*) is also caused by proteolytic activity of alkaline proteinase (Boye and Lanier, 1988).

Muscle proteins

The proteins of muscle can be categorized into three groups (Hultin et al., 1985): (1) sarcoplasmic proteins are globular and soluble in water or dilute salt; (2) myofibrillar proteins are the contractile proteins which are fibrous and soluble in salt solution; (3) stroma proteins are insoluble fractions. After being washed and refined, surimi constitutes mainly myofibrillar proteins. Approximately 55-60% of myofibrillar proteins is myosin (Bechtel, 1986). Actin is the second major component comprising 15-30% of myofibrillar proteins. Monomer form of actin with molecular weight of 43,000 daltons is called globular actin (G-actin). Actin molecules polymerize together and form actin filament

referred to as fibrous actin (F-actin). Other small fractions of protein associated with either actin or myosin are tropomyosin, troponin complex, actinins, M-proteins, and C-proteins (Asghar and Pearson, 1980).

Myosin is an elongated molecule with molecular weight of 470,000 daltons (Bechtel, 1986). It is composed of two large subunits called heavy chain and four small subunits called light chain. Each heavy chain consists of a long α-helical and globular region with a molecular weight of 200,000 daltons. A long α-helix of each myosin heavy chain are wound around each other and forms the rod portion (Hultin et al., 1985). The globular regions are responsible for ATPase activity and contain actin binding site. Molecular weight of each light chain ranges from 16,000 to 27,500 daltons, depending on the source of the myosin (Bechtel, 1986). When myosin is digested with trypsin, two major fragments are obtained (Asghar and Pearson, 1980; Bechtel, 1986). The largest fragment is commonly known as heavy meromyosin containing both myosin globular heads and a portion of the myosin rod. The other major fragment is light meromyosin which is the helical rod portion of the myosin molecule. Further proteolytic digestion of heavy meromyosin yields S-1 fragment, the individual myosin head, and S-2 fragment, the small portion of myosin rod.

Gelation of surimi

According to Mulvihill and Kinsella (1990), protein gels can be defined as "three-dimensional matrices or networks in which polymer-polymer and polymer-solvent interactions occur in an ordered manner resulting in the immobilization of large amounts

of water by a small proportion of protein". Ferry (1948) proposed that formation of protein gels involves the following mechanisms:

$$x\ P_n \rightarrow x\ P_d \rightarrow (P_d)_x$$

where x is the number of protein molecules, P_n is the native protein, and P_d is denatured protein. Based on this mechanism, gelation is a two-stage process involving denaturation and aggregation. Denaturation is a process in which native proteins undergo conformational changes, including alterations of hydrogen bondings, hydrophobic interactions, and ionic linkages (Mulvihill and Kinsella, 1990). Denaturation is a prerequisite step for protein gelation (Hermansson, 1979). The following process is aggregation in which denatured protein molecules align themselves and interact each other at specific points to form a three-dimensional network.

Surimi is a minced, washed and dewatered fish flesh that contains a relatively high concentration of myofibrillar proteins. In surimi paste preparation, surimi is comminuted with sodium chloride and water. Addition of salt is required to extract myofibrillar proteins from the fish muscle (Niwa, 1992). This is achieved through disrupting intermolecular electrostatic forces among myofibrillar proteins (Goodno and Swenson, 1975; Sano et al., 1990a). In addition, salt destabilizes the native structures of proteins prior to thermal denaturation (Park and Lanier, 1990). Interactions between myosin and actin upon chopping yield a macromolecule called actomyosin (Niwa, 1992). Three-dimensional gel network is formed when the solubilized actomyosin is heated. Thermal denaturation of actomyosin begins at 30-35°C in which native tropomyosin and troponin dissociate from F-actin (Ziegler and Acton, 1984). Then, helical structure of F-actin

unravels to single chain at 38°C. When temperature reaches 40-45°, four myosin light chain subunits dissociates from the globular head of myosin heavy chain. Concomitantly, conformational changes of globular head occur. As temperature increases to 45-50°C, actin-myosin complex starts to dissociate from each other. Helical regions of myosin heavy chain then unfold and transform to random coil structure. Finally, conformation of G-actin monomer alters at temperature greater than 70°C.

Among myofibrillar proteins, myosin is the major component capable to aggregate orderly and form gel networks (Samejima et al., 1969). According to Ferry (1948), the aggregation is a process following denaturation. However, aggregation of myosin proceeds prior to the final stage of denaturation (Wright and Wilding, 1984; Chan et al., 1992). Chan et al. (1992) reported that the discrete regions of myosin molecule exhibiting less thermal stability initially unraveled and then aggregated before the entire molecule completely denatured. Based on the results from dynamic viscoelasticity and spectrophotometric measurements, Sano et al. (1990a,b) reported that gel formation of carp myosin first began to develop by interaction of the light meromyosin at 30-45°C. The second step of gel formation involved aggregation among heavy meromyosin by hydrophobic interactions at 50°C. However, Taguchi et al. (1987) suggested that aggregation of heavy meromyosin S-1 occurred to a greater extent than that of light meromyosin at 30 to 40°C. They proposed that myosin gelation was initiated by interaction of heavy meromyosin S-1 at 30-40°C, followed by the thermal unfolding and interaction of light meromyosin to form gel network at 50°C. This gelation mechanism is in agreement with the findings of Gill and Conway (1989) who reported that the tail region of cod myosin, rather than the head, participated in noncovalent interaction at 40-

50°C. Chan et al. (1993) studied the role of myosin fragments in cod and herring and suggested that myosin molecules initially aggregated through interaction of heavy meromyosin S-2, at 30-40°C. Further aggregation at 40-55°C was mediated by interaction of light meromyosin. The results from these studies indicates the important role of discrete regions of myosin molecules in forming gel networks. Discrepancies in the role of myosin fragments between the studies of Sano et al. (1990a,b) and the others are probably due to the structural differences in these discrete portions among different fish species (Chan et al., 1993).

The synergistic role of actin in gelation of myofibrillar proteins is also observed (Ishioroshi et al., 1980; Samejima et al., 1982; Yasui et al., 1982). The optimal weight ratio of myosin to actin that yields the highest shear modulus is 15:1. Yasui et al. (1982) explained that the complex formed between F-actin and myosin behaved as a cross-linker between the rod portion of myosin molecules, which consequently increased rigidity of the gel. They also reported that tropomyosin and troponin did not affect gelation of myofibrillar proteins.

Hydrogen bonding and hydrophobic interaction mainly contribute to gel structure of surimi, but the role of disulfide bonding is insignificant (Liu et al., 1982; Park et al., 1994). In contrast, Samejima et al. (1981) reported that globular head segments of rabbit myosin formed disulfide linkages providing extra cross-linking to gel structure. Hydrophobic interactions are enhanced during heating when myosin structures unfold and expose their hydrophobic amino acids to environment (Howe et al., 1994). During the cooling period, surimi gel matrix is stabilized and strengthened by hydrogen bonding (Howe et al., 1994).

Nondisulfide covalent bonds are also involved in gelation of surimi made from some fish species. Preincubation of Alaska pollock surimi pastes at 25°C for 1-24 hr prior to heating to 90°C improves textural properties of surimi gel (Numakura et al., 1985; Joseph et al., 1994). This phenomenon is called "setting" and also observed in surimi made from other fish species, such as Atlantic croaker (Kim, 1987; Kamath et al., 1992) as well as Southern blue whiting and hoki (MacDonald et al., 1994). The optimum setting temperature is species specific (Kamath et al., 1992). An improvement of surimi gels is due to polymerization of myosin molecules between γ-carboxyamide groups of glutamine and ϵ-amino groups of lysine residues (Numakura et al., 1987; Nishimoto et al., 1988). The cross-linking of myosin is Ca^{+2}-dependent reaction and is catalyzed by endogenous transglutaminase (Seki et al., 1990; Kimura et al., 1991). Recently, the addition of microbial transglutaminase produced by *Streptoverticillium mobaraense* also increases ϵ-(γ-glutamyl)lysine crosslinks as well as textural properties of pollock surimi (Sakamoto et al., 1995) and surimi-based products (Seguro et al., 1995). Unique characteristic of microbial transglutaminase is its Ca^{+2}-independent catalytic activity (Sakamoto et al., 1995)

Ohmic heating

Ohmic heating occurs when alternating electric current (50 or 60 Hz) passes through electrically conducting food product (de Alwis and Fryer, 1990). Heat is generated within the food due to its electrical resistance, resulting in a relatively rapid heating rate. Rate of temperature increase in ohmic process ranges from 1-5°C/sec (de Alwis and Fryer, 1992), whereas that at the surface of a can in conventional sterilization

is about 0.2°C/sec (Datta and Hu, 1992). A number of applications have been found in areas, such as blanching (Mizrahi et al., 1975; Vigerstrom, 1976), thawing (Naveh et al., 1983), and sterilization (Biss et al., 1989). Most studies have indicated that the ohmic process reduces processing time and results in superior product quality to those processed by conventional means. However, practical application in such processes has been very limited due to the problems associated with process and equipment design (Sastry and Palanippan, 1992a).

Recently, ohmic heating has been introduced commercially as a means of sterilizing particulate foods (Biss et al., 1989). In conventional aseptic processing of particulate foods, the heat is transferred to the solid particles from the heated liquid phase. Heat penetration is relatively slow and non-uniform because heat transfer is controlled by conduction. Thus, the product is normally overprocessed to ensure commercial sterility, resulting in destruction of flavors and nutrients, and mechanical damage to the particulates (Sastry and Palaniappan, 1992a). This problem can be overcome with ohmic heating in which particulate and liquid portions are simultaneously heated if the electrical conductivities of both phases are similar (de Alwis and Fryer, 1992). The ohmic heating process developed by APV Baker consists of a series of cylindrical electrodes connected to a 50 Hz three-phase supply (Parrot, 1992). The food is pumped through the electrodes and is rapidly sterilized. The use of multiple electrodes provides a uniform electric field in each section.

Inactivation of microorganisms in the ohmic process is mainly attributed to thermal effect, rather than the nonthermal effect associated with field strength

(Palaniappan et al., 1992). Rate of heat generation in ohmic heating for a homogenous material can be written as (de Alwis and Fryer, 1992):

$$Q = \sigma E^2 \quad (1.1)$$

where Q is rate of heat generation per unit volume, σ is electrical conductivity (S/m), and E is voltage gradient (V/m). Electrical conductivity is the critical property affecting rate of heat generation. In the case of very low or zero electrical conductivity (insulator) , the resistance of the food will be so high that a desired heating rate cannot be achieved because of negligible current flow (Fryer and Li, 1993). Furthermore, the sample with infinite electrical conductivity cannot be heated due to insignificant resistance. Thus, feasible region of electrical conductivity is required for ohmic operation (Stirling, 1987; de Alwis and Fryer, 1992).

For solid foods, electrical conductivity measured at 60 Hz linearly increases with temperature (Palaniappan and Sastry, 1991a). However, electrical conductivity of the purified proteins, such as keratin, bovine serum albumin, and collagen, increase with temperature according to the Arrhenius equation (Pethig, 1979). The varied trend is due to the differences in the mode of conduction. Electrical conductivity of food is mainly caused by the movement of free ions (Na^+, Cl^-), whereas conductivity of the purified compound is induced by the electrons or ions associated in the molecule. Voltage gradient enhances diffusion of cell fluid in food, especially vegetables, which consequently increases electrical conductivity (Halden et al., 1990; Palaniappan and Sastry, 1991a,b; Schreier et al., 1993). Ajibola (1985) measured electrical conductivity of cassava and reported that electrical conductivity increased linearly with moisture content. However, exponential trend is reported in the purified proteins (Pethig, 1979).

For liquid (orange and tomato juices), electrical conductivity also increases with temperature, and it decreases with increased pulp content due to of the presence of nonpolar compounds in the pulp (Palaniappan and Sastry, 1991b).

Orientation of particle also affects electrical conductivity (de Alwis and Fryer, 1992; Sastry and Palaniappan, 1992b). In static ohmic process, when a low conductive particle aligns in parallel direction in high conductivity medium, it is heated slower than the medium because most of current density passes through the medium, rather than the particle (de Alwis and Fryer, 1992). However, if the particle aligns perpendicular to the current path, it will be heated at a faster rate. This is because the current through the medium is blocked by particle. When solid particle is more conductive than the liquid, it is heated at a faster rate in parallel direction and slower rate in perpendicular orientation. The effect of orientation is relatively small for cubic and spherical particle (Sastry and Palaniappan, 1991b). Fryer and Li (1993) reported that electric current tended to concentrate around the corner and sharp edges of the particle, leading to high heat generation in such areas.

Besides aseptic process of particulate foods, several attempts were made to use ohmic heating in the meat industry. de Alwis and Fryer (1990) summarized the application of ohmic heating in cooking frankfurters, meat patties, and coagulation of meat products. The design of ohmic heating devices were varied according to the shape and type of the product. In general, these meat products are packed into a non-conductive container and are contacted between two electrodes. Luijerink (1962) developed the ohmic apparatus to cook non-homogenous meat products. The sample was placed between several adjacent electrode pairs connected to a temperature sensor. The

temperature at each compartment was automatically controlled accordingly by adjusting power supply. The use of ohmic heating in solid foods has been very limited due to contamination problems caused by sparking and burning of the electrodes. Good contact between the electrode and product, which minimizes the scorching, has been suggested by various means, such as applying edible electrical conducting gel at electrode interface (Theimer and Heinze, 1977), using spring-loaded contacts, and assuring appropriate penetration of the product by the electrode (de Alwis and Fryer, 1990).

Appropriate electrode design is essential to prevent contamination from the electrode. Stirling (1987) reported that platinised titanium electrodes have been commercially used in ohmic heating process without contamination problems. Flow of alternating current in ohmic process occurs by migration of ions through ionic double layer, which is formed by electrostatic alignment of solution ions on the electrode surface of the opposite charge (Oldham and Myland, 1994). Potential across ionic double layer increases as current density increases. When the potential is increased to a certain level, it will initiate electrochemical reactions on the electrode surface and/or in food materials (Stirling, 1987). Therefore, it is necessary to determine the optimum current density in such a way that electrochemical reactions are prevented and a desired heating rate is achieved. For the platinised titanium electrode, electrochemical reactions in saturated NaCl solution is noticed at current density of 8,000 A/m^2, therefore, the maximum for this type of electrode is recommended at 4,000 A/m^2 (Stirling, 1987).

Destruction kinetics of foods

Chemical kinetics is the study of changes in concentration of a reactant molecule with time (Wang, 1992). The general rate equation can be expressed as:

$$\frac{dP}{dt} = kP^n \qquad (1.2)$$

where dP/dt is the rate of reaction, n is reaction order, P is concentration of reactant, and k is rate constant. The reaction order can be either integer or noninteger. Reaction order determined in food kinetics does not represent the mechanism of the reaction because reactions in food systems are very complex (Arabshahi and Lund, 1985; Wang, 1992). Nonenzymatic browning reactions can be described by zero order (Cohen et al., 1994; Peterson et al., 1994). Many other deteriorative reactions in food, such as loss of vitamins and microbial death can be represented by a first-order model (Mishkin et al., 1984; David and Merson, 1990; Fontana et al., 1993; Johnson et al., 1995). In addition, kinetic model of denaturation of whey proteins is described with n = 1.4 (Dannenberg and Kessler, 1988).

The study of destruction kinetics of food are usually encountered with thermal lag problems because instantaneous heating cannot be achieved. Degradation kinetics of food during sterilization can be obtained by either an isothermal or a nonisothermal procedure (Lenz and Lund, 1980). In isothermal experiments, the samples are heated at various temperatures for various times. The effect of the heating and cooling phase of the thermal curve is compensated for by the use of a control which is only heated and cooled. Time-temperature effects during holding can be evaluated by subtracting out the heating and cooling effect. Rate constant is simply determined from equation (1.2) using either

differential or integral method described by Hill and Grieger-Block (1980). For the nonisothermal procedure, the heat-up and cool-down phases are used as part of the final data analysis. Heating time is converted to the dimensionless group, Fourier number (Lenz and Lund, 1977a,b). Lethality of microorganism or nutrient is calculated from integration of the Arrhenius equation over Fourier heating time. Kinetic parameters (rate constant and activation energy) are obtained by an iterative procedure (Lenz and Lund, 1980). Lenz and Lund (1980) suggested that when the half-life of the component is short, the nonisothermal method is recommended to derive kinetic parameters. In contrast, isothermal procedure can be used to analyze kinetics of compounds whose half-life is long, and effect of thermal lag is insignificant.

In addition to the method described by Lenz and Lund (1977 a,b), the effect of thermal lag in kinetic analysis can be accounted for by equivalent time which is the time required in instantaneous heating (Davies et al., 1977; Perkin et al., 1977). The derived equation for equivalent time is based on the principle of thermal death time (TDT), which expresses rate constant and its temperature dependence in terms of decimal reduction time (D-value) and z value, respectively (Perkin et al., 1977). Perkin et al. (1977) applied this concept to analyze thermal inactivation kinetics of *Bacillus stearothermophilus* spores heated in glass capillary at ultra high temperature range. They concluded that the effect of heat-up period was negligible up to 135°C. Above this temperature, the heating period became more significant and the calculation of equivalent time provided more reliable kinetics. Calculation of thermal lag effect based on TDT concept has also been applied to determine kinetics for other microorganisms (Palaniappan et al., 1992) and enzymes (Wicker and Temelli, 1988; Awuah et al., 1993). Lenz and Lund (1977b) indicated that

the TDT provided accurate kinetic parameters only in the small temperature ranges. Therefore, the TDT model is satisfactory for the compounds with high activation energy of destruction (microorganism). Kinetics for those with low activation energy of destruction (enzyme or nutrient) can be determined using the Arrhenius equation.

The equivalent point method is another approach to determine the effect of thermal lag (Swartzel, 1982). This method is derived from Arrhenius relationship of rate constant. The equivalent time and temperature represent the condition where the effect on constituent concentration of the nonisothermal system would be equivalent to that of the instantaneous isothermal condition. Thermal effect is defined as (Swartzel, 1982):

$$G = \int_0^t \exp\left[\frac{-E_a}{R}\left(\frac{1}{T(t)} - \frac{1}{T_{ref}}\right)\right] dt \qquad (1.3)$$

where t is heating time, E_a is activation energy, R is gas constant, and T_{ref} is reference temperature (121.1°C for sterilization process). Thermal effects are calculated at heat-up, holding, and cool-down periods, and are totaled for the three sections for each selected E_a value. With a set of E_a values, equivalent time and temperature are obtained from the following equation (Nunes and Swartzel, 1990):

$$G^{total} = t_E \exp\left[\frac{-E_a}{R}\left(\frac{1}{T_E} - \frac{1}{T_{ref}}\right)\right] \qquad (1.4)$$

G^{total} is total thermal reduction, t_E is equivalent time, T_E is equivalent temperature, Both equivalent time and temperature can be accurately estimated using either nonlinear least squares regression or weighted least squares regression (Nunes and Swartzel, 1990). Equivalent time and temperature are independent of activation energy (Swartzel, 1982;

Swartzel, 1984). Therefore, kinetic parameters (rate constant and activation energy) are not directly obtained from this method.

A different approach to analyze kinetics of food destruction under nonisothermal conditions is a dynamic method (Mishkin et al., 1984; Rhim et al., 1989; Cohen et al., 1994). The physical or chemical changes of the interested compound are monitored at each time interval during the course of reaction. Then, reaction order and activation energy are estimated from the following relationship:

$$\frac{dP}{dt} = k_0 \left[e^{\frac{-E_a}{RT(t)}} \right] P^n \qquad (1.5)$$

Information required to determine kinetic parameters in the above equation is temperature history and the changes in quality factors with respect to time (dP/dt). Accuracy of kinetic parameters obtained from this method highly depends on the approximation of dP/dt. This method is found to be very sensitive to the experimental errors (Rhim et al., 1989). Rhim et al. (1989) recommended the use of equivalent point method (Swartzel, 1982) to obtain more accurate kinetic parameters at a wide range of temperatures. A similar dynamic approach was also applied to determine degradation of ascorbic acid in potato during dehydration process (Mishkin et al., 1984).

Chapter2

OHMIC HEATING MAXIMIZES GEL FUNCTIONALITY OF PACIFIC WHITING SURIMI

Jirawat Yongsawatdigul, Jae W. Park, Edward Kolbe,
Yaser AbuDagga, and Michael Morrissey

Journal of Food Science
Institute of Food Technologists, Chicago, IL
1995, Volume 60, No.1: 1-5

Abstract

Surimi without enzyme inhibitors containing 78% moisture and 2% NaCl was heated conventionally and ohmically to 90°C after holding at 55°C for 0, 1, 3 and 5 min. Gels heated slowly in a water bath exhibited poor gel quality, while the ohmically heated gels without holding at 55°C showed more than a twofold increase in shear stress and shear strain over conventionally heated gels. Degradation of myosin and actin was minimized by ohmic heating, resulting in a continuous network structure. Ohmic heating with a rapid heating rate was an effective method for maximizing gel functionality of Pacific whiting surimi without enzyme inhibitors.

Key Words: ohmic heating, surimi, proteolysis, whiting

Introduction

Pacific whiting (*Merluccius productus*) is the most abundant fishery resource off the West Coast of the U.S. with an allowable biological catch of 140,000 to 250,000 metric tons for the last three years (NMFS, 1993). The major portion of the harvested fish is used to produce surimi, a washed and dewatered fish mince, which is further utilized as a raw ingredient for surimi seafood products (Pacheco-Aguilar et al., 1989). Due to the presence of heat-stable endogenous proteinase in the flesh (Nagahisa et al., 1981; Patashnik et al., 1982; Erickson et al., 1983), Pacific whiting surimi normally exhibits undesirable textural properties unless food grade enzyme inhibitors are added (Chang-Lee et al., 1989; Morrissey et al., 1993). The proteinase responsible for textural degradation was purified and characterized as cathepsin L with an optimum temperature at 55°C (Seymour et al., 1994). A gel weakening at around 55-60°C has also been reported in threadfin bream (*Nemipterus bathybius)* (Toyorahara and Shimizu, 1988), Atlantic menhaden (*Brevoorti tyrannus*) (Boye and Lanier, 1988), arrowtooth flounder (*Atheresthes stomias*)(Greene and Babbitt, 1990), white croaker (*Micropogon opercularis*) and oval filefish (*Navodon modestus*) (Toyohara et al., 1990).

Beef plasma proteins, egg white, and other food grade enzyme inhibitors have been used to inhibit proteolytic activity (Nagahisa et al., 1981; Chang-Lee et al., 1989; Hamann et al., 1990; Porter, 1990; Morrissey et al., 1993). However, these enzyme inhibitors used commercially have some negative effects such as high cost, off-odor, off-color and labeling concerns. Consequently, processing alternatives to overcome the textural deterioration of Pacific whiting surimi are needed.

Ohmic heating is a method in which alternating electric current is passed through an electrically conducting food product (Biss et al., 1989). Heat is internally generated, resulting in a rapid heating rate. Because heat is simultaneously generated in liquid and solid phases, the temperature increase in the product is uniform, compared with the conventional process in which heat is applied at the external boundary (de Alwis and Fryer, 1990; Parrot, 1992).

To minimize proteolysis, thermal inactivation of the enzyme should be quickly achieved (Patashnik et al., 1982). Greene and Babbitt (1990) demonstrated that textural degradation caused by proteolysis in arrowtooth flounder was notably reduced when cooked by microwaves. However, microwave processes can create non-uniform heating patterns because of differences in dielectric properties of the sample and non-uniform microwave field distribution (Decareau, 1985). In addition, microwave heating rates are so rapid that conductive heat transfer cannot uniformly take place (Datta and Hu, 1992). Since ohmic heating provides a rapid heating rate with more uniform temperature distribution than microwave heating, it could be an alternative to overcome the proteinase problem. Our objective was to investigate the feasibility of ohmic heating to maximize gel functionality of whiting surimi without enzyme inhibitors.

Materials and Methods

Unfrozen Pacific whiting (*Merluccius productus)* surimi (100 kg) without food grade enzyme inhibitors and cryoprotectants was obtained from Point Adams Packing Co.(Hammond, OR). Surimi was packed in polystyrene foam containers with ice and delivered to the OSU Seafood Laboratory (Astoria, OR) within 30 min. Each 17 kg of

surimi was mixed with cryoprotectants, 4% sucrose, 4% sorbitol (ICI Specialties, New Castle, DE), and 0.3% sodium tripolyphosphate (B.K. Ladenburg Corp., Cresskill, NJ) using a Hobart vertical mixer(Hobart Manufacturing Co., Troy, OH) for 2 min. Mixing was carried out in a cold room (3°C). Mixed surimi was then divided into ≈ 500-g portions and vacuum-packed in plastic trays. Samples were frozen and stored in a -30°C room.

Surimi gel preparation

Frozen surimi samples were thawed at room temperature (≈23°C) for 2 hr and cut into small pieces. Moisture content of surimi was determined using the microwave procedure described by Morrissey et al. (1993). One kg of surimi was chopped for 1.5 min in a Stephan vertical vacuum cutter (model UM 5 universal, Stephan Machinery Co., Columbus, OH) at low speed. Salt was added and mixed with surimi another 1.5 min. Then, ice was added to adjust final moisture to 78%, and the sample was further chopped at high speed under vacuum (600 mm Hg) for 3 min. The paste was maintained below 8°C during chopping. An aliquot of the paste (≈300 g) was stuffed into stainless steel cooking tubes (1.9 cm i.d. x 17.5 cm long) and heated in a 90°C water bath for 15 min. Change in temperature at the geometric center of the tube sample was measured using a T-type thermocouple, and recorded every 30 sec in a datalogger (model 21x, Campbell Scientific Inc., Logan, UT). The remainder was stuffed into PVC tubes (1.9 cm i.d. x 20.5 cm long) and heated using an ohmic heating apparatus. Surimi gels heated by both heating methods were kept at 4°C for torsion tests, scanning electron microscopy, and gel electrophoresis within 24 hr. Gel preparation was replicated twice.

Ohmic heating apparatus

An ohmic heating apparatus was developed from two rhodium-coated stainless steel electrodes at each end of a PVC tube (1.9 cm i.d. × 20.5 cm long), a variable transformer (Powerstat F246, Newark Electronics, Eugene, OR), a current transducer (CT5-010A, Syntex Co., Wilsonville, OR), and a voltage transducer (VT-240A, Syntex Co., Wilsonville, OR). To minimize electrical hazards, the sample tube and electrodes were housed inside a plexiglass enclosure equipped with an open-snap action switch, which was activated when the plexiglass box was closed.

A 1.6-mm-diameter hole was drilled at the center of the PVC tube, and a saddle (diameter = 1.9 cm) was glued in that position to make a thermocouple port. To measure temperature at the geometric center of the sample, a 30-gauge teflon-sealed type-T thermocouple (OMEGA Engineering Inc., Stamford, CT) with a compression fitting (OMEGA Engineering Inc., Stamford, CT) was used. Sixty-Hz alternating current at 240 V was supplied to a variable transformer. Voltage output ranged from 0 to 280 V. Voltage and current transducers were used to measure voltage across and current through the sample, respectively.

Ohmic heating procedure

A sample tube containing surimi paste was placed on the sample holders (Fig. 2.1). An electrode was inserted into each end of the tube to obtain a sample length of ≈15 cm. One of the electrode support rods was connected to an air cylinder (Power Transmission Products, Albany, OR). Pressure of 448 kPa was applied to the sample to maintain an

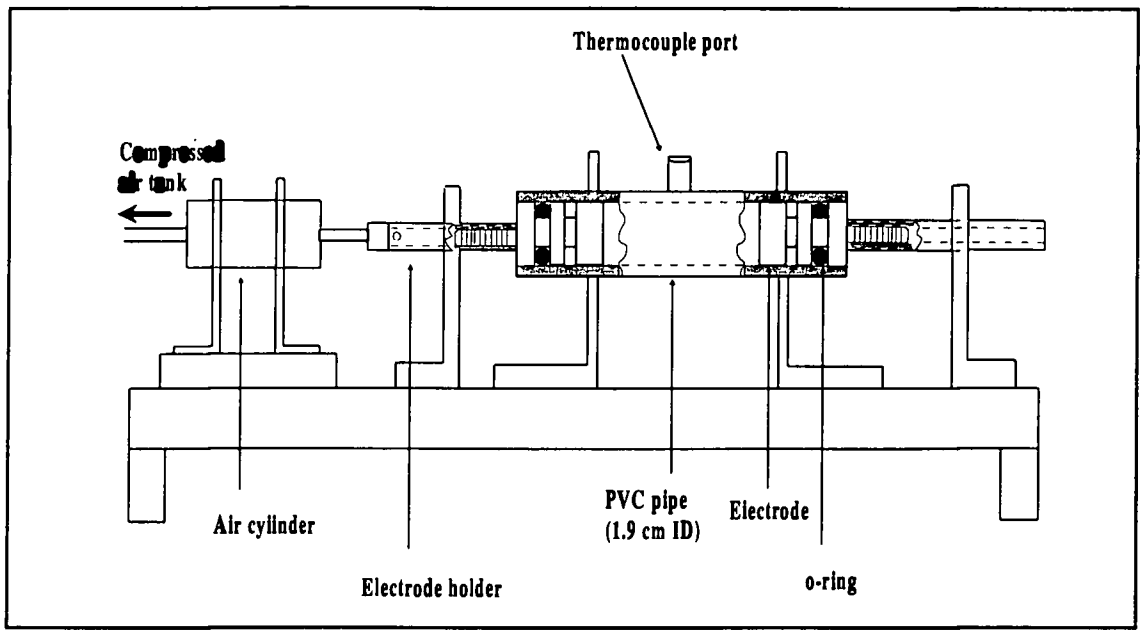


Figure 2.1. Diagram of ohmic heating apparatus.

air-free contact between the electrodes and the paste. Based on preliminary studies, gel strength of surimi and corrosion of the electrodes increased with applied voltage gradient. However, at voltage gradient >13.3 V/cm, corrosion was so severe that it interrupted electrical circuits. Therefore, the apparatus was operated at the voltage gradient of 13.3 V/cm (applied voltage of 200 V). The pastes were heated to 55°C, and were held at 55°C for 0, 1, 3, and 5 min. Temperature was controlled by manually adjusting the variable transformer. After each holding time was achieved, the sample was heated to 90°C by re-applying 200 V. Each heating treatment was replicated 3 times. Temperature, voltage, and current changes during heating were recorded at 1 sec intervals on a datalogger (model 21X, Campbell Scientific, Inc., Logan, UT).

Torsion test

Torsion failure tests were performed as described by NFI (1991). Gels were equilibrated to and tested at room temperature. Ten hourglass samples were used for each treatment. Shear stress and shear strain at failure were calculated from equations described by Hamann (1983).

SDS-PAGE

Unheated surimi paste and heated samples were analyzed by SDS-PAGE. Twenty seven mL of heated (95°C) 5% sodium dodecyl sulfate solution was added to 3 g sample. The mixtures were homogenized for 1 min at a speed setting of 3-4 by a Polytron (Brinkmann Instruments, Westbury, NY). The homogenates were incubated in an 80°C water bath for 1 hr, and were centrifuged at 7,000 × g (Sorvall, DuPont Co., Newton, CT) for 10 min at room temperature. The protein concentration of supernatants was measured by the Lowry method (Lowry et al., 1951), using bovine serum albumin (Sigma Chemical Co., St. Louis, MO) as a standard.

Electrophoresis was carried out according to the procedure of Laemmli (1970). Stacking gels and separating gels were 4% (w/v) and 10% (w/v) polyacrylamide, respectively. The amount of protein loaded on the polyacrylamide gel was 60 μg. The separated proteins were stained with 0.125% Coomassie brilliant blue R-250 (Bio-Rad, Richmond, CA), and destained in a solution containing 25% ethanol and 10% acetic acid.

Scanning electron microscopy

Small samples of heated gels were cut with a razor blade and fixed for 2 hr in 1% osmium tetroxide solution buffered with 0.125 cacodylate buffer pH 7.2. Samples were immersed for 20 min in 1,1 dimethoxypropane to remove unreacted osmium tetroxide and water. They were then soaked in absolute acetone twice, (10 min each). The samples were freeze-dried (model 500, Refrigeration for Science Inc., Island Park, NY) at a vacuum of 750 mm Hg and -73°C for 6 hr. Dried samples were mounted on aluminum specimen stubs (Ted Pella Inc., Redding, CA) using DUCO cement (Devcon Corp., Wood Dale, IL), and coated with gold in a sputter (model S150B, Edwards High Vacuum, West Sussex, England) for 45 sec at 10 kV and 40 mA. The samples were examined at 7,500× using a scanning electron microscope (Amray 1000A, Bedford, MA) at an accelerating voltage of 10 kV.

Statistical analysis

Since there were no significant differences ($P > 0.05$) between two replications, data were evaluated by one-way analysis of variance using STATGRAPHIC Version 6.0 (Manugistics Inc., Rockville, MD). Differences within heating methods were determined using the least significant difference (LSD) multiple range test (Box et al., 1978)

Results and Discussion

Effects of heating methods on gel strength and myosin heavy chain

Shear stress and shear strain values of surimi gels heated conventionally (CON) were compared with those heated ohmically (Figs. 2.2A,B). Shear stress and shear strain are correlated with surimi gel hardness and cohesiveness, respectively (Hamann and MacDonald, 1992). Hardness of OH0 and OH1 gels was significantly higher than that of CON, OH3, and OH5 gels ($P<0.05$). No significant differences occurred in cohesiveness between CON and OH3 gels ($P>0.05$). However, cohesiveness of these two gels was significantly lower than that of OH0 and OH1, and higher than that of OH5 gels ($P<0.05$). Since an acceptable surimi gel should have a strain value of at least 2.0 (Hamann et al., 1990), textural properties of surimi gels heated in a 90°C water bath and those held ohmically at 55°C for 3 and 5 min were very poor. Low shear stress and shear strain in OH3 and OH5 illustrated the effect of proteinase activity at 55°C on textural characteristics of Pacific whiting surimi gel. Those gels heated ohmically to 90°C with a voltage gradient of 13.3 V/cm (200 V) and those held in ohmic heating at 55°C for 1 min showed good textural properties with strain values of 2.81 and 2.50, respectively. These were more than twice the value of conventionally heated surimi gels. Based on torsion test results, Pacific whiting gels without enzyme inhibitors heated ohmically without holding at 55°C were slightly better than those heated conventionally with incorporation of enzyme inhibitors such as beef plasma proteins, egg white, and potato extract. These had a maximum strain value around 2.5 (Morrissey et al., 1993).

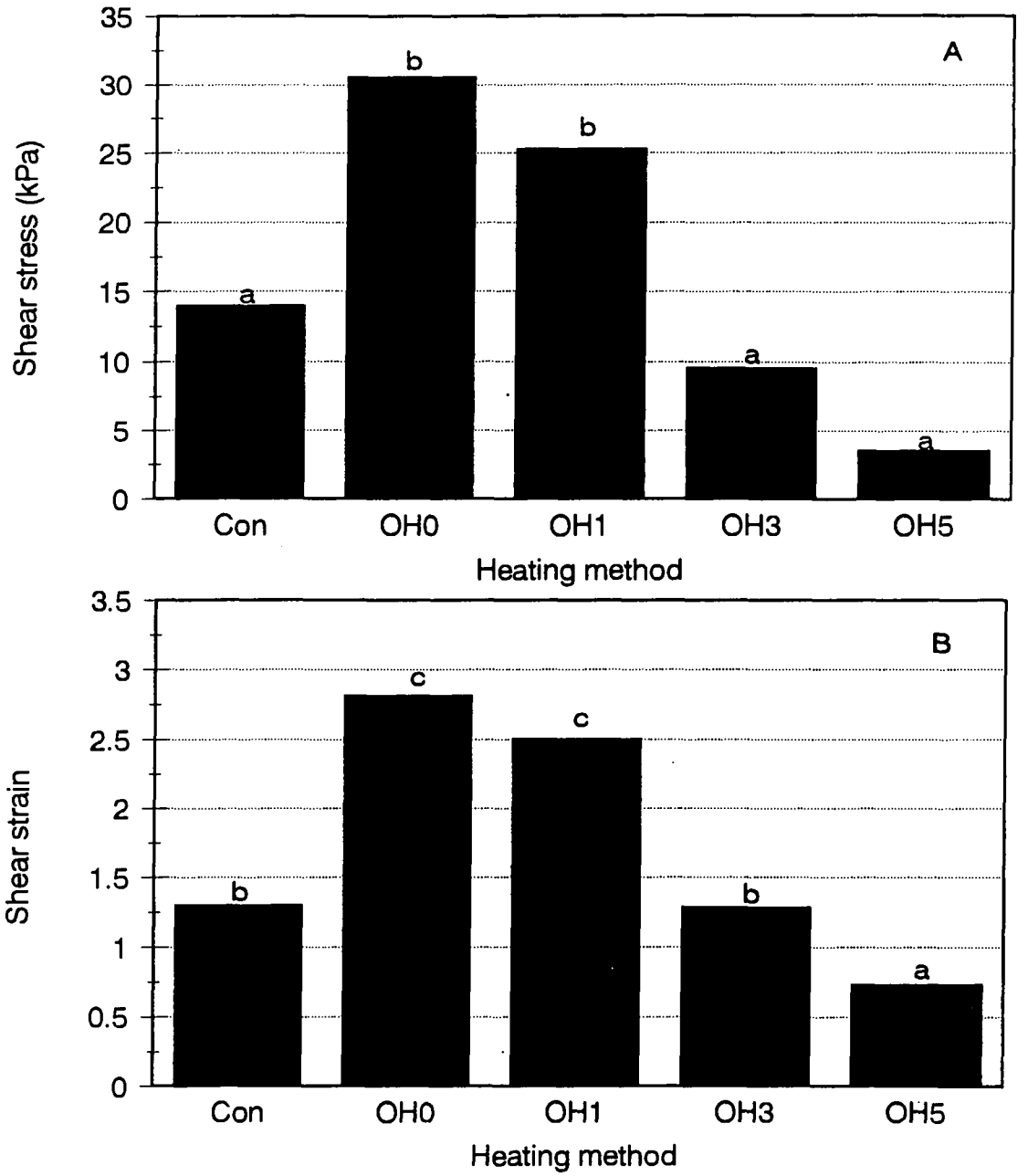


Figure 2.2. Shear stress (A) and shear strain (B) of surimi gels heated by various heating methods. Means with the same letter (a,b,c) are not significantly different ($P > 0.05$). CON = conventional heating (heated in a 90°C water bath for 15 min). OH0 = heated ohmically with voltage gradient of 13.3 V/cm (applied voltage = 200 V) to reach 90°C. OH1, OH3, and OH 5 = heated ohmically with voltage gradient of 13.3 V/cm and held for 1, 3, and 5 min, respectively, at 55°C before heating to 90°C.

Seymour et al. (1994) illustrated that activity of the proteinase purified from Pacific whiting gradually increased as temperature increased until peaking at 55°C. It then started decreasing and reached a minimum at 70°C. The myosin and actin have been reported as a substrate of endogenous proteinase found in threadfin-bream surimi (Toyohara and Shimizu, 1988; Toyohara et al., 1990), and of cathepsin L purified from rabbit skeletal muscle (Okitani et al., 1980) and Pacific whiting (An et al., 1994). Temperature profiles of conventional heating (Fig. 2.3), indicate that surimi was slowly heated in a 90°C water bath and was exposed to a temperature range of 40-60°C for ≈2 min. Moreover, it took about 6 min to reach 70°C at which the proteinase could be inactivated. Due to the slow heating rate, the enzyme was activated and started degrading the myosin before thermal inactivation occurred. Since the myosin plays an important role in gel network formation (Niwa, 1992), severe degradation of the myosin heavy chain (Fig. 2.4) resulted in low gel strength (Figs. 2.2A, B). When holding time at 55°C in ohmic heating was prolonged, integrity of the myosin heavy chain and actin were diminished (Fig. 2.4). The OH0 and OH1 samples exhibited higher intensity of myosin and actin than the CON sample, while less intensity was observed in the OH3 and OH5. As surimi was subjected to optimum temperature of the enzyme for a longer period of time, more degradation of myosin and actin occurred. For the samples held at 55°C for 5 min, the myosin heavy chain and actin almost disappeared. These results suggest that gel-weakening associated with breakdown of the myosin heavy chain and actin in Pacific whiting surimi was due to proteolytic activity. The highest gel strength and the most intense myosin heavy chain and actin bands were observed in OH0 gels that

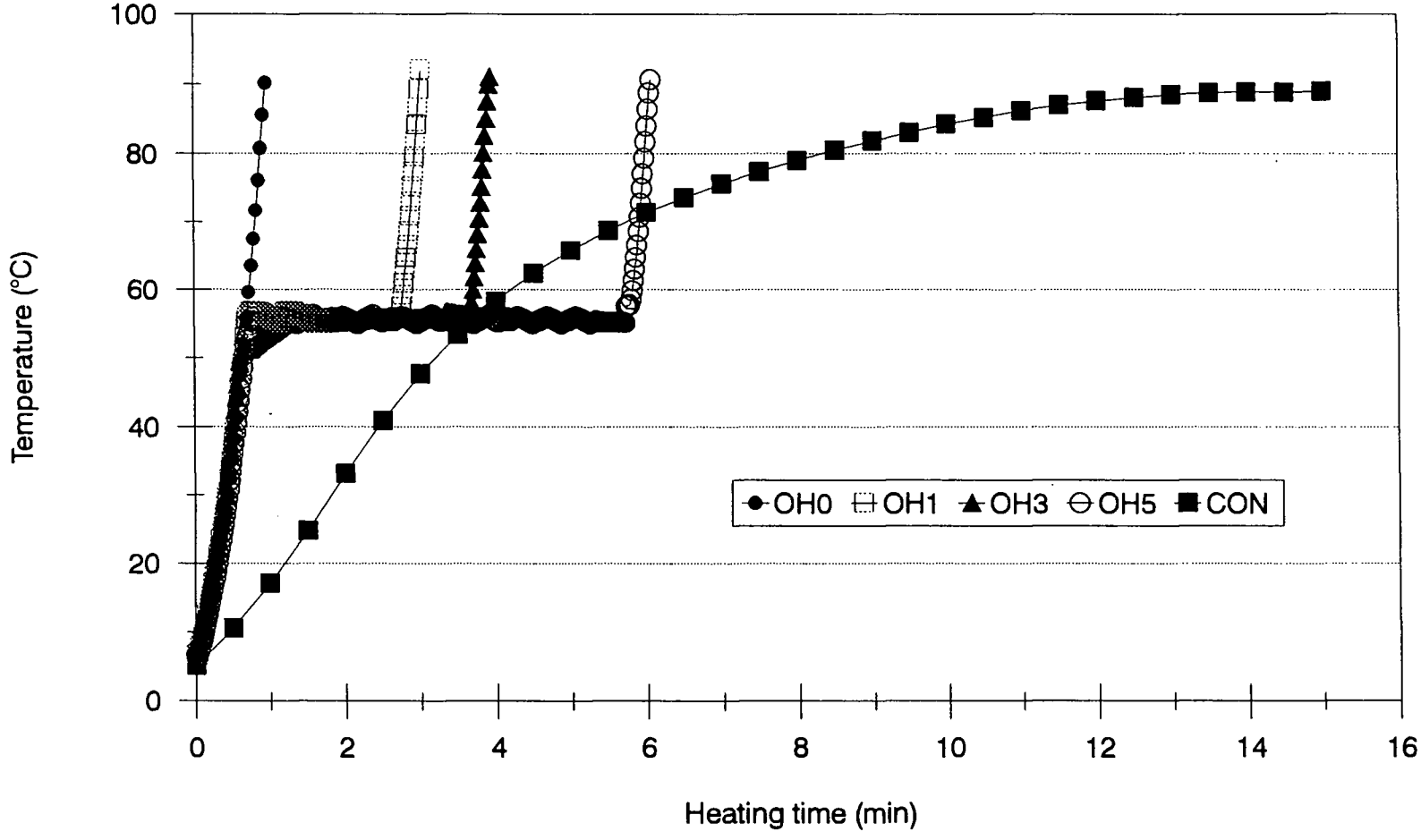


Figure 2.3. Temperature profiles of surimi gels heated by various heating methods (abbreviations indicated in Fig. 2.2).

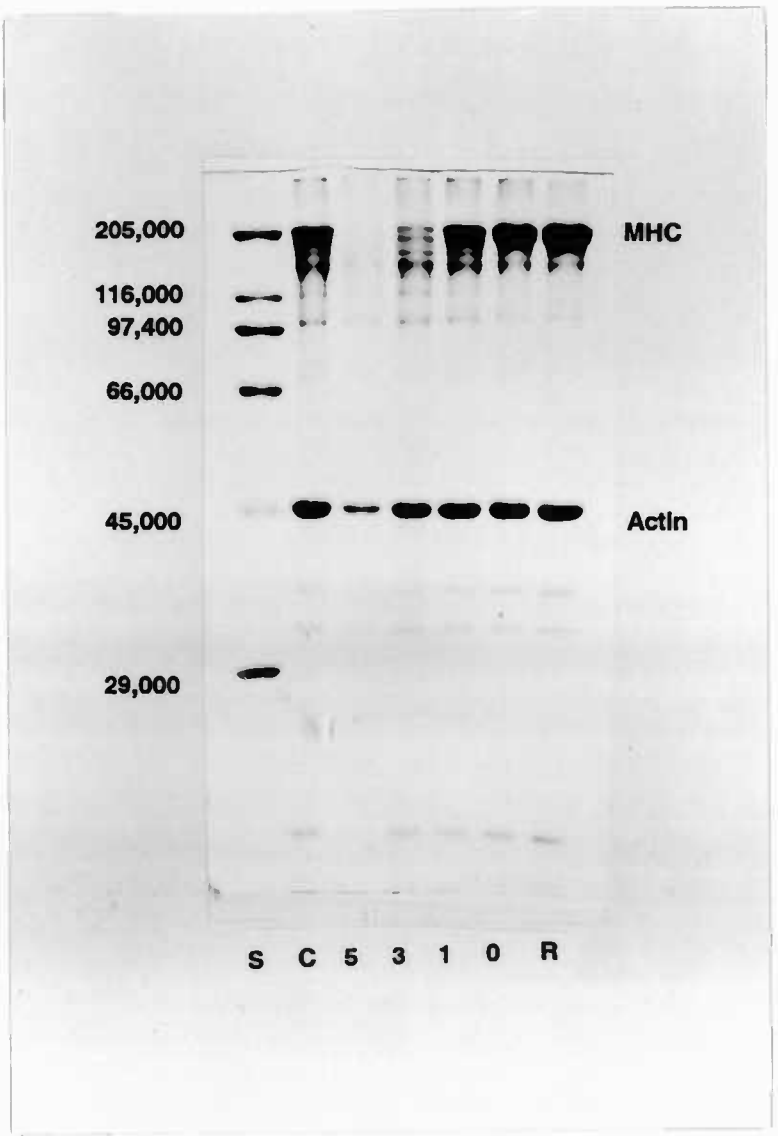


Figure. 2.4. SDS-PAGE separation pattern of surimi gels heated by various heating methods: (R) unheated surimi paste; (0) surimi heated ohmically with 13.3 V/cm to 90°C; (1) surimi heated ohmically with 13.3 V/cm and held at 55°C for 1 min before heating to 90°C; (3) surimi heated ohmically with 13.3 V/cm and held at 55°C for 3 min before heating to 90°C; (5) surimi heated ohmically with 13.3 V/cm and held at 55°C for 5 min before heating to 90°C; (C) surimi heated conventionally; (S) High molecular weight standard; MHC: Myosin heavy chain.

were heated to 90°C within 1 min. The myosin and actin bands of the OH0 were comparable to those of unheated surimi paste (Fig. 2.4). Probably rapid thermal inactivation minimized degradation of the myosin and actin.

The effect of ohmic heating on surimi gel quality was also reported by Shiba (1992). Gel strength of surimi made from walleye pollock, white croaker, and sardine was increased when ohmically heated at a heating rate of 47°C/min was applied. Shiba and Numakura (1992) illustrated that walleye pollock surimi (diameter = 3 cm, length = 18 cm) heated by ohmic heating within 1 min exhibited a 33.1% increase in gel strength and a 38.1% increase in myosin heavy chain content in comparison with that heated conventionally 50 min. Gel strength and the myosin heavy chain content were decreased when the samples were heated ohmically with a slow heating rate. Since there is no substantial evidence of the presence of endogenous proteinase(s) in walleye pollock, an increased gel quality may not be solely affected by rapid inactivation of the proteinase(s). However, ohmic heating with a rapid heating rate can evidently improve surimi gel quality.

Although the intensity of the myosin heavy chain band of CON gels was visually greater than that of OH3 gels, no differences ($P > 0.05$) occurred in shear strain and shear stress between those two gels. Toyohara and Shimizu (1988) also found a similar discrepancy. They indicated that the intensity of myosin did not always accordingly correlate with gel strength. We could hypothesize that, once the myosin was degraded by the enzyme to a specific level i.e. in CON sample, the gel network could not be properly formed regardless of degree of degradation. This would agree with the fact that intact myosin is required for gel formation (Ziegler and Acton, 1984). When the myosin

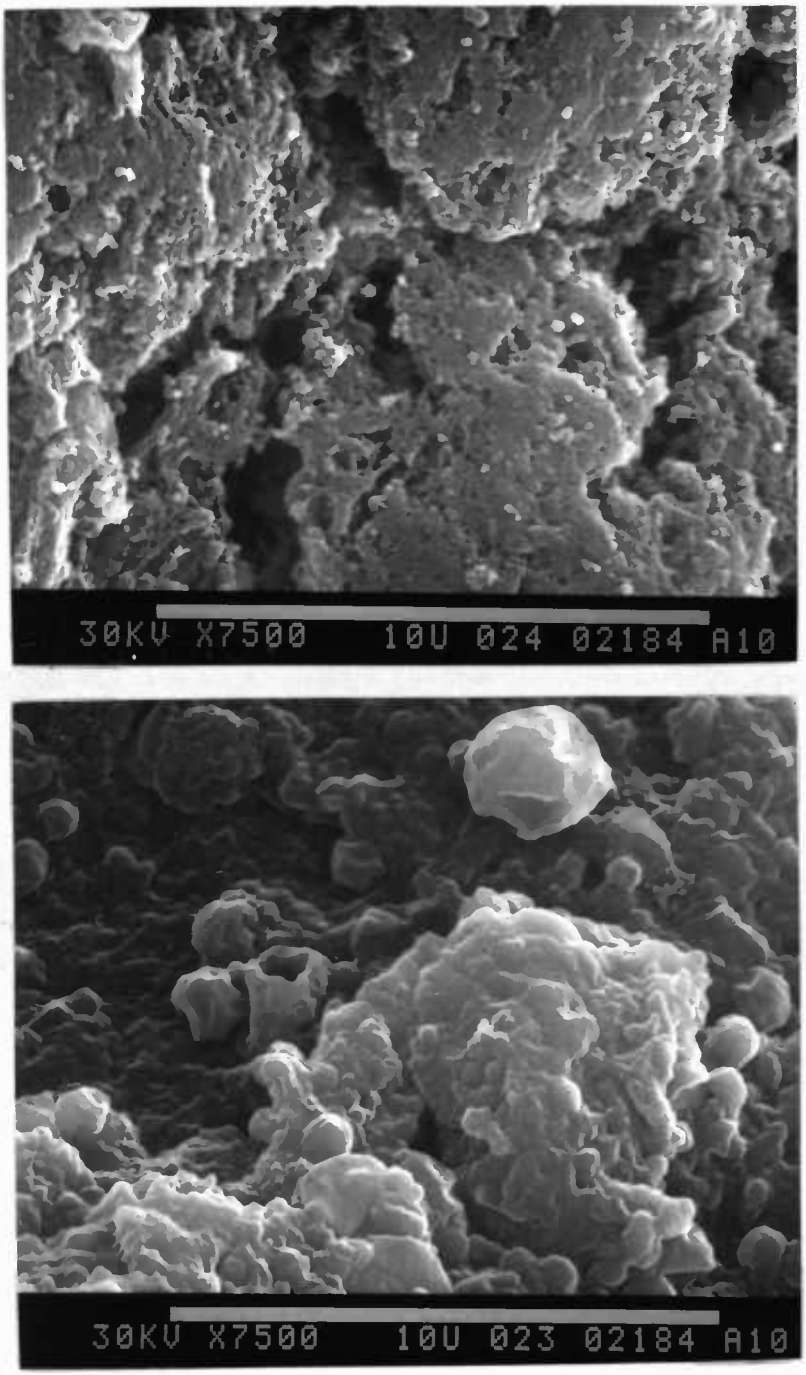
is severely hydrolyzed to smaller protein fragments (OH3 and OH5 in Fig. 2.4), a gel network was hardly developed, resulting in extremely low gel strength and very mushy texture.

Effects of heating methods on gel microstructure

Scanning electron micrographs of surimi gels heated by conventional and ohmic heating were compared (Fig. 2.5a,b). Microstructure of CON gels showed a sponge-like structure with several voids (Fig. 2.5a). The protein particles appeared to be randomly aggregated, without ordered network formation. This corresponded to a poor gel strength observed in the conventionally heated samples. On the other hand, OH0 gels involved formation of a continuous network exhibiting some definite degree of order (Fig. 2.5b). The micrographs suggested that poor gel network formation resulted from degradation of myofibrillar proteins caused by proteolysis.

Conclusions

Pacific whiting gels rapidly heated to 90°C by ohmic heating with a voltage gradient of 13.3 V/cm demonstrated superior shear stress and shear strain. Degradation of the myosin heavy chain and actin was significantly minimized. Scanning electron micrographs showed a continuous gel structure corresponding to its gel strength. A decreased gel strength associated with poor gel network and degradation of the myosin heavy chain and actin was noticed as holding time at optimum temperature of the proteinase, 55°C, was prolonged. Surimi gels were susceptible to proteolysis under



A

B

Figure 2.5. Scanning electron micrographs of surimi gels heated by water bath and ohmic heating. (A) = CON; (B) = OH0 (abbreviations indicated in Fig. 2.2).

conventional heating since a slow heating rate allowed the enzyme to degrade more myosin heavy chain and actin.

Acknowledgement

This work was supported by grant No. NA36RG0451 (project no. R-SF-2) from the National Oceanic and Atmospheric Administration to the Oregon State University Sea Grant College Program and by appropriations made by the Oregon State Legislature. The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA or any of its subagencies.

Chapter 3

ELECTRICAL CONDUCTIVITY OF PACIFIC WHITING SURIMI PASTE DURING OHMIC HEATING

Jirawat Yongsawatdigul, Jae W. Park, and Edward Kolbe

Journal of Food Science
Institute of Food Technologists, Chicago, IL
1995, Volume 60, No.5: 922-925,935

Abstract

Electrical conductivities of Pacific whiting surimi paste containing various moisture (75, 78, 81, and 84%) and added salt (1, 2, 3, and 4%) were measured using an ohmic heating apparatus at alternating current of 3.3, 6.7, and 13.3 V/cm. Electrical conductivity of surimi significantly increased with temperature and salt content and slightly increased with moisture content. Electrical conductivity was found to be linearly correlated with temperature ($r^2 = \sim 0.99$). Generally, voltage gradient did not significantly affect electrical conductivity. However, variations of electrical conductivity with voltage gradient observed at combinations of high moisture (81, 84%) and NaCl content (3, 4%) were probably caused by electro-chemical reactions at electrode surfaces. The empirical model of electrical conductivity predicted values within 15.6% of independent experimental results.

Key Words: Ohmic heating, electrical conductivity, surimi, whiting.

Introduction

Surimi made from Pacific whiting (*Merluccius productus*) normally undergoes textural degradation due to the presence of a heat-stable endogenous proteinase in the flesh (Chang-Lee et al., 1989; Patashnik et al., 1982). The proteinase has been identified as cathepsin L with an optimum temperature at 55°C (Seymour et al., 1994). A breakdown of myofibrillar proteins caused by proteolysis, inhibits the proper development of a three-dimensional gel structure (Niwa, 1992). As a result, the gel forming ability of Pacific whiting surimi is poor unless food grade enzyme inhibitors, such as beef plasma proteins, egg white, and potato extract are added (Chang-Lee et al., 1990; Morrissey et al., 1993; Porter, 1990). In conventional heating methods, heat is transferred from the heating medium to the product interior by means of both convection and conduction. Yongsawatdigul et al. (1995) illustrated that temperature at the center of a cylindrical sample (I.D.=1.9 cm, length=17.9 cm) of whiting surimi paste heated in a 90°C water bath took approximately 10 min to reach 70°C. The proteinase is completely inactivated at this temperature (Seymour et al., 1994). For two minutes of this process, surimi was exposed to a temperature range in which the proteinase is the most active: 40-60°C. Thus, a typical slow heating rate activates the proteinase to degrade myofibrillar proteins before the proteinase can be thermally inactivated. To minimize proteolytic activity without the use of enzyme inhibitors, the proteinase would need to be thermally inactivated as quickly as possible. This could be achieved through a rapid heating methods as reported by Greene and Babbitt (1990) and Yongsawatdigul et al. (1995).

Ohmic heating is a method in which an alternating electric current is passed through an electrically conducting food product (de Alwis and Fryer, 1992). Heat is internally generated due to electrical resistance of the food sample, supporting a rapid heating rate. Moreover, ohmic heating can provide a uniform temperature distribution because both liquid and solid phases are heated simultaneously (Parrott, 1992). These advantages have led to development of commercial ohmic sterilization for particulate foods (Biss et al., 1989; de Alwis and Fryer, 1990). Application of ohmic heating in seafoods has also been investigated. Gel strength of surimi made from walleye pollock, white croaker, threadfin bream, and sardine was improved when samples were ohmically heated, as compared with samples heated in a 90°C water bath (Shiba, 1992; Shiba and Numakura, 1992). Pacific whiting surimi heated ohmically with an applied voltage gradient of 13.3 V/cm exhibited good gel quality (shear stress of 30.5 kPa; shear strain of 2.8), while those gels heated conventionally had shown shear stress and strain values of 13.9 kPa and 1.3, respectively (Yongsawatdigul et al., 1995). Improved gel functionality was accompanied by significant retention of myosin heavy chain, indicating that the endogenous proteinase was quickly inactivated by the rapid heating rate associated with ohmic heating.

To optimally design the ohmic process, electrical conductivity of food materials during ohmic heating must be elucidated, because it is a critical parameter influencing the rate of heat generation, as described by Palaniappan and Sastry (1991a) and de Alwis and Fryer (1992). Although electrical conductivities measured using alternating current (50 or 60 Hz) of various food products have been studied (Halden et al., 1990; Palaniappan and Sastry, 1991a, b; Schreier et al., 1993), information about electrical conductivity of

seafoods, particularly surimi paste, is still unknown. Therefore, the objectives of this study were: (1) to investigate the effects of temperature, voltage gradient, moisture, and added salt content on the electrical conductivity of Pacific whiting surimi paste and (2) to establish an empirical model of electrical conductivity as a function of compositional variables.

Materials and Methods

Surimi paste preparation

Pacific whiting (*Merluccius productus*) surimi was taken from a process line of a local manufacturer and mixed with 4% sucrose, 4% sorbitol (ICI Specialties, New Castle, DE), and 0.3% sodium tripolyphosphate (B.K. Ladenburg Corp., Cresskill, NJ) at the OSU Seafood Laboratory. No food grade enzyme inhibitors were added. The samples were frozen and kept in a -30°C cold room throughout the experiment. Frozen surimi samples were thawed at room temperature (~23°C) for 2 hr and cut into small pieces (about 3 cm cubes). Sixteen batches of surimi paste representing four different moisture contents (75, 78, 81, and 84% wet basis) and added NaCl (Mallinckrodt Inc., Paris, KY) contents (1, 2, 3, and 4%w/w) were randomly prepared. One kg of partially thawed surimi was chopped for 1.5 min in a Stephan vertical vacuum cutter (model UM 5 universal, Stephan Machinery Co., Columbus, OH). Salt was added and mixed with surimi for another 1.5 min. Then ice was added to adjust final moisture content to desired level and the sample was further chopped at high speed under vacuum of 600 mmHg for 3 min. Final moisture content of each batch was determined using the

standard oven method (AOAC, 1990). The paste was stuffed into PVC tubes (1.9 cm I.D. x 20.5 cm long) and heated using the ohmic heating apparatus described below.

Electrical conductivity measurement

A sample tube containing surimi paste was placed on the sample holders as shown by Yongsawatdigul et al. (1995). An electrode was inserted into each end of the tube to obtain a sample length of 15 cm. A minimum pressure that can maintain an air-free contact between the rhodium-coated stainless steel electrodes and the paste, 448 kPa, was applied to the sample. The sample was heated to 90°C using alternating current of 60 Hz at applied voltages of 50, 100, and 200 V, corresponding to voltage gradients of 3.3, 6.7, and 13.3 V/cm, respectively. Temperature at the geometric center of the samples was continuously measured with a T-type thermocouple covered with teflon to prevent interference from the electrical field. Voltage and current were measured using a voltage and current transducers, respectively. Temperature, voltage and current data were recorded on a datalogger (model 21X, Campbell Scientific, Inc., Logan, UT) at 10, 2, and 1 sec intervals, according to the applied voltage gradient of 3.3, 6.7, and 13.3 V/cm, respectively. Electrical conductivity of surimi paste during ohmic heating was measured twice at each voltage gradient.

The accuracy of the voltage and current transducers was tested against a portable multimeter (John Fluke Manufacturing, Everett, WA). The voltage was applied to the circuit connected with a known 500 Ω resistor. Voltage and current transducers were calibrated so that they provided the same readings as those obtained from the multimeter. Since surimi paste is a homogenous material and electric field was assumed to be uniform

along the sample tube, temperature variation throughout the sample tube was neglected.

Data analysis

Electrical conductivities of the samples were calculated from voltage and current data using the equation described by Palaniappan and Sastry (1991a):

$$\sigma = \left(\frac{1}{R}\right)\left(\frac{L}{A}\right) \tag{3.1}$$

where,

σ = electrical conductivity (S/m)

L = sample length (m)

A = cross-sectional area of the sample (m^2)

R = resistance of the sample (ohm)

Since electrical conductivity linearly increased with temperature at all salt-moisture levels, the general model of electrical conductivity as a function of temperature was expressed as:

$$\sigma = a_0 + b_0 T \tag{3.2}$$

where a_0 is the estimated intercept, b_0 is the estimated slope, and T is temperature (°C). Initially, the effect of voltage gradient (V), moisture (M) and added salt (N) content on the estimated parameters (a_0 and b_0) was evaluated using split plot design (Peterson, 1985). Whole plots were assigned to M and N. Three levels of V were assigned as a subplot factor. The estimated values were subjected to analysis of variance using the

General Linear Models (GLM) procedure of the Statistical Analysis System (SAS Institute, Inc., 1990).

An empirical for electrical conductivity was developed from equation 3.2. Each estimated parameter (a_0 and b_0) was modeled as a function of compositional characteristics (M and N) . Total 5 independent variables including 2 main effects (M and N), 1 interaction term (MN), and 2 quardatic terms (M^2, N^2), were analysed for their statistical significance using stepwise regression (SAS Institute, Inc., 1990). The empirical models for a_0 and b_0 were then substituted into equation 3.2 to obtain electrical conductivity model. Since residuals plot of each model indicated the assumption of constant variance was violated, weighted regression was applied using the inverse of sum of square errors as a weighting factor. Mean square error of the electrical conductivity model was calculated (Dunn and Clark, 1974). Predictability of the model was tested using electrical conductivity of surimi paste obtained from independent experiment. Tested levels of moisture and salt were 75, 84% and 1, 2, 3, 4%, respectively.

Results and Discussion

Surimi is a washed and dewatered minced flesh consisting primarily of myofibrillar proteins. Due to 3 cycles of washing and dewatering, ionic constituents originally present in the fish are dissolved out with wash water, resulting in a low ion content of surimi (Lin, 1992). The sodium content of Pacific whiting surimi without enzyme inhibitors was found to be 0.002% (Chung et al., 1993); this is relatively low compared to the amount of NaCl used in this study (1-4%). For this reason, the effect of added NaCl content was studied instead of total ion content.

Table 3.1. Means of estimated parameters of electrical conductivity modeled as a function of temperature.

Moisture (%)	Salt (%)	Coeff.(°C)$^{-1}$ Voltage gradient(V/cm)			Intercept (S/m) Voltage gradient (V/cm)		
		3.3	6.7	13.3	3.3	6.7	13.3
75	1	0.030±0*	0.030±0	0.028±0	0.540±0.003	0.606±0.039	0.649±0.062
	2	0.052±0.002	0.049±0	0.050±0.001	1.099±0.041	1.204±0.007	1.267±0.033
	3	0.074±0.001	0.067±0.004	0.069±0.002	1.704±0.018	1.904±0.020	1.921±0.037
	4	0.092±0	0.090±0	0.104±0.003	2.264±0.028	2.533±0.009	2.160±0.071
78	1	0.033±0.001	0.031±0	0.029±0.001	0.613±0.007	0.645±0.025	0.696±0.024
	2	0.053±0.001	0.051±0.001	0.051±0.004	1.257±0.014	1.326±0.026	1.449±0.042
	3	0.080±0	0.072±0.002	0.087±0.003	1.653±0.059	1.869±0.032	1.894±0.042
	4	0.099±0.003	0.096±0.001	0.100±0	2.285±0.176	2.651±0.069	2.709±0.065
81	1	0.032±0.001	0.031±0	0.029±0.001	0.629±0.009	0.682±0.034	0.767±0.010
	2	0.058±0	0.054±0	0.057±0.002	1.327±0.016	1.381±0.038	1.499±0.028
	3	0.083±0.003	0.076±0.003	0.082±0.003	1.933±0.023	2.239±0.256	1.984±0.079
	4	0.112±0.005	0.110±0.003	0.131±0.001	2.540±0.046	2.556±0.046	2.404±0.125
84	1	0.036±0.001	0.034±0.001	0.032±0	0.678±0.034	0.758±0.038	0.832±0.024
	2	0.060±0.001	0.057±0	0.056±0	1.433±0.028	1.623±0.004	1.708±0.008
	3	0.074±0.001	0.081±0.001	0.088±0.002	2.006±0.056	2.433±0.062	2.418±0.301
	4	0.092±0.002	0.107±0.001	0.106±0.008	3.343±0.107	3.495±0.112	3.051±0.268

* 0 indicates values less than 0.001

Linear relationship between temperature and electrical conductivity was evident with r^2 ranging from 0.987 to 0.999 (Table 3.1). The changes of electrical conductivity of Pacific whiting surimi paste with respect to temperature at various added NaCl and moisture contents are shown in Fig. 3.1 and Fig. 3.2. Since variation between two replicates as shown in Table 3.1 was small, the raw data from either one were presented in Fig. 3.1 and Fig. 3.2. Moisture and salt content significantly affected the estimated values of temperature coefficients (a_0 and b_0) ($P<0.05$), indicating that the electrical conductivity was influenced by compositional characteristics. Electrical conductivity significantly increased as temperature and added salt content increased, and slightly increased as moisture content increased. As salt content in surimi paste increased, the number of ions (Na^+ and Cl^-) available for conducting electrical current increased, which in turn increased electrical conductivity of surimi. An increased electrical conductivity at high temperature was due to ionic mobility (Palaniappan and Sastry, 1991a). This linearly increasing trend agreed with the results reported by Fryer et al. (1993) and Palaniappan and Sastry (1991a, b). However, electrical conductivity of the purified proteins, such as wool, collagen, and elastin varied with temperature according to the Arrhenius-type equation (Pethig, 1979). Differences in the trend could be due to the differences in electrical conducting mode of the materials. Ionic constituents played a major role in conducting electrical currents through the surimi paste and other food materials during ohmic heating. On the other hand, electrical conductivity studied in the purified proteins was mainly attributed to negative and positive charges associated with the protein molecules (Pethig, 1979). According to Shiba (1992), impedance of surimi paste linearly decreased as it was heated ohmically from 5°C to 50°C, indicating an

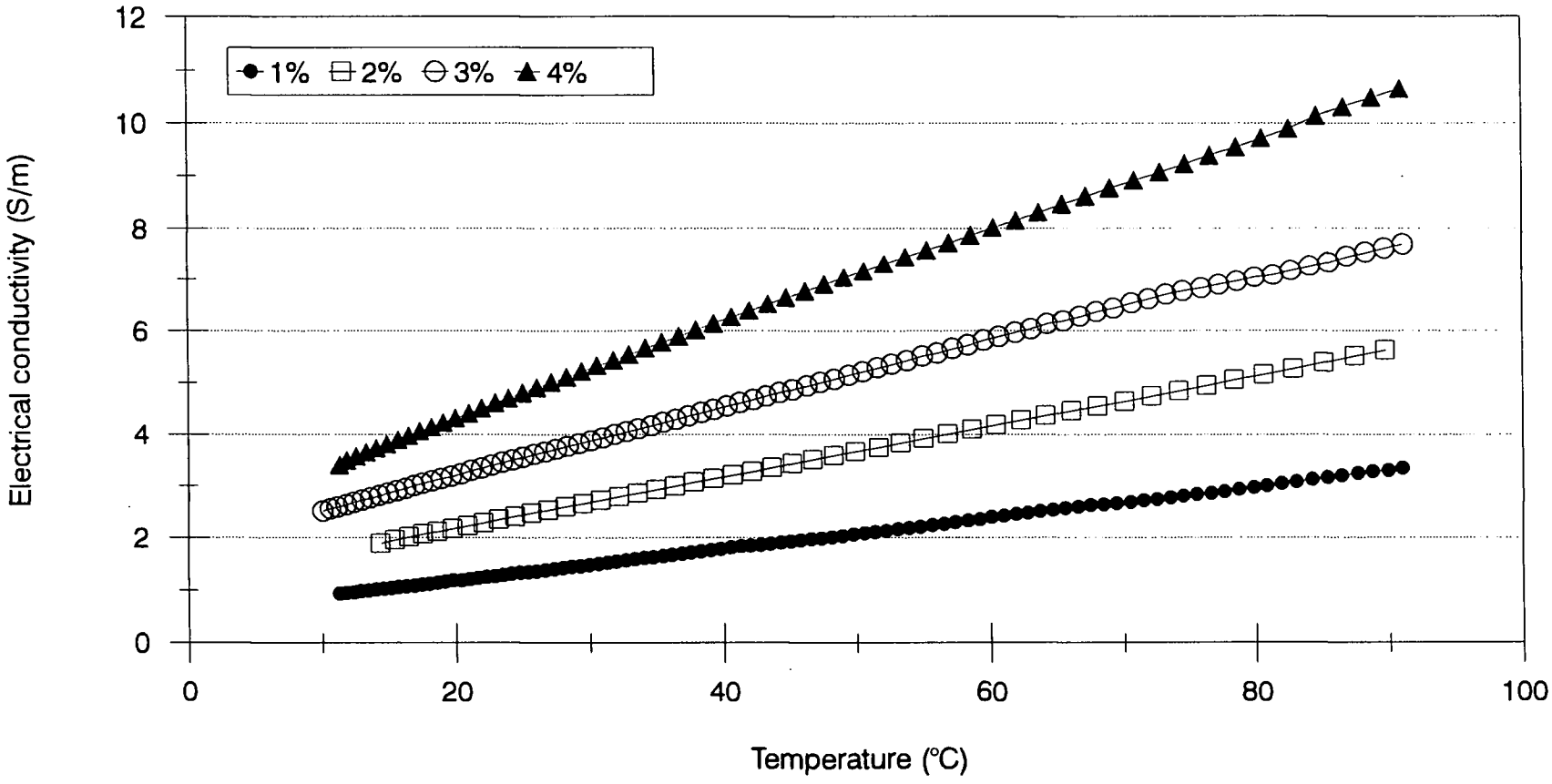


Figure 3.1. Effect of salt content on electrical conductivity of surimi paste containing 75% moisture content, measured at 6.7 V/cm.

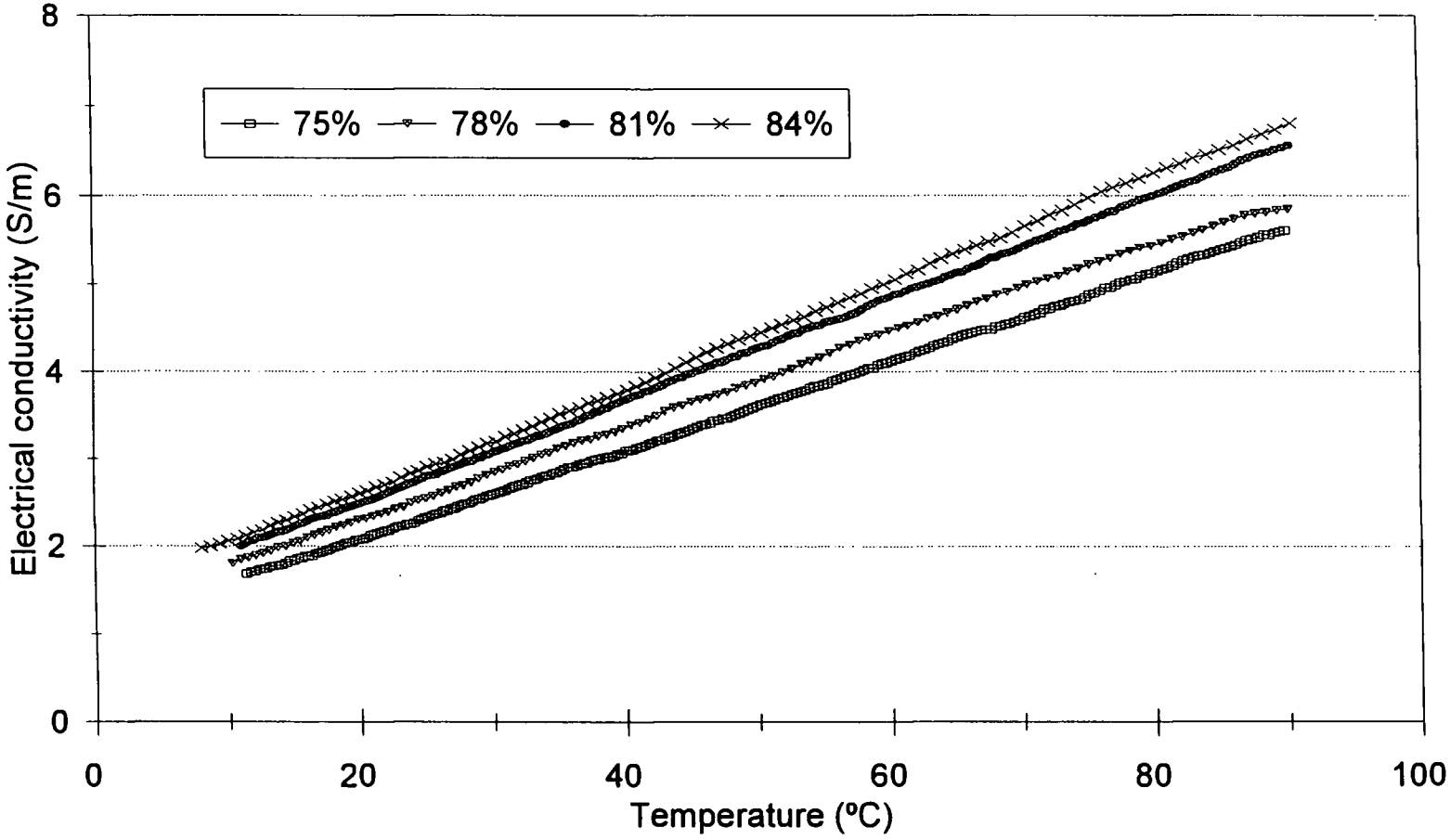


Figure 3.2. Effect of moisture content on electrical conductivity of surimi containing 2% salt at voltage gradient of 3.3 V/cm.

increase of electrical conductivity with increased temperatures. However, electrical conductivity of surimi at temperatures greater than 50°C cannot be compared with our results because electric current in Shiba's (1992) study was controlled below 3 A in order to achieve a desired heating rate.

Electrical conductivity tended to increase with increased moisture content (Fig. 3.2). Ion solvation could increase when more water molecules are available, leading in an increased ionic mobility. An increase in electrical conductivity with increased moisture content was also observed in various proteins, such as hemoglobin, keratin, and bovine plasma albumin (Pethig, 1979). This was due to ionic conduction effects associated with salts in hydrated proteins. Hydration of proteins allowed protons and other ionic species to migrate around the surface of the hydrated proteins (Pethig, 1979). Our study demonstrated that changes in electrical conductivity were greater with respect to salt content than moisture content (Fig. 3.1 and Fig. 3.2) in the studied ranges.

The estimated values of temperature coefficients at various voltage gradients of each moisture-salt combination were very similar (Table 3.1). However, analysis of variance of temperature coefficients revealed that the interaction effect N*V was significant ($P < 0.05$). The effect of voltage gradient appeared to be predominant in the sample containing 3 and 4% NaCl (Figs. 3.3A,B). The effect of voltage gradient on electrical conductivity of fruit and vegetable products was reported by Palanippan and Sastry (1991b) and Halden et al. (1990). High applied electric field enhanced cell fluids motion within plant cells and ruptured cell membrane, resulting in a release of cell fluids (Halden et al., 1990). As a result, electrical conductivity of fruits and vegetables increased with increasing voltage gradient. The differences in electrical conductivity at

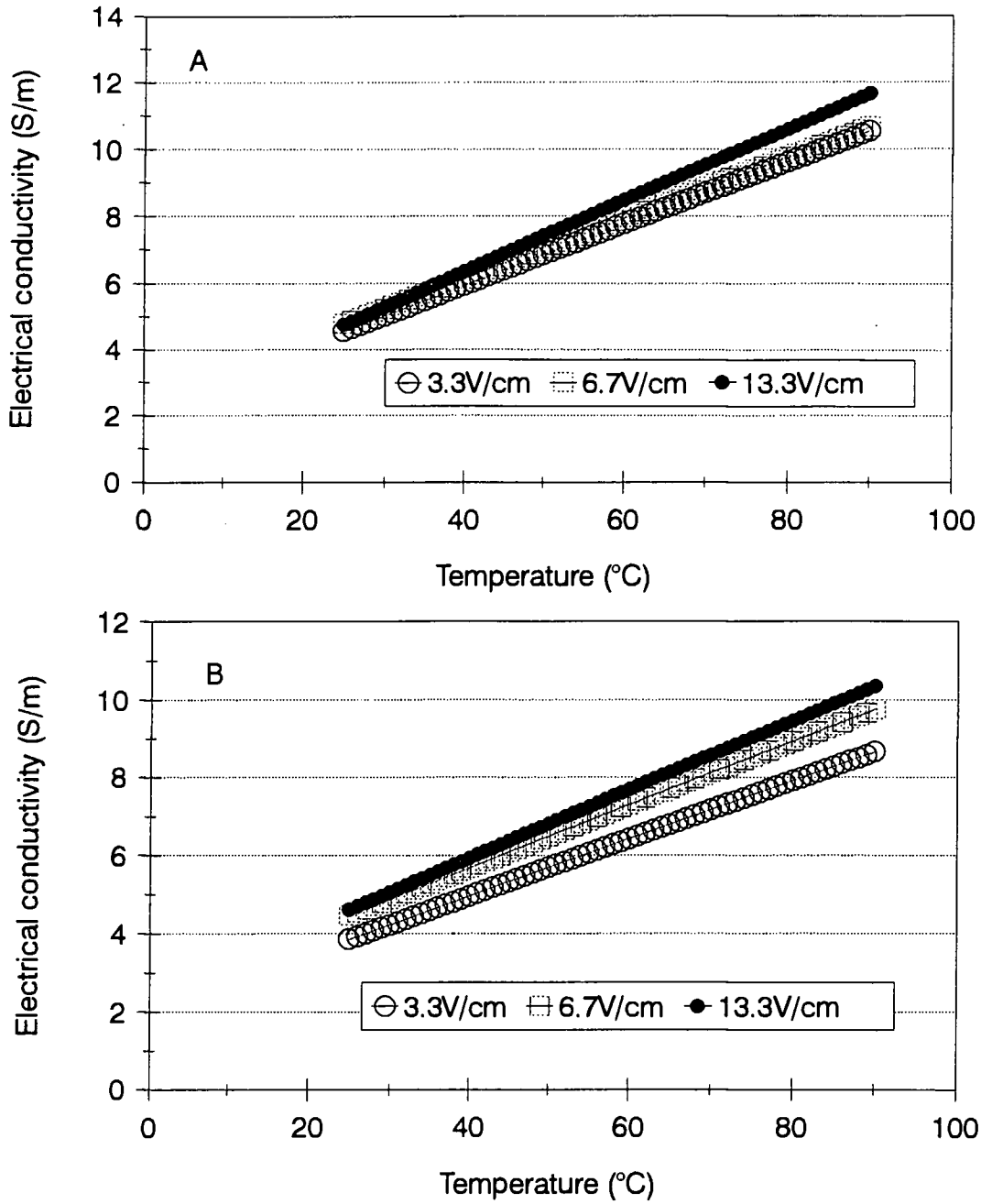


Figure 3.3. Effect of voltage gradient on electrical conductivity. (A) 75% moisture and 4% salt; (B) 84% moisture and 3% salt.

varied voltage gradients observed in this study were unlikely to arise from the enhanced motion of the cell fluids because surimi paste was finely comminuted and was a homogeneous material which was quite different from vegetables or fruits that retained the integrity of plant cells during ohmic heating. Dependence of electrical conductivity on voltage gradient could be caused by corrosion of the electrodes which normally occurred in the samples containing 3 and 4% NaCl. Faradaic current, which was a direct current generated by electrolytic reactions, was observed when current density greater than 8,000 A/m^2 passed in a platinised-titanium electrode immersed in saturated sodium chloride solution (Stirling, 1987). Thus, an electric current measured under corroded condition included both applied alternating current and Faradaic current(Oldham and Myland, 1994). To minimize corrosion of the electrode, the maximum current density was recommended at 4,000 A/m^2 (de Alwis and Fryer, 1992). Fig. 3.4 illustrates relationship between applied voltage and electric current of surimi pastes at 25°C. For the sample containing 4% NaCl and 84% moisture, electrode corrosion and deviation from the Ohm's law were observed when electric current exceeded 1 A, corresponding to a current density of about 3,500 A/m^2. On the other hand, Ohm's law was followed for the sample containing 1% NaCl and 75% moisture, when the total current was less than 1 A and electrode corrosion was not noticed. To obtain accurate electrical conductivity values, it is critical to measure conductivity in such a way that electrochemical reactions at the electrodes do not occur.

A model for electrical conductivity of Pacific whiting surimi as a function of temperature (T), added NaCl content (N) and moisture content (M) was developed. The effect of voltage gradient was not included in the model because it appeared to be

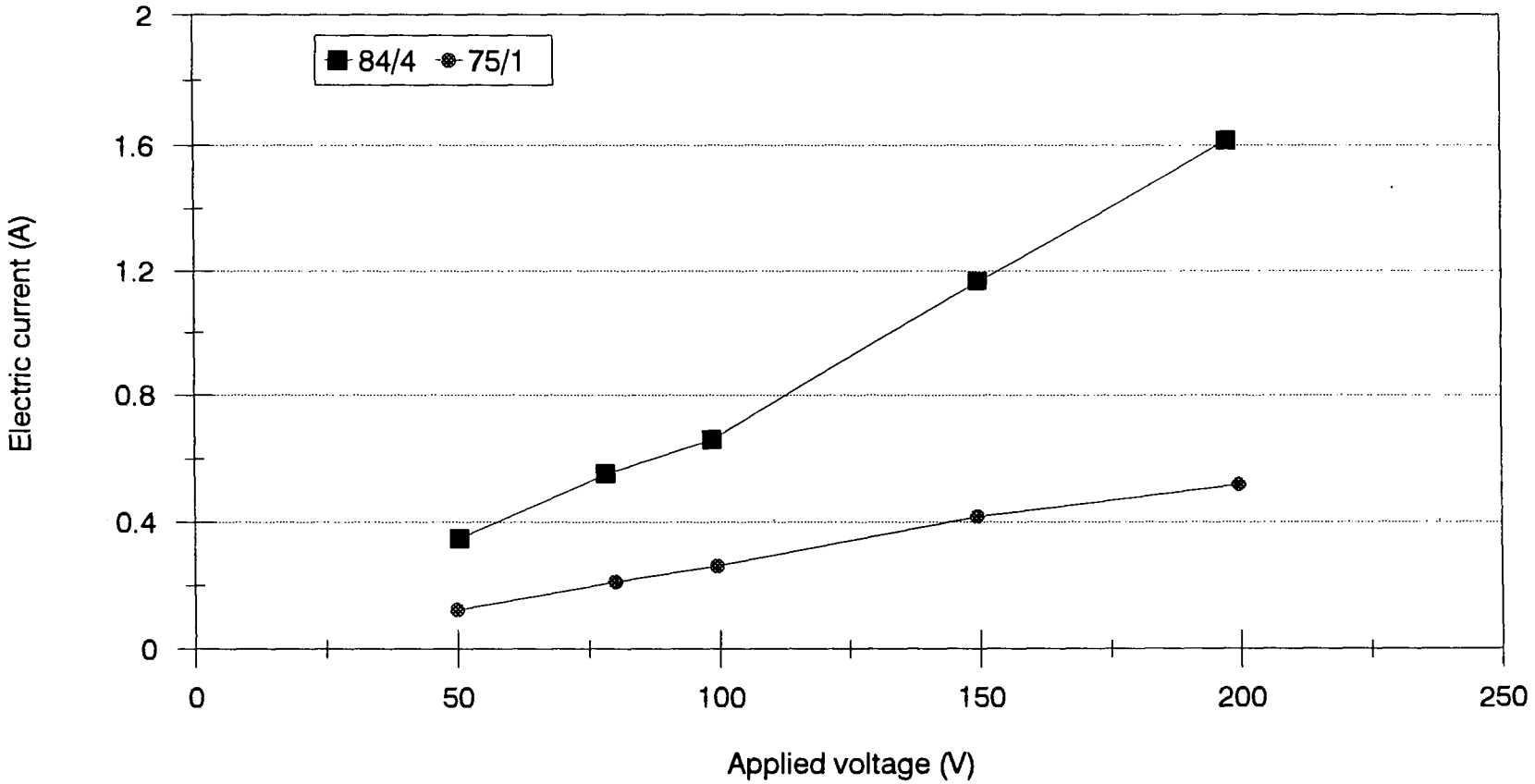


Figure 3.4. Relationship between electric current and applied voltage at different compositional characteristics of surimi: 84/4: surimi paste containing 84% moisture and 4% salt at 25°C, 75/1: surimi paste containing 75% moisture and 1% salt at 25°C.

interfered by the electrode corrosion, rather than be affected by property of the sample. From stepwise regression, significant variables ($P<0.001$) for models of intercept (a_0) and slope (b_0) can be written as:

$$a_0 = a_{01} + a_{02}N + a_{03}MN$$
$$b_0 = b_{01} + b_{02}N + a_{03}MN + b_{03}N^2$$

where a_{0i} and b_{0i} were estimated parameters shown in Table 3.1. Therefore, the final model of electrical conductivity as a function of compositional characteristics and temperature can be described as:

$$\sigma = (a_{01} + a_{02}N + a_{03}MN) + (b_{01} + b_{02}N + a_{03}MN + b_{04}N^2)\,T$$

Table 3.2. Estimated coefficients of a_0 and b_0 as a function of moisture and salt content.

Variable	Estimated coefficient ($x10^3$)	Standard error ($x10^3$)
Model for intercept (a_0)		
a_{01}	34.94	4.53
a_{02}	-1,008.54	29.45
a_{03}	20.94	0.41
Model for slope (b_0)		
b_{01}	7.83	0.42
b_{02}	-1.83	0.90
b_{03}	0.31	0.01
b_{04}	0.26	0.11

Mean square error of the electrical conductivity model was estimated to be 0.082 (degree of freedom = 5,989). Positive coefficient of TN indicated that an increase in electrical conductivity with temperature was greater at higher salt content. Although the effect of moisture content was not significant, a positive coefficient of MN suggested that an increase in electrical conductivity with salt content was greater in the samples containing higher moisture content. Predictability of the model is illustrated in Figs. 3.5a-b. The model satisfactorily predicted electrical conductivity of Pacific whiting with an error ranging from 0-15.6%. Error of prediction was relatively large in the sample containing 3-4% NaCl and 84% moisture content at high temperature (Fig. 3.5b). This was probably due to electrode corrosion problem as discussed previously. Although the model was primarily developed for Pacific whiting surimi paste, it could be used to estimate electrical conductivity of surimi made from other fish species. This inference was based on the fact that ions content of surimi made from various fish could be similar due to the washing and dewatering process. Furthermore, electrical conductivity of surimi-based seafoods, such as surimi-based crabmeat, could be predicted from this model because the main factors affecting electrical conductivity of those products are only salt and moisture content.

Conclusions

Electrical conductivity was highly dependent on temperature and added salt content, and was slightly dependent on moisture content. The effect of applied voltage was insignificant. The changes of electrical conductivity affected by heating temperature and compositional characteristics of the sample indicated the importance of such factors

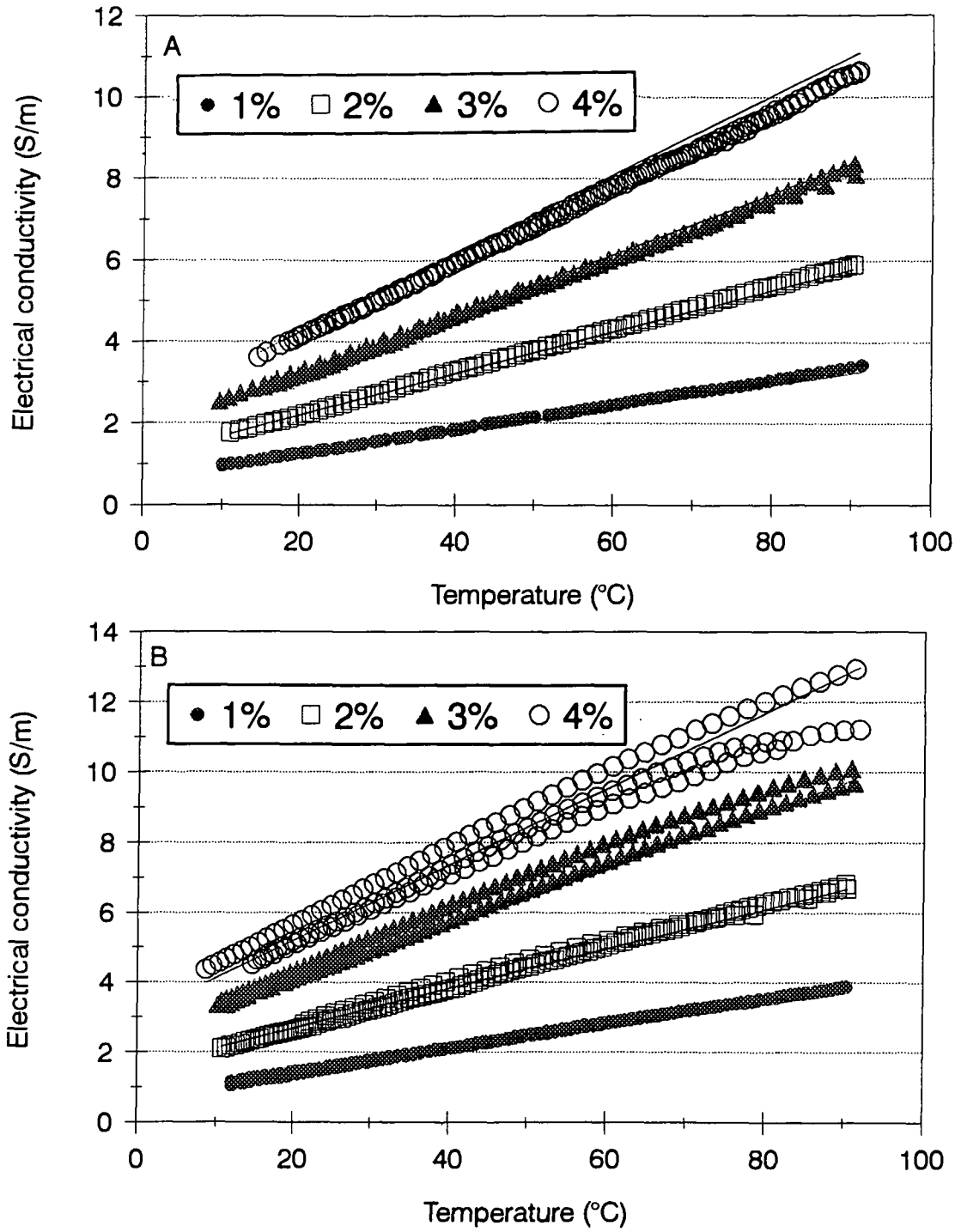


Figure 3.5. Predictability of electrical conductivity model as compared with experimental values. (A) 75% moisture; (B) 84% moisture; line: predicted values; symbol: experimental values.

in the design and operation of ohmic heaters for surimi-based seafood products. The empirical model obtained from this study adequately predicted the electrical conductivity of whiting surimi based on temperature, salt and moisture content.

Acknowledgement

This work was supported by grant No. NA36RG0451 (project no. R-SF-2) from the National Oceanic and Atmospheric Administration to the Oregon State University Sea Grant College Program and by appropriations made by the Oregon State Legislature. The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA or any of its subagencies.

The authors thank Mr. Y. AbuDagga for assistance with hardware assembly and operation.

Chapter 4

DEGRADATION KINETICS OF GEL TEXTURE AND MYOSIN HEAVY CHAIN OF PACIFIC WHITING SURIMI

Jirawat Yongsawatdigul, Jae W. Park, and Edward Kolbe

Department of Food Science and Technology
OregonState University
Corvallis, OR,
January 1996.

Abstract

Degradation kinetics of whiting surimi gel texture and of whiting myosin heavy chain (MHC) were examined over a temperature/time range (40-85°C, 1-35 min). Changes in both textural properties and MHC of whiting surimi gel were mainly affected by proteolytic activity of endogenous proteinase. A decrease of failure shear stress and shear strain followed first order kinetics. The kinetic models developed using either isothermal or nonisothermal principles provided similar kinetic parameters. Degradation rate of gel texture increased with temperature, reaching a maximum at 55°C. It then decreased to the minimum at 70°C. Activation energy (E_a) values for the activation and inactivation were in the range of 2.0×10^3-2.3×10^3 and 2.0×10^2-4.7×10^2 kJ/mol, respectively.

A kinetic model of MHC degradation was derived using the nonisothermal concept. Degradation of MHC was best described with an apparent reaction order of 1.4. Changes of degradation rate with temperature exhibited similar trend as that of textural degradation. Maximum degradation rate was estimated to be at 57°C and the minimum rate was at 75°C. E_a values of activation and inactivation temperature range were 2.1×10^3 and 1.3×10^3 kJ/mol, respectively. The kinetic model satisfactorily estimated MHC content with an averaged error of 10.8%. Degradation of actin did not show any particular trend.

Key words: kinetics, gel texture, myosin degradation, proteolysis

Introduction

Pacific whiting (*Merluccius productus*) surimi without enzyme inhibitors normally undergoes texture degradation during slow heating (Chang-Lee et al., 1989; Morrissey et al., 1993; Yongsawatdigul et al., 1995). This is due to the presence of heat stable endogenous proteinase(s) that exhibit high hydrolytic activity on muscle myosin (Erickson et al., 1983; Patashnik et al., 1982). One of the endogenous proteinases purified from Pacific whiting flesh is cathepsin L, which has the highest proteolytic activity towards a synthetic substrate, N-carbobenzoxy-Phe-Arg-7-amido-4-mehtylcoumarin (Z-Phe-Arg-NMec), at pH 5.5 and 55°C (Seymour et al., 1994). The degradation pattern of myofibrillar proteins in the presence of the purified cathepsin L was consistent with that of surimi incubated at 55°C (An et al., 1994). This indicated that cathepsin L was responsible for a gel weakening phenomenon in whiting surimi.

In the formation of gels by conventional heating methods, heat is transferred within the solid sample by means of conduction from the outside boundaries. Rate of heat transfer is typically slow and depends on various factors, such as temperature of heating medium, geometry, and thermal properties of the sample. When heated in a 90°C water bath, temperature at the geometric center of whiting surimi paste in stainless steel tubes (i.d. = 1.9 cm) increased from 10 to 80°C within 8 min (Yongsawatdigul et al., 1995). Such a slow heating process allowed the proteinase(s) to be active for a relatively long period of time. As a result, substantial loss of myosin heavy chain (MHC) was evident concomitant with very poor textural characteristics (Yongsawatdigul et al., 1995). Rapid heating methods, such as microwave and ohmic heating, have been reported to effectively minimize textural degradation of fish muscle caused by endogenous

heat stable proteinases (Greene and Babbitt, 1990; Yongsawatdigul et al., 1995). For both heating methods, heat is internally generated, rather than externally transferred from the heating medium, therefore, temperature of the sample quickly increases. As a result, the proteinase is thermally inactivated in a rapid manner.

High quality of whiting surimi gel (shear stress and shear strain of 30 kPa and 2.8, respectively) was obtained when heated ohmically from 10 to 90°C within 1 min (Yongsawatdigul et al., 1995). On the other hand, as the time of ohmic heating was prolonged to 80 min over the same temperature range, very soft and mushy gels were obtained, accompanying complete disappearance of MHC and a reduced actin band (Yongsawatdigul and Park, 1996). It is clear that heating time and temperature are critical factors controlling textural properties of whiting surimi. This trend also extends to other fish species that exhibit similar problems with endogenous heat stable proteinase as shown for threadfin bream (*Nemipterus bathybius*) (Toyohara and Shimizu, 1988), Atlantic menhaden (*Brevoorti tyrannus*) (Boye and Lanier, 1988), and arrowtooth flounder (*Atheresthes stomias*) (Greene and Babbitt, 1990). To obtain desired whiting surimi gel properties without the use of inhibitors, proper heating rates and temperature need to be determined. Therefore, the kinetics of gel weakening and MHC degradation during heating should be understood.

In systems where the sample size is so small that instantaneous heating and cooling can be assumed, kinetic parameters can be derived using isothermal approach. A reaction rate constant, k, is obtained from the relationship between concentration and time at different temperatures. The temperature dependence of the rate constant is then modeled using either the Arrhenius equation or other function forms (Saguy and Karel,

1980). However, the Arrhenius equation can be applied over a wide temperature range and often provides an accurate estimate (Lenz and Lund, 1977b; Saguy and Karel, 1980). Example applications include kinetic studies of denaturation of whey protein (Dannenberg and Kessler, 1988), thermal inactivation of enzymes and inhibitors (Park et al., 1988; Roa et al., 1989), and loss of ascorbic acid in orange juice (Johnson et al., 1995).

However, when instantaneous heating and cooling is not possible, i.e. physico-chemical changes during heat-up and cool-down periods are significant, kinetic parameters cannot be accurately determined using an isothermal approach. In such cases, a thermal lag correction is required (Davies et al., 1977; Perkin et al., 1977; Wicker and Temelli., 1988; Awuah et al., 1993). An equivalent isothermal process time can be calculated using the equations derived from the thermal death time (TDT) model (Davies et al., 1977; Perkin et al., 1977, Wicker and Temelli, 1988; Palaniappan et al., 1992) or from the Arrhenius model (Lenz and Lund, 1977a,b; Swartzel, 1982).

Since the TDT method is only appropriate for first-order reactions (Ramaswamy and Abdelrahim, 1992), it cannot be applied to derive kinetic parameters for degradation of MHC and gel texture whose reaction orders are unknown. The kinetic analysis in this study was also complicated by the fact that activation and inactivation of the endogenous proteinase took place as whiting surimi was heated to 90°C. This was in contrast with degradation kinetics of microorganisms and nutrients during thermal processing in which the rate of degradation only increased with temperature. Therefore, the existing methodology developed for thermal processing could not be directly applied to this study. The objective of this research was to develop kinetic models describing the changes of

gel texture and MHC of whiting surimi when heated to 90°C, using the Arrhenius model as a fundamental basis.

Materials and Methods

Surimi gel preparation

Unfrozen Pacific whiting (*Merluccius productus)* surimi without enzyme inhibitors and cryoprotectants was obtained from Point Adams Packing Co. (Hammond, OR). Surimi was processed from the fish caught within 18 hr. The surimi was mixed with cryoprotectants as described by Yongsawatdigul et al. (1995). The samples were stored at -30°C throughout the experiment and used within 6 mo.

Surimi pastes with 78% moisture content and 2% salt concentration, were prepared as described by Yongsawatdigul et al. (1995). The paste was stuffed into chlorinated polyvinyl chloride (CPVC) tubes (1.9 cm i.d. × 20.5 cm long) and heated using an ohmic heating apparatus. This device described in detail by Yongsawatdigul et al. (1995), was developed from two rhodium-coated stainless steel electrodes, a variable transformer, current and voltage transducers and a temperature controller (Model CN3201, OMEGA Engineering Inc., Stamford, CT) connected to a solid state on-off relay which enabled the control of temperature as a constant or ramp-increase function. To minimize electrical hazards, the sample tube and electrodes were housed inside a plexiglass enclosure equipped with an open-snap action switch, which was activated when the plexiglass box was closed. Temperatures at the geometric center of the sample were measured by a 30-gauge teflon-sealed thermocouple (Type-T) and were recorded on a

datalogger (model 21X, Campbell Scientific, Inc., Logan, UT). Temperature variation during the constant-temperature holding period was ±0.5°C.

Table 4.1. Heating regimes at various temperatures.

Holding Temp. (°C)	Initial heat-up (sec)	Holding time (min)	Final heat-up (sec)
40	27	0,5,10,15,25,35	40
45	32	0,3,5,8,12,15	36
50	36	0,1,2,3,5,7	32
55	41	0,1,2,3,4,5	28
60	45	0,1,2,3,4,5	24
65	46	0,1,2,3,4,5	20
70	48	0,0.5,1,2,2.5,3	16
75	51	0,0.5,1,2,2.5,3	12
80	54	0,0.5,1,2,2.5,3	8
85	58	0,0.5,1,2,2.5,3	4

Preliminary studies indicated no changes in shear stress, shear strain, and MHC content occurred in surimi gels held up to 1 hr at 30°C. Therefore, the samples were heated to temperatures ranging from 40 to 85°C and increased at the maximum allowable rate corresponding to a voltage gradient of 13.3 V/cm (Yongsawatdigul et al., 1995); the samples were held at these temperatures for various times. When the holding times were achieved, the samples were then heated rapidly to 90°C using the same voltage gradient applied for initial heating. Heating regimes at various temperatures are shown in Table 4.1. Control samples were prepared by heating surimi paste continuously from 10°C to

90°C at 13.3 V/cm with no intermediate holding temperature. To obtain adequate number of gel samples for torsion testing, three sample tubes were individually heated for each holding time. Once cooked, surimi gels were cooled in ice water and kept at 4°C for torsion tests and gel electrophoresis. One batch of surimi paste (1.5 kg) was used for each studied temperature and its control. The experiment was performed in duplicate

Determination of the effective concentration of E-64

Textural properties of surimi gel could be affected by kinetics of gelation and/or proteinase. Therefore, it was necessary to identify the primary reaction governing final gel structure so that kinetic models could be reasonably interpreted. To study the effect of gelation kinetics on textural properties, the enzymatic reaction was inhibited using 1-(L-*trans*-epoxysuccinylleucylamino)-4 -guanidinobutane (E-64). E-64, a cysteine proteinase inhibitor, was chosen because it completely inhibited the activity of cathepsin L purified from whiting flesh, which is presumably responsible for textural degradation (Seymour et al., 1994). Autolysis test on surimi was conducted to determine the effective concentration of E-64. A solution of 1 mM E-64 (Sigma Chemical Co., St. Louis, MO) was prepared. A 1.5 g surimi paste sample containing 2% NaCl and 78% moisture content was mixed with various volumes of a 1 mM E-64 solution to obtain the final concentration of 357, 286.5, 178.5, 107.2, 35.7 μg/g of surimi. The mixture was incubated in a 55°C water bath for 1 hr (Morrissey et al., 1993). Blank samples containing all components were kept in ice and analyzed to account for oligopeptides present in surimi. The control was the surimi paste without addition of E-64. Autolytic

reaction was stopped by adding 5% cold trichloroacetic acid (TCA) solution. Different amounts of cold TCA ranging from 12.3 to 13.5 mL was added to adjust the final volume to 15 mL. The mixture was incubated at 4°C for 15 min and centrifuged at 6,100 × g for 15 min. The TCA-soluble proteins expressed as mmoles of tyrosine released were analyzed for the oligopeptide contents by Lowry's assay (Lowry et al., 1951). Duplicate samples for each concentration were run. The inhibitory effect of E-64 was calculated as follows:

$$\%Inhibition = \frac{[(Tyr_{con} - Tyr_{con,blank}) - (Tyr_{E\text{-}64} - Tyr_{E\text{-}64,blank})]}{[(Tyr_{con} - Tyr_{con,blank})]} \times 100$$

where Tyr_{con} was tyrosine content of the control, $Tyr_{con,blank}$ was tyrosine content for the control kept in ice, $Tyr_{E\text{-}64}$ was tyrosine content of the sample with E-64, and $Tyr_{E\text{-}64,\ blank}$ was tyrosine content of the sample with E-64 kept in ice.

Surimi gel with E-64

Since the autolysis test indicated that inhibitory effect of E-64 at various concentrations (35.7-357 μg/g of surimi) was about 97-100% (not shown), E-64 at concentration of 35.7 μg/g of surimi was used in surimi gel preparation. A batch of 1,200 g surimi was prepared with 2% NaCl to form a paste. A solution of 1 mM E-64 was added to the paste along with ice water to attain 35.7 μg/g of surimi. Chopping and stuffing procedures were as described above. The paste was heated ohmically to 40 and 55°C with the same heating regimes as shown in Table 4.1. Moisture content of surimi

paste determined by the AOAC (1990) method was 80.4 ± 0.2% (average ± standard deviation). Surimi gels were cooled in ice water and kept at 4°C for torsion tests.

Torsion test

Torsion failure tests were performed at room temperature, 18-24 hr after gel preparation as described by NFI (1991). Ten hourglass-shaped samples for each treatment were equilibrated to room temperature before torsion. Shear stress and shear strain at failure were calculated from equations described by Hamann (1983).

SDS-PAGE

Unheated and heated samples were solubilized in 5% sodium dodecyl sulfate solution (95°C) as described by Morrissey et al. (1993). Electrophoresis was carried out according to the procedure of Laemmli (1970). Stacking gels and separating gels were made of 4% (w/v) and 10% (w/v) polyacrylamide, respectively. The samples treated at each individual temperature were applied on each SDS-PAGE gel along with the unheated surimi paste and the control. The amount of protein applied on polyacrylamide gel was 40 μg. Rabbit MHC (Sigma Chemical Co., St. Louis, MO) was used as a molecular weight standard, and loaded on each SDS-PAGE gel at concentrations of 5, 10, 20, 30, and 40 μg. Rabbit myosin was used instead of whiting myosin because of its purity and known concentration. Protein patterns were scanned using an HP ScanJet II scanner (Hewlett-Packard Co., Minneapolis, MN) equipped with NIH Image Software 1.54 (NIH, Washington, DC). MHC of the samples was quantified using a standard

curve established from the density of rabbit MHC bands. Change of actin with respect to time at any temperature was expressed as the ratio of the area of its density to that of the control sample. Gel electrophoresis and densitometric analyses were conducted in duplicate.

Kinetic modeling of textural degradation

Changes of textural properties were initially assumed to follow first order reaction. The kinetic models were determined using two different approaches described as follows:

1. The kinetic models for degradation of shear stress and shear strain were developed using isothermal concept. The model can be expressed as:

$$lnP = lnP_0 - kt \qquad (4.1)$$

where P = textural properties of surimi gel at time t

t = holding time (min)

k = apparent rate constant (min^{-1})

P_0 = textural properties of the sample heated to 90°C using voltage gradient of 13.3 V/cm (control).

This was based on the fact that temperature history of every sample during heat-up periods would be identical to that of the control (Fig. 4.1). Therefore, the effect of any thermal lag on a sample would be equivalent to that on the control during the heat-up period. Thus, the ratio of P to P_0 would represent the effect of proteolysis during the holding time only. The effect of P reduction during the thermal lag period would be the

same for sample and control and reflected in the value of P_0. Rate constants at each holding temperature were obtained using weighted least square regression between ln (P/P_0) and holding time. The inverse of the square of residuals from the unweighted regression was used as a weight factor (Hill and Grieger-Block, 1980; Arabshahi and Lund, 1985). Rate constant values were modeled as a function of temperature using the Arrhenius model, then kinetic parameters (activation energy, E_a ,and frequency factors, k_0) were calculated.

2. The second approach applied nonisothermal principles to derive the kinetic models. P_0 was defined in this approach to represent textural properties of the sample that could be heated instantaneously with no proteolysis. Even though the control was heated, using a maximum allowable voltage gradient (13.3 V/cm), from 10 to 90°C within 1 min (Fig. 4.1), heat-up period was not instantaneous and inhibition of proteolytic activity was not completed. Thus, the second approach is to develop kinetic models which account for degradation during heat-up periods. For nonisothermal conditions, equation (4.1) is rewritten as:

$$\ln[P] = \ln[P_0] - \int_0^t k[T(t)]\, dt \qquad (4.2)$$

where the apparent rate constant is now a function of temperature, and temperature a function of time. If instantaneous heating and cooling were achieved, equation (4.2) could be modified to:

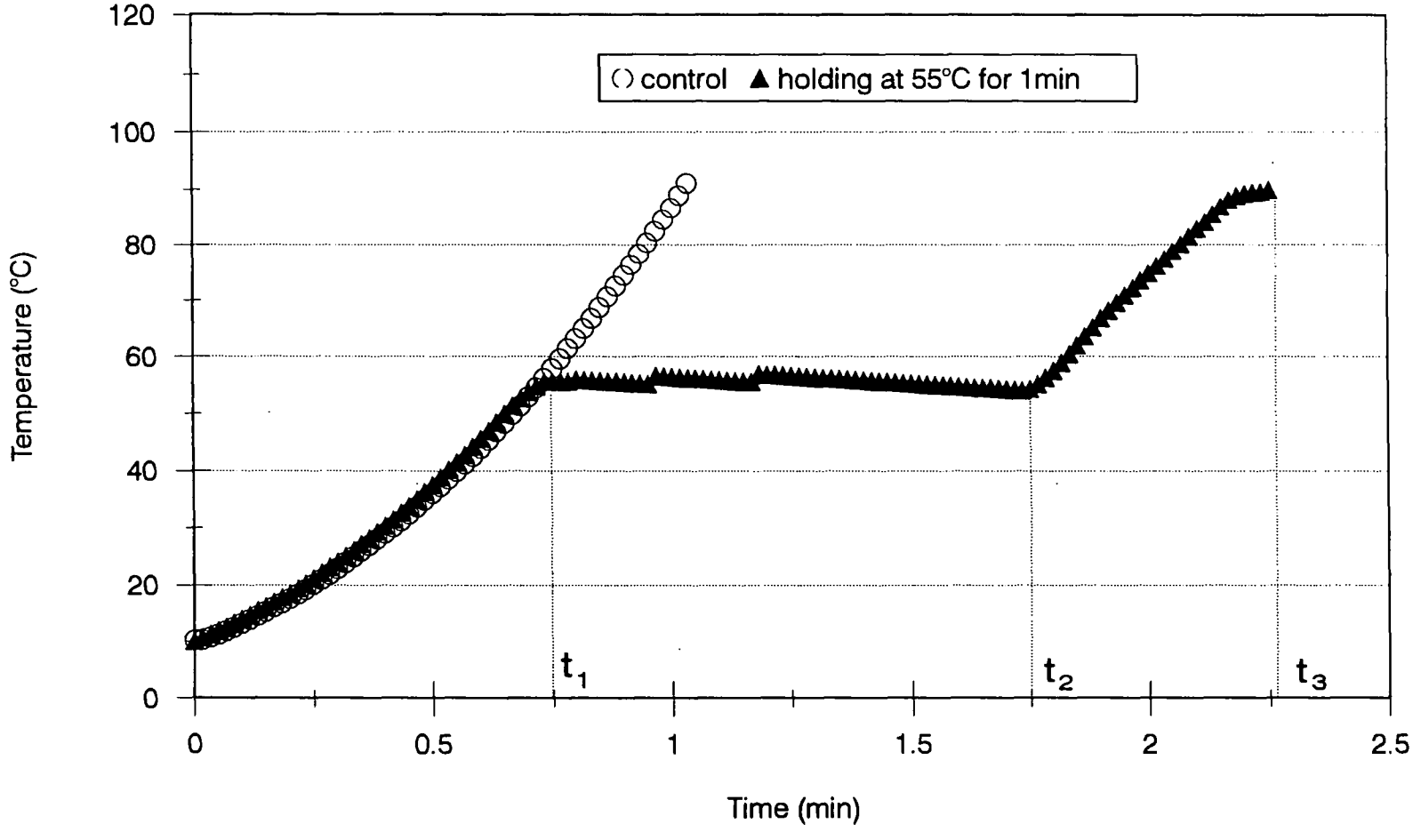


Figure 4.1 Representative temperature profiles of surimi paste heated by ohmic means at 2 different heating regimes (applied voltage gradient = 13.3 V/cm).

$$\ln\left[\frac{P}{P_0}\right] = -k_{Ti}\, t_{aTi} \tag{4.3}$$

where k_{Ti} = apparent rate constant at temperature T_i if there was no thermal lag

t_{aTi} = equivalent holding time at temperature T_i

t_{aTi} is the summation of the holding time and the equivalent heat-up time from the initial temperature to T_i and from T_i to the final temperature (90°C). To determine t_{aTi} for a nonisothermal process, equation (4.2) and (4.3) can be combined and rewritten in term of the Arrhenius model as:

$$k_{Ti}\, t_{aTi} = k_0 \int_0^t e^{\frac{-E_a}{RT(t)}}\, dt \tag{4.4}$$

where k_0 = frequency factor (min^{-1})

E_a = activation energy (J/mol)

R = gas constant (8.315 J/mol K)

T = absolute temperature (K)

Based on temperature profiles shown in Fig. 4.1, equation (4.4) can be rewritten as:

$$t_{aTi} = \frac{1}{k_{Ti}}\left[(k_0)_{ac,inc}\int_0^{t_1} e^{\frac{(-E_a)_{ac,inc}}{RT(t)}}\, dt + k_{Ti}(t_2 - t_1) + (k_0)_{ac,inc}\int_{t_2}^{t_3} e^{\frac{(-E_a)_{ac,inc}}{RT(t)}}\, dt\right] \tag{4.5}$$

Where t_1 is the time when the sample first reaches the holding temperature (T_i), t_2 the time when holding is completed, and t_3 the finished heating time (Fig. 4.1). The subscripts ac, inc represent values of the kinetic parameters during activation and inactivation of the proteinase, respectively. When temperatures are in the range of

activation, $(E_a)_{ac}$ and $(k_0)_{ac}$ are substituted in the integral terms, while $(E_a)_{inc}$ and $(k_0)_{inc}$ are applied in the inactivation range. The integral terms of equation (4.5) indicate the proportional of loss of textural properties during heat-up periods, 0-t_1 and t_2-t_3. It can be seen that if the thermal lag effect is insignificant, equivalent holding time will be the same as holding time.

Because the true values of P_0, t_{aTi}, k_{Ti}, $(E_a)_{ac,inc}$ and $(k_0)_{ac,inc}$ are unknown, iterative procedure was adopted to determine these values. The results from the first approach (k_{Ti}, $(E_a)_{ac,inc}$ and $(k_0)_{ac,inc}$) were used as initial values to calculate t_{aTi} and P of control was first used as P_0. Since temperature was assumed to be uniform throughout the sample, the two integral parts were numerically solved by Simpson's rule (Forsythe et al., 1977) using time-temperature data recorded during the heating-up periods. Using the calculated t_{aTi} , the new k_{Ti}'s were estimated from equation (4.3). When k_{Ti}'s at all studied temperatures were determined, $(E_a)_{ac,inc}$and $(k_0)_{ac,inc}$ were calculated using an Arrhenius plot. Iterative calculation was performed about 3-5 cycles until the difference in k_{Ti} from the previous cycle was ≤ 0.0001.

The new P_0 was calculated using the following equation:

$$\ln P_0 = \ln P + \int_0^t k[T(t)]\, dt \qquad (4.6)$$

The P value represented the textural properties of the control. The integral term indicating thermal effect of the control was numerically solved by Simpson's rule using the calculated k_{Ti}'s and $(E_a)_{ac,inc}$. The P_0 for each individual batch was calculated and used to reestimate k_{Ti} and $(E_a)_{ac,inc}$ as described above. Iterative calculation was performed

until the difference in P_0 within individual batch was less than 0.01. The P_0 values normally converged in 3 cycles.

Kinetic modeling of MHC degradation

The kinetic model of MHC degradation was developed using a nonisothermal approach. Reaction order of MHC degradation was first determined using a differential method described by Fogler (1992). In this approach, MHC content as a function of time was fitted to either a 3rd or 4th degree polynomial function, and then the function was differentiated to obtain the rate of myosin degradation (dP/dt). Reaction order was determined from a plot of ln(dP/dt) vs ln(P) (Hill and Grieger-Block, 1980; Fogler, 1992). Degradation of MHC held at 55, 60, and 65°C for longer than 3 min was so severe that accurate measurement of MHC content could not be obtained. Therefore, reaction order was determined at all temperatures except at these temperatures because the 3rd and 4th polynomial functions could not be truly represented by 4 data points. When reaction order was determined, t_{aTi} and the kinetic parameters were then calculated by the iterative procedures described previously. The MHC contents were then plotted against the calculated t_{aTi}'s and dP/dt was recalculated to determine the new reaction order. Iterative calculation for determining reaction order converged after 3 cycles. A final kinetic model was used to estimate the MHC content of the control. Differences between the estimated and experimental values were evaluated by paired t-test using STATGRAPHIC Version 6.0 (Manugistic Inc., Rockville, MD).

Results and Discussion

Effect of E-64 on textural properties

Shear stress and shear strain of surimi gels with E-64 heated at 2 different heating regimes are shown in Figs. 4.2A,B. At each holding temperature, there were no differences in both shear stress and shear strain of whiting surimi gels held at any holding times ($P > 0.05$). Although the role of E-64, if any, as a gelling enhancer or suppressor on gelation of myofibrillar proteins has not been documented, it was reasonable to neglect such effects because the amount of E-64 was less than 10^{-5} of the amount of surimi. The effect of E-64 on textural properties of surimi was mainly due to its inhibitory function. The result at 55°C implied that concentration of E-64 used in this study was sufficient to inhibit textural degradation caused by proteolysis. E-64, a cysteine proteinase inhibitor, suppressed textural breakdown at 55°C, confirming that proteinase responsible for proteolysis at elevated temperatures was cysteine proteinase. Activity of proteinase purified from Pacific whiting was also inhibited (99%) by 0.01 mM E-64 (Seymour et al., 1994). Moreover, Saeki et al. (1995) reported that a breaking strength of chum salmon surimi incubated at 40, 50, or 60°C up to 6-8 hr prior to heating to 90°C was improved by addition of E-64. They concluded that cysteine proteinase(s) was involved in gel weakening of chum salmon.

In whiting surimi without enzyme inhibitors, degradation of MHC catalyzed by the endogenous proteinase(s) also occurred simultaneously with its gelation process. The extent of these two reactions were time- and temperature-dependent. Therefore, both gelation and enzyme kinetics could play a vital role in governing the final textural

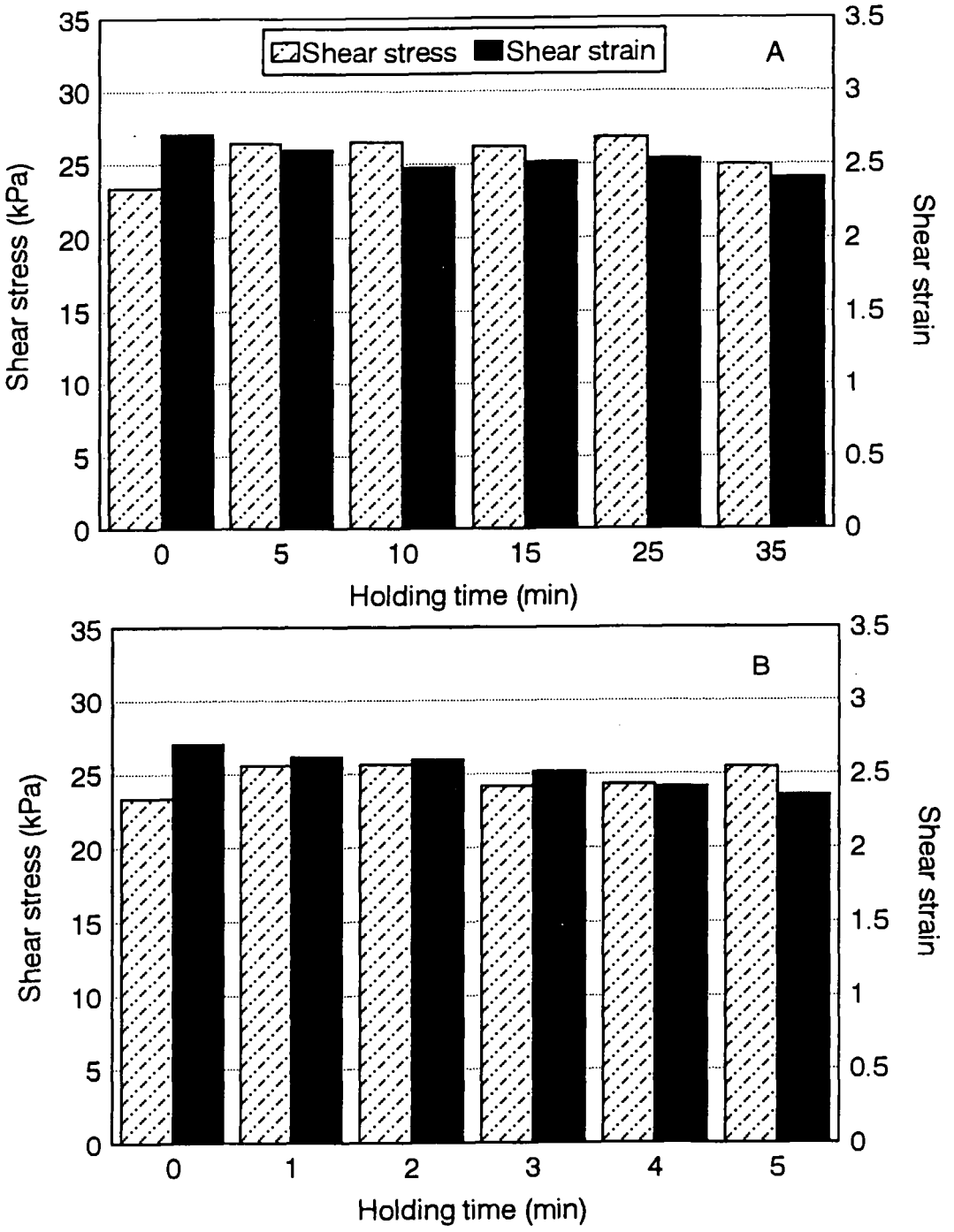


Figure 4.2. Textural properties of surimi gels with addition of E-64. (A) Held at 40°C; (B) Held at 55°C. No statistical differences were found among various holding times at each holding temperature ($P>0.05$).

properties of surimi gels. Aggregation process would be favored when heating time was prolonged, resulting in formation of strong gel networks (Hermansson, 1979; Foegeding et al., 1986a; Arntfield and Murray, 1992). Although textural degradation caused by the proteinase was inhibited by E-64, in our case increasing holding times at 40°C, a process which would supposedly promote aggregation, did not significantly increase shear stress and shear strain of whiting surimi gel (Fig. 4.2A). This indicated that changes in gel texture of whiting surimi with respect to the experimental time range were not influenced by kinetics of gelation.

Kinetic of textural degradation

Changes in both shear stress and shear strain with respect to time at various temperatures are shown in Figs. 4.3A-B and 4.4A-B, respectively. Because there was no difference between 2 experimental runs ($P>0.05$), averages of 2 replications are presented. Failure shear stress and shear strain of the samples held at various times at any temperatures were normalized by those of the control. Textural properties were plotted against holding time. Textural breakdown of surimi gels started to occur at 40°C and the most severe deterioration was at 55-60°C. Shear stress and shear strain of the samples held for 5 min at 55, 60, and 65°C could not be evaluated because the samples were very soft and mushy. Textural properties of the samples held at 75 and 80°C did not significantly change with holding times ($P>0.05$). This indicated that textural degradation of whiting surimi gel would be minimized if surimi was heated quickly to 75°C.

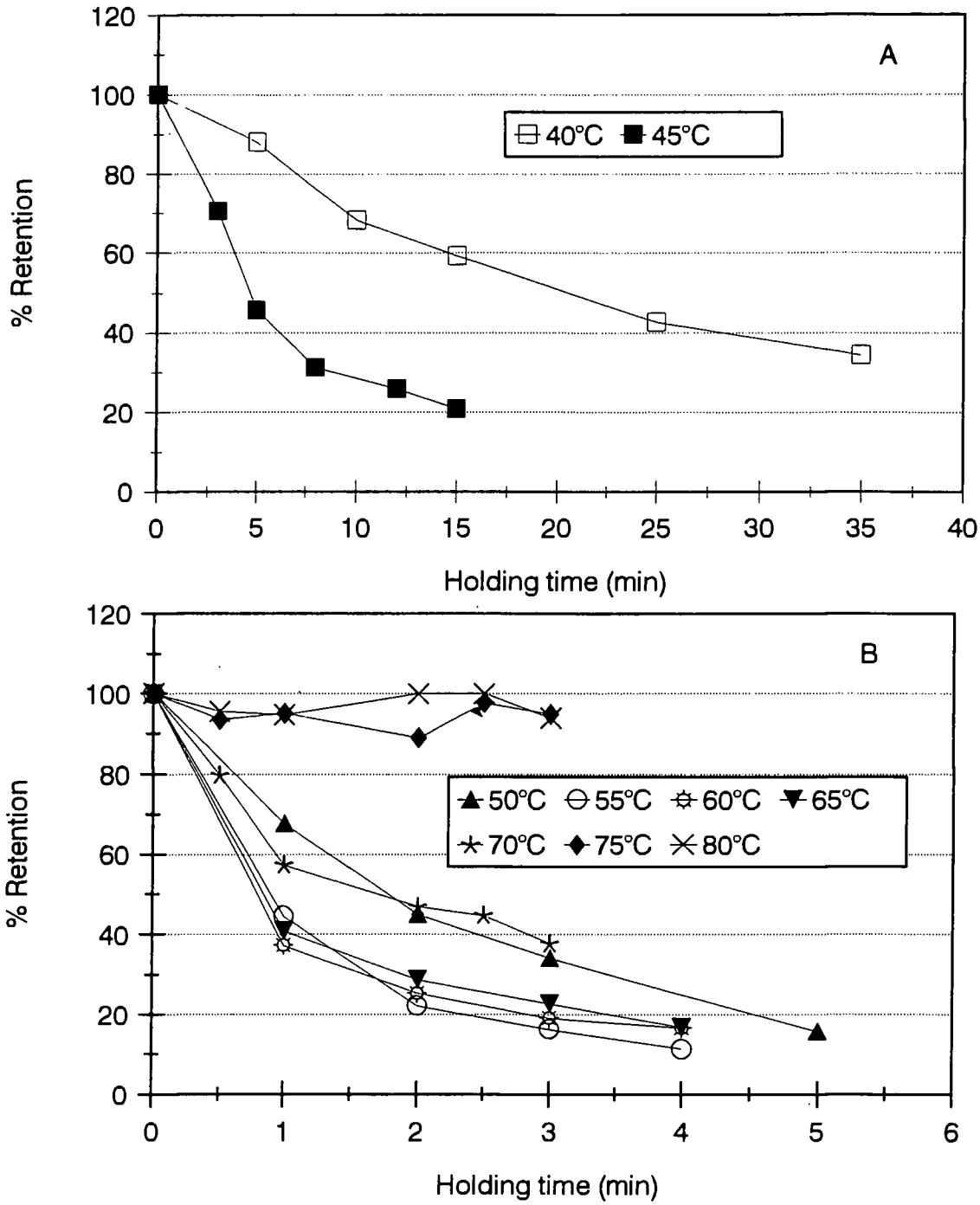


Figure 4.3. Effects of holding time and temperature on retention of shear stress of whiting surimi. (A) at 40-45°C; (B) at 50-80°C.

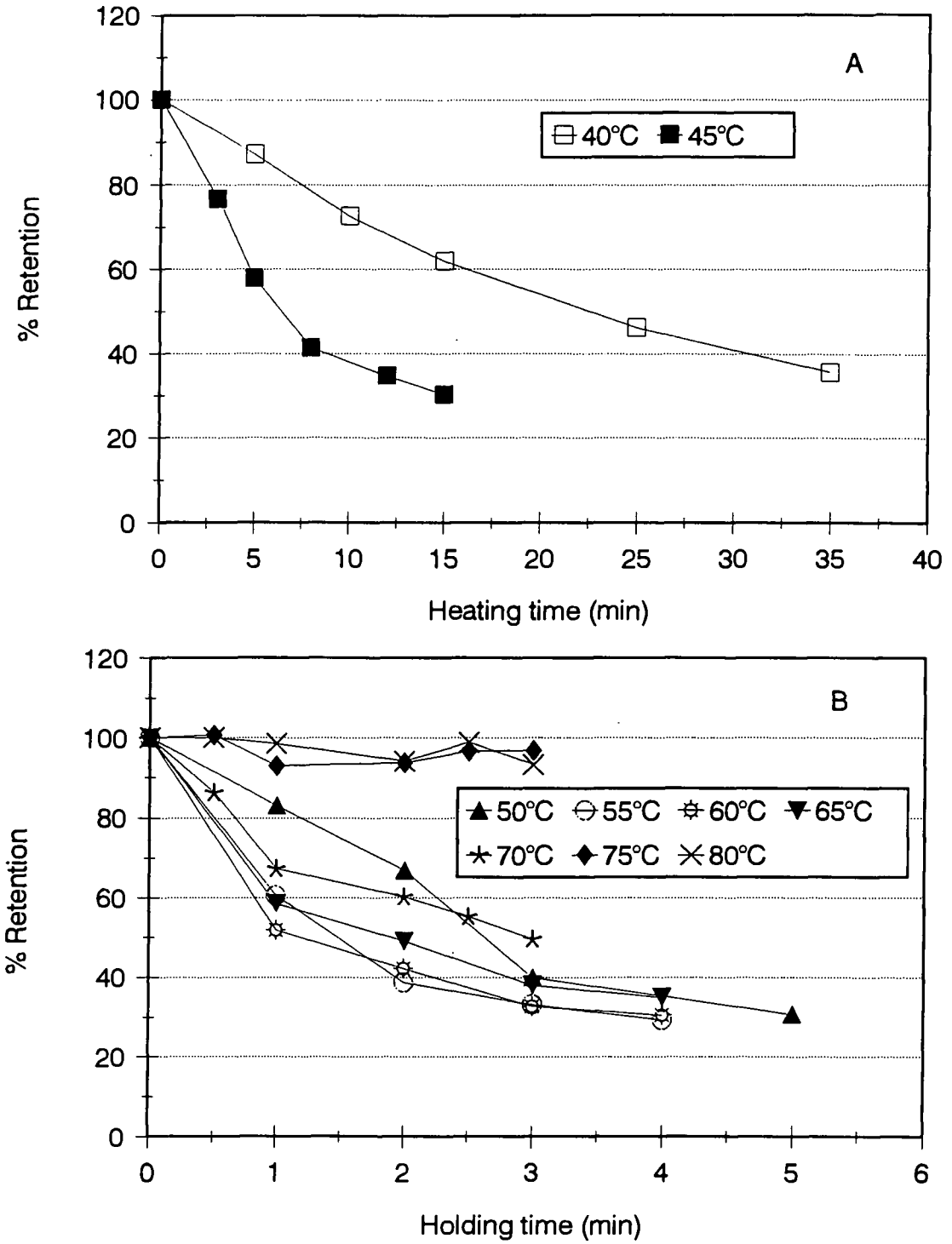


Figure 4.4. Effects of holding time and temperature on retention of shear strain of whiting surimi. (A) at 40-45°C; (B) at 50-80°C.

From Figs. 4.3A-B and 4.4A-B, changes in shear stress and shear strain of whiting surimi did not reach equilibrium state. But it decreased with time and elastic gel became mushy so that textural properties could not be measured. When retention of shear stress and shear strain was less than 10% and 30%, respectively, the samples exhibited no gel properties. Therefore, changes in shear stress and shear strain of whiting gel were modeled using the ratio of P to P_0. Apparent rate constants of textural degradation at each temperature obtained from 2 different analyses are shown in Table 4.2. The apparent rate constants of both shear stress and shear strain obtained from using either isothermal ($P_0 = P_{control}$) or nonisothermal conditions ($P_0 = P_{cal}$) were similar. Therefore, only the results calculated from nonisothermal model are shown in the plots of $\ln(P/P_0)$ versus equivalent holding time (Figs. 4.5A-B for shear stress and in 4.6A-B for shear strain). It is seen that first order reaction sufficiently described the changes of textural properties in whiting surimi (Table 4.2, Figs. 4.5A-B and 4.6A-B). Since heating rates of the control and samples were identical, textural degradation of the control was equivalent to that of the sample during heat-up periods. Kinetic analysis based on isothermal condition was also reported by Lenz and Lund (1980). The advantage of using isothermal condition to derive the kinetic model is that apparent rate constants and other kinetic parameters can be readily determined from the experimental data. The kinetic model is independent of heating rate during heat-up periods as long as the control and sample are subjected to the identical temperature history. However, the definition of P_0 is not universal and must be specified, depending on the conditions used in the experiment.

Table 4.2. Apparent rate constants for kinetic of textural degradation.

Temp. (°C)	Shear stress				Shear strain			
	$P_0 = P_{control}$		$P_0 = P_{cal}$		$P_0 = P_{control}$		$P_0 = P_{cal}$	
	k (min^{-1}) (Std error)	r^2	k_{Ti} (min^{-1}) (Std error)	r^2	k (min^{-1}) (Std error)	r^2	k_{Ti} (min^{-1}) (Std error)	r^2
40	0.0370 (0.0012)	0.988	0.0366 (0.0012)	0.989	0.0292 (0.0005)	0.997	0.0292 (0.0005)	0.997
45	0.1077 (0.0050)	0.976	0.1092 (0.0048)	0.980	0.0821 (0.0046)	0.967	0.0828 (0.0044)	0.971
50	0.3560 (0.0067)	0.997	0.3659 (0.0022)	0.999	0.2269 (0.0038)	0.978	0.2044 (0.0144)	0.957
55	0.5376 (0.0185)	0.988	0.5419 (0.0193)	0.987	0.3046 (0.0144)	0.978	0.3152 (0.0134)	0.982
60	0.4267 (0.0576)	0.873	0.4341 (0.0548)	0.887	0.2840 (0.0386)	0.871	0.2886 (0.0368)	0.948
65	0.4157 (0.0075)	0.997	0.4175 (0.0066)	0.998	0.2582 (0.0213)	0.942	0.2614 (0.0129)	0.978
70	0.3046 (0.0160)	0.986	0.3088 (0.0164)	0.986	0.2462 (0.0424)	0.891	0.1997 (0.0227)	0.950

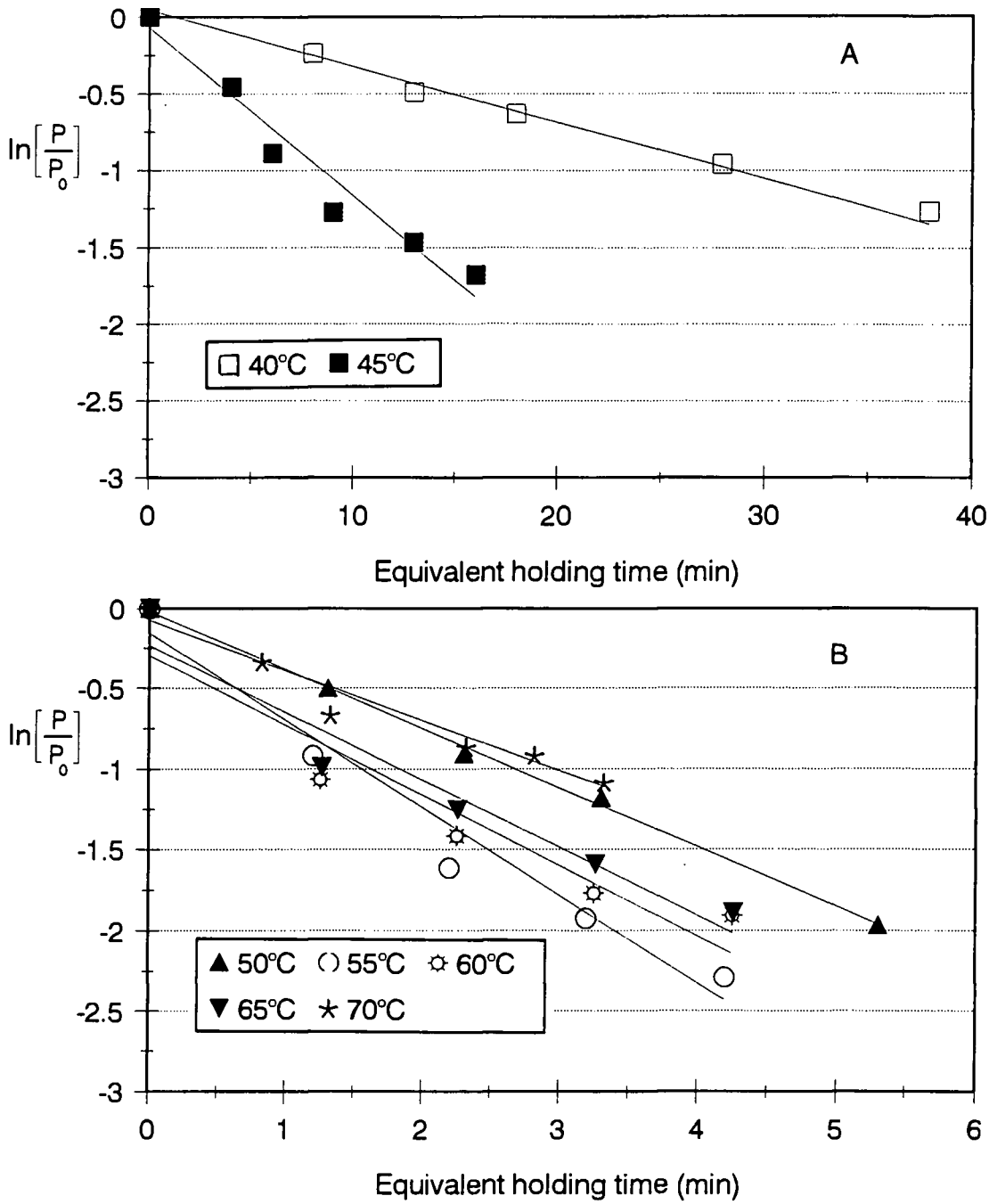


Figure 4.5. Graphical representation of changes in shear stress as a first order reaction determined using $P_0 = P_{cal}$. (A) at 40-45°C; (B) at 50-70°C.

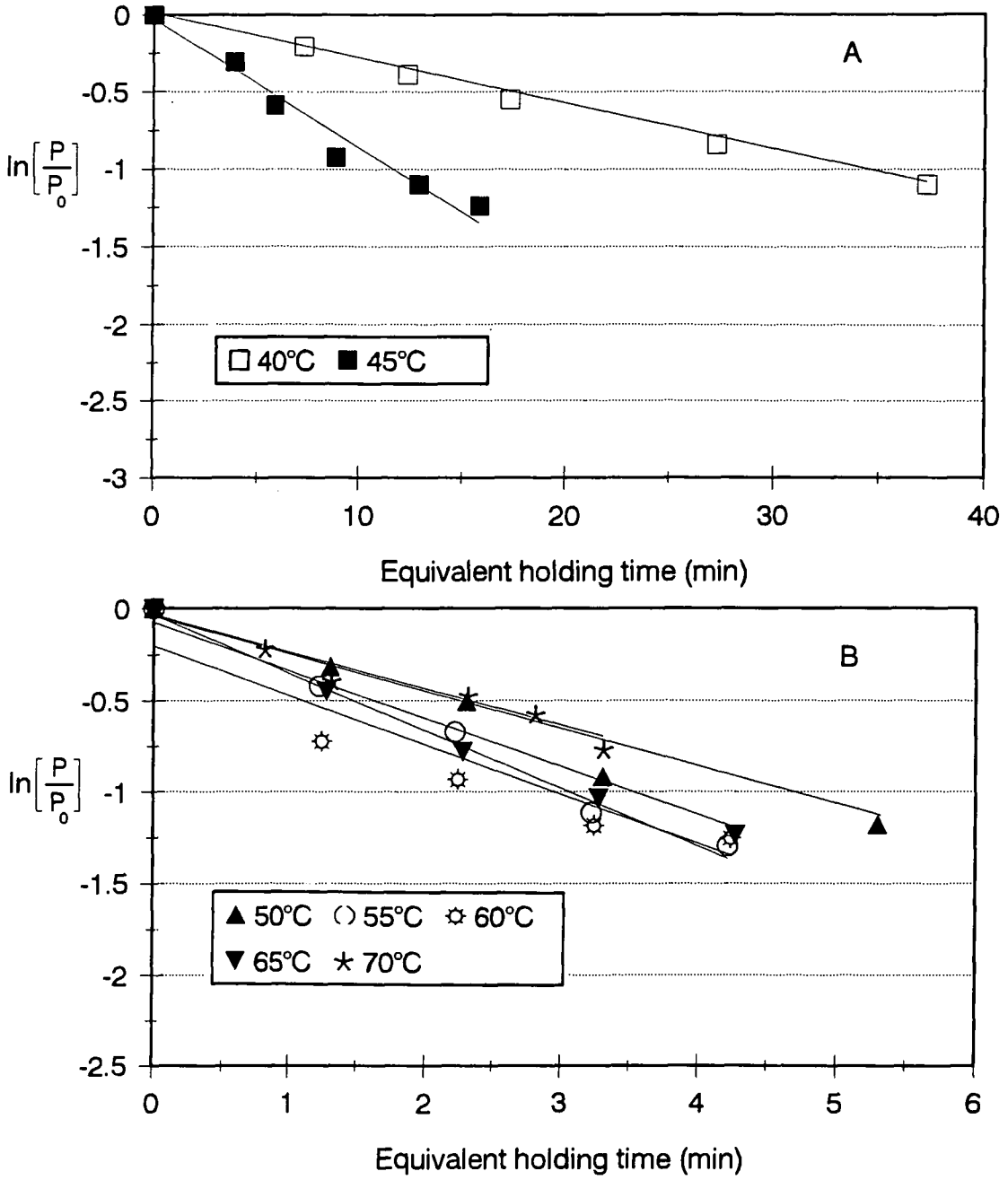


Figure 4.6. Graphical representation of changes in shear strain as a first order reaction determined using $P_0 = P_{cal}$. (A) at 40-45°C; (B) at 50-70°C.

Table 4.3 shows the corrected times at various temperatures calculated using the following equation:

$$t_{corr} = t_{aTi} - t \quad (4.7)$$

where t_{corr} is corrected time (min), t_{aTi} is equivalent holding time (min), and t is holding time (min). The corrected times indicate the equivalent thermal effect during heat-up periods at each temperature. For instance, thermal effect on shear stress during heat-up periods of the sample held at 40°C was equivalent to the thermal effect occurring at 40°C for 2.99 min. When comparing corrected time with the holding time (Table 4.1), the thermal effect during heat-up periods was more pronounced at higher temperatures (55-70°C), especially at relatively short holding times (0.5-3 min).

Table 4.3. The corrected times at various temperatures.

Temp (°C)	Corrected time (min)	
	Shear stress index	Shear strain index
40	2.99	2.33
45	1.04	0.87
50	0.30	0.30
55	0.20	0.22
60	0.25	0.24
65	0.26	0.27
70	0.35	0.31

The calculated P_0 obtained from iterative calculations for shear stress and shear strain are presented in Table 4.4. The "calculated P_0" indicated a theoretical maximum value of shear stress and shear strain that could have been obtained if textural degradation

Table 4.4. Experimental and theoretical values of shear stress and shear strain of samples heated ohmically using 13.3 V/cm (control).

Run	Shear stress (kPa)		Shear strain	
	Experimental value	Calculated P_0	Experimental value	Calculated P_0
1	30.24 ± 1.62	33.76	2.59 ± 0.06	2.77
2	34.62 ± 1.73	38.65	2.80 ± 0.08	3.00
3	28.91 ± 2.14	32.27	2.68 ± 0.09	2.88
4	24.60 ± 1.58	27.46	2.53 ± 0.06	2.72
5	27.32 ± 1.26	30.50	2.51 ± 0.04	2.69
6	29.91 ± 1.69	33.39	2.69 ± 0.07	2.89
7	26.09 ± 1.67	29.13	2.63 ± 0.10	2.82
8	29.75 ± 1.53	33.21	2.48 ± 0.07	2.66
9	25.74 ± 1.32	28.74	2.77 ± 0.11	2.97
10	28.15 ± 1.03	31.43	2.58 ± 0.09	2.77
11	29.69 ± 1.44	33.15	2.77 ± 0.09	2.97
12	28.86 ± 1.66	32.22	2.48 ± 0.07	2.66
13	26.69 ± 1.18	29.80	2.62 ± 0.12	2.81
14	29.65 ± 1.34	33.10	2.73 ± 0.06	2.93
15	27.07 ± 1.08	30.22	2.61 ± 0.10	2.80
16	27.67 ± 1.65	30.89	2.55 ± 0.10	2.74
17	30.78 ± 1.58	34.36	2.73 ± 0.09	2.93
18	27.99 ± 1.26	33.76	2.75 ± 0.11	2.95
19	28.24 ± 1.38	31.53	2.64 ± 0.08	2.83
20	27.84 ± 1.18	31.08	2.72 ± 0.06	2.92

had not occurred. Shear stress and shear strain of whiting surimi decreased by ~10 and ~7%, respectively, when it was heated from 10 to 90°C at voltage gradient 13.3 V/cm. It should be noted that whiting surimi retained good gel quality despite the approximate 10% textural degradation.

Figs. 4.7A-B illustrate the plot of ln k_{Ti} of shear stress and shear strain versus the reciprocal of the absolute temperature, respectively. The k_{Ti}'s were calculated from the nonisothermal approach. It was evident that there were two different temperature ranges in which the trends of apparent rate constant (k_{Ti}) were markedly different. The break in straight line was at about 55°C for both shear stress and shear strain. Rate of textural degradation increased with temperature and reached a maximum at 55°C. Then, it gradually decreased until 70°C. Rate of textural degradation could not be monitored at ≥ 75°C. The rate of textural changes resembled the temperature profile of purified proteinase activity from Pacific whiting (Seymour et al., 1994). An increase in rate of textural degradation with temperature up to 55°C could be due to enzyme activation, while a decrease in rate of degradation at higher temperature was probably caused by thermal denaturation of the endogenous proteinase. This supported the results from our E-64 study that proteolysis by the endogenous proteinase predominantly controlled final gel texture. Table 4.5 gives the values of E_a and r^2 determined from two different approaches. E_a values of shear stress and shear strain at the temperature ranging from 40-55°C and 55-70°C were comparable, implying that proteolysis affected these two parameters to a similar extent.

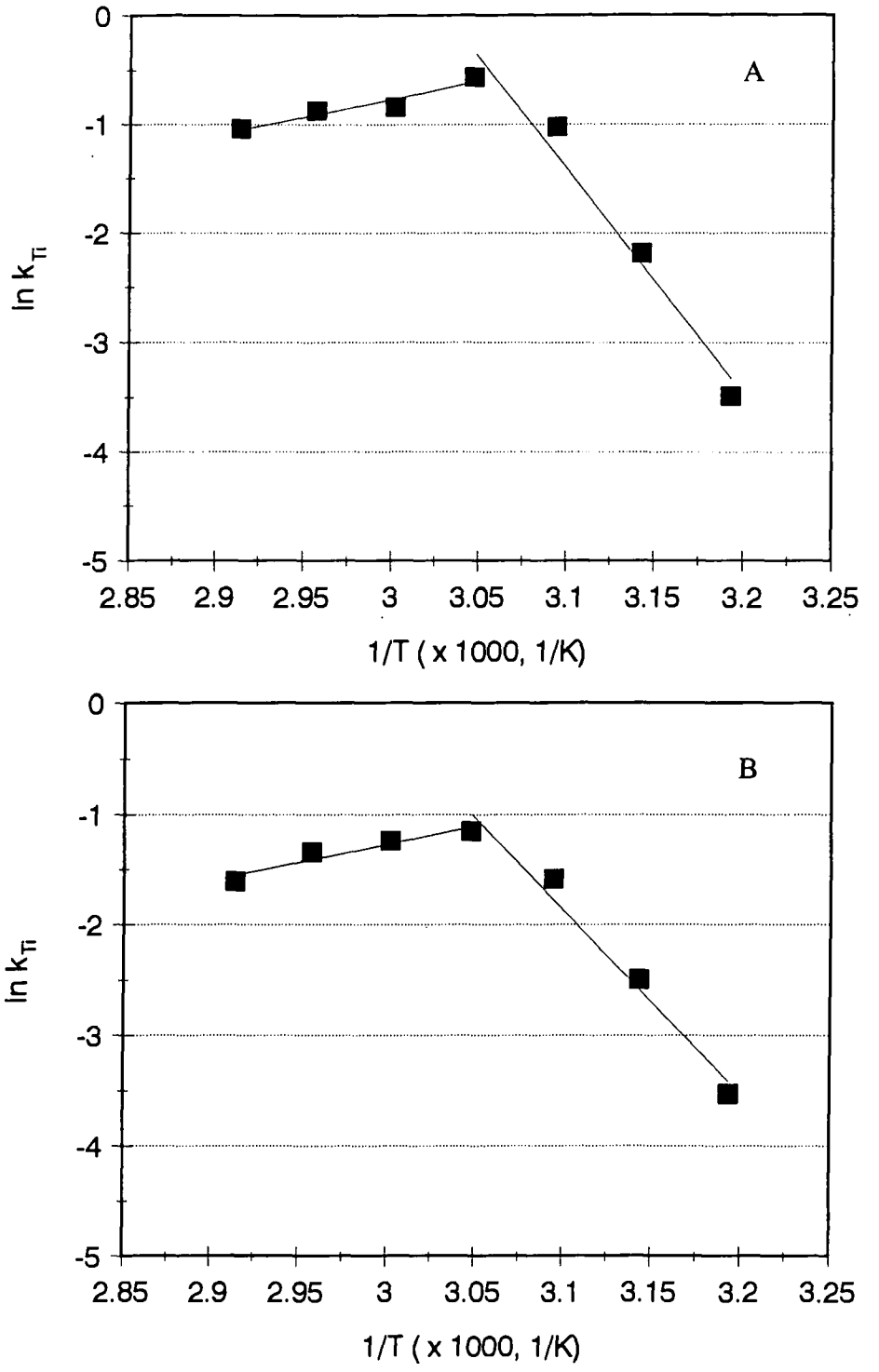


Figure 4.7. Effect of temperature on the apparent rate constant, k_{Ti}, of textural degradation. (A) shear stress; (B) shear strain.

Table 4.5. Activation energy values for kinetics of textural degradation.

Temp. (°C)	Shear stress				Shear strain			
	$P_0 = P_{control}$		$P_0 = P_{cal}$		$P_0 = P_{control}$		$P_0 = P_{cal}$	
	$E_a \times 10^{-3}$ (kJ/mol)	r^2	$E_a \times 10^{-3}$ (kJ/mol)	r^2	$E_a \times 10^{-3}$ (kJ/mol)	r^2	$E_a \times 10^{-3}$ (kJ/mol)	r^2
40-55	2.28	0.972	2.30	0.967	2.00	0.958	1.99	0.975
55-70	0.47	0.914	0.47	0.926	0.20	0.987	0.40	0.912

Kinetic of MHC degradation

Standard curves exhibited good linear relationship (r^2 = 0.982-0.999) between the area of densitogram and concentration of MHC standard (not shown). MHC degradation showed similar patterns to the changes in shear stress and shear strain (Figs. 4.8A-B). Degradation of MHC was significant from 40 to 75°C, and it increased with time at all temperatures. Degradation of MHC was in the range of 15-25% when the samples were held at 80 and 85°C for 0.5 min, but no changes of MHC content were observed at holding times beyond that time. Thus, it was reasonable to assume that degradation of MHC at 80 and 85°C was mainly due to the effect of thermal lag during heat-up periods. Yongsawatdigul and Park (1996) demonstrated that gelation of whiting surimi did not involve covalent cross-linking of MHC. Therefore, a decrease of MHC observed on SDS-PAGE in this study was mainly due to proteolysis of the endogenous proteinase. Degradation of MHC at 75°C suggested that proteinase *in situ* exhibited higher thermal stability than the purified proteinase, which exhibited the minimum activity at 70°C (Seymour et al., 1994; An et al., 1995). This could be because proteinase *in situ* was surrounded by and/or bound to its substrate, which tended to stabilize the enzyme structure and protect the proteinase towards thermal denaturation (Stauffer, 1989).

The overall reaction order determined from the iterative calculation procedure was 1.4. The kinetic model was expressed as:

$$\frac{1}{C_t^{0.4}} - \frac{1}{C_0^{0.4}} = 0.4\, k_{Ti}\, t_{a_{Ti}} \qquad (4.7)$$

where C_t is MHC content at time t (μg), C_0 is initial MHC content (μg), k_{Ti} is apparent rate constant at temperature T_i ($\mu g^{-0.4}$ /min), and t_{aTi} is equivalent holding time (min).

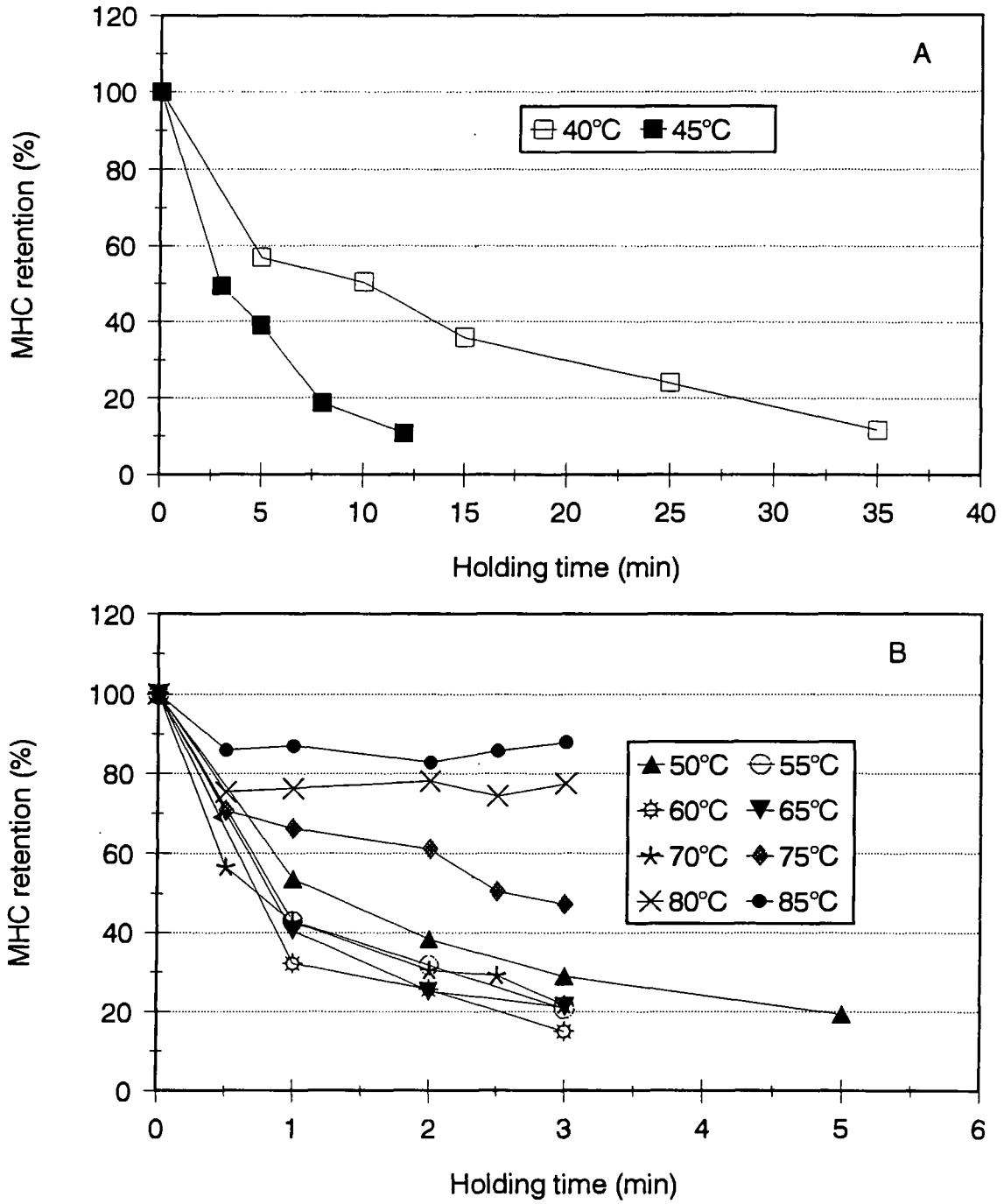


Figure 4.8. Effects of holding time and temperature on degradation of MHC. (A) at 40-45°C; (B) at 50-85°C.

The kinetic model was derived using MHC content of unheated surimi paste as the initial concentration, instead of using MHC content of the control. This was because MHC content of the unheated paste truly represented initial concentration of MHC and could be experimentally obtained. The derived kinetic model (equation 4.7) reasonably estimated MHC content of control samples. Based on paired t-test results, the estimated values of individual batch were not different from the experimental values ($P=0.296$, t value$=1.075$, degree of freedom = 18). Difference between the estimated and experimental value of all 19 samples was averaged to be 10.8%. The kinetic model can be employed to determine MHC content of whiting surimi (78% moisture and 2% NaCl) when temperature history of the sample is known.

Average value of MHC content of unheated surimi paste was 23.10 ± 5.34 μg, while that of the control sample was 17.51 ± 3.71 μg. Degradation of MHC of the sample heated ohmically at voltage gradient of 13.3 V/cm was calculated to be 24.2%. Degradation of MHC in the control sample was in agreement with the assumption that degradation of MHC at 80 and 85°C ranging from 15-25% (Fig. 4.8B) was mainly due to the thermal lag effect during heat-up periods. Based on the calculated P_0 shown in Table 4.4, shear stress and shear strain of the control samples decreased about 10 and 7% from the calculated values, respectively, when approximately 24% of MHC was hydrolyzed. The plots of equation (4.7) shown in Figs. 4.9A-B and r^2 shown in Table 4.6 indicated that degradation of MHC was adequately explained by the kinetic model.

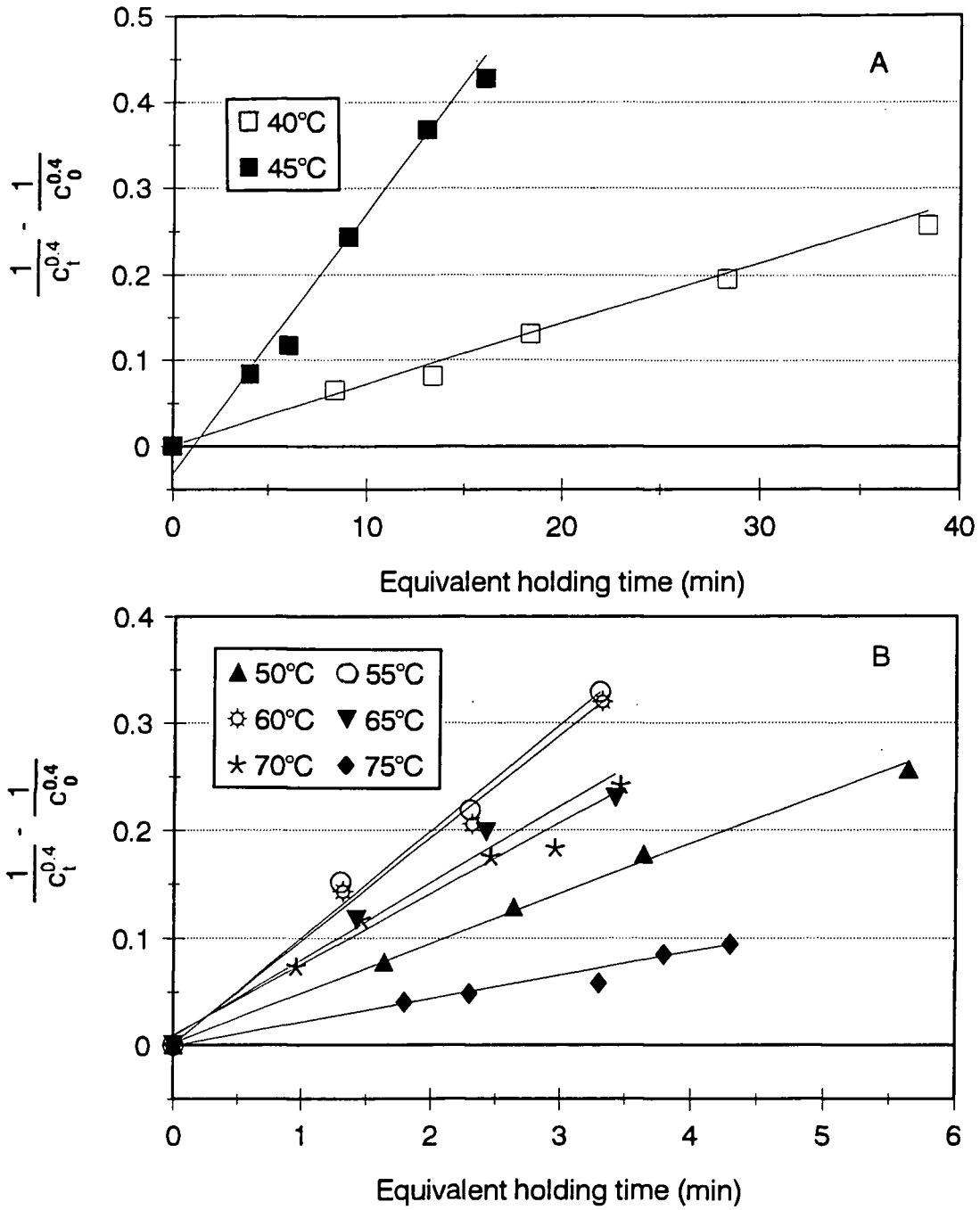


Figure 4.9. Graphical representation of changes in degradation of MHC at reaction order of 1.4. (A) at 40-45°C; (B) at 50-75°C.

Table 4.6. Apparent rate constants and corrected times for kinetic of MHC degradation.

Temp (°C)	Corrected time (min)	k_{Ti} ($\mu g^{-0.4}$/min) (Std error)	r^2
40	3.37	0.0178 (0.0005)	0.980
45	1.01	0.0760 (0.0016)	0.989
50	0.64	0.1161 (0.0012)	0.997
55	0.30	0.2485 (0.0023)	0.998
60	0.32	0.2402 (0.0037)	0.996
65	0.42	0.1894 (0.0067)	0.977
70	0.46	0.1647 (0.0048)	0.979
75	1.30	0.0556 (0.0010)	0.995

Rate of MHC degradation at various temperatures exhibited similar trends as those of shear stress and shear strain (Fig 4.10). From intersection of the lines shown in Fig. 4.10, the maximum rate of MHC degradation was estimated to be at 57°C. Rate of degradation markedly increased in the range of 40-57°C with E_a of 2.1×10^3 kJ/mol and then decreased in the range of 57-75°C with E_a of 1.3×10^3 kJ/mol. Since degradation

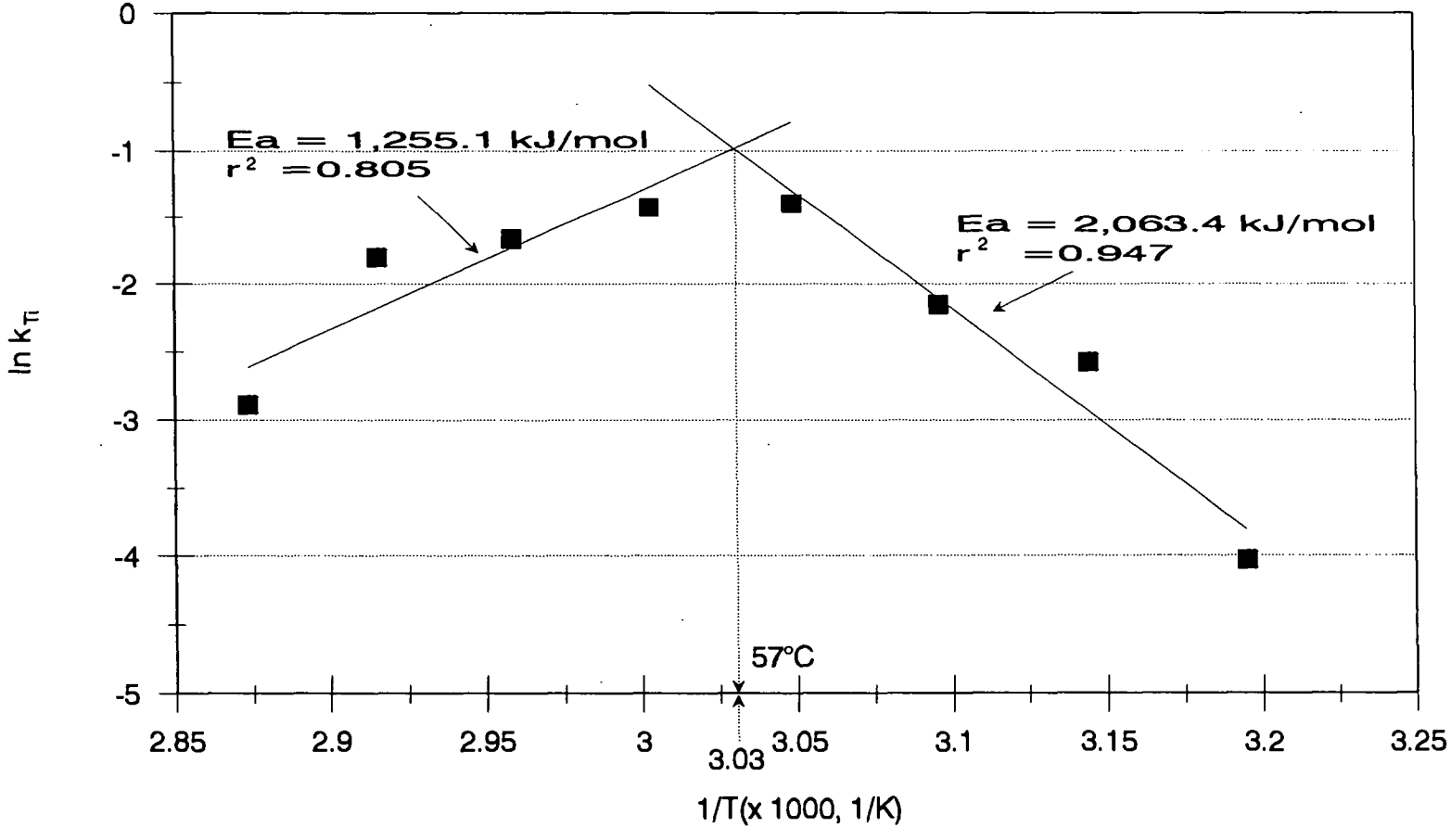


Figure 4.10. Effect of temperature on the apparent rate constant, k_{Ti}, of MHC degradation.

of MHC was mainly caused by activity of endogenous proteinase, E_a in the range of 40-57°C and 57-75°C could be interpreted as energy required for thermal activation and inactivation of the endogenous proteinase *in situ*, respectively. Higher thermal stability of the endogenous proteinase *in situ* was evident when E_a of MHC degradation in the inactivation temperature range (57-75°C) was compared to that of cathepsin L purified from whiting flesh, which was reported to be 320.2 kJ/mol (An et al.,1996). Interestingly, E_a of MHC degradation in the activation temperature range was similar to that of textural degradation. This suggested that proteolytic activity affected both MHC and textural properties to a similar extent during the enzyme activation range. However, E_a of thermal inactivation obtained from degradation of MHC was about 4 times greater than that obtained from textural degradation, indicating that degradation of MHC was more temperature-sensitive in the range of 57-75°C. An increase in temperature had greater effect on the rate of MHC degradation than on textural degradation. This seemed to suggest that MHC content was not the only factor controlling textural properties of surimi. Degradation of actin was observed at 50, 55, 60, 65 and 70°C (Fig. 4.11). The extent of proteolysis of actin was highest at 55°C and minimum loss occurred at temperature above 70°C. However, rate of actin degradation was much slower than that of MHC. Approximately 80% of MHC was hydrolyzed as surimi was held at 55°C for 3 min (Fig. 4.8B), while loss of actin from the same sample was about 30% (Fig. 4.11). This agreed with the result of An et al. (1994) indicating that the endogenous proteinase exhibited greater hydrolytic activity toward myosin than other myofibrillar proteins and least activity towards actin.

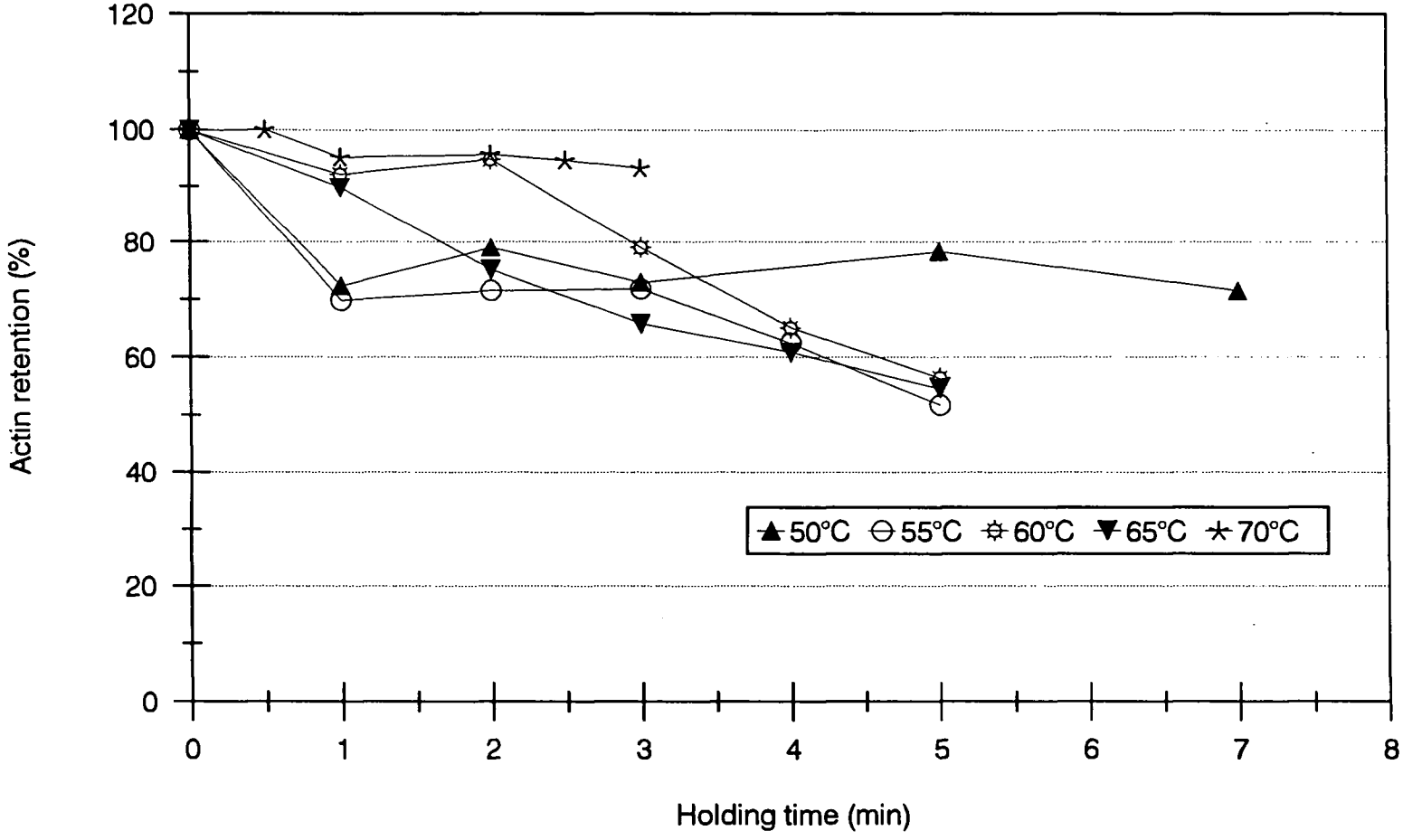


Figure 4.11. Effects of holding time and temperature on degradation of actin at 50-70°C.

Although actin was not directly involved in forming the gel network structure, it has been reported to increase gel rigidity when mixed with myosin at the myosin:actin mole ratio of 1.5-2.0 (Yasui et al., 1980). Therefore, textural properties of surimi gel would be affected by quantities of both myosin and actin. Even though degradation of both MHC and actin was noticed at the activation temperature range, 40-55°C (Figs. 4.8B and Fig. 4.11), textural degradation appeared to be well correlated with MHC degradation as it was evident by the similar E_a values of both reactions. This could be because MHC was a limiting factor governing final gel structure when both MHC and actin were severely hydrolyzed. However, when only MHC degradation was predominant, textural properties did not proportionally vary with MHC content. Surimi gels did not exhibit any changes in shear stress and shear strain at 75°C although its MHC retention was as low as 47.2% (Fig. 4.8B). It was unlikely that such a low retention level was a critical MHC content required to form gel. This was because the similar extent of MHC degradation at 50°C (held for 1 min) resulted in about 20-30% reduction in both textural parameters (Fig. 4.8B). Fig. 4.11 revealed that approximately 30% loss of actin was observed in the sample held at 50°C for 1 min, while no degradation of actin was observed at temperature exceeding 70°C. The differences in actin content between the samples heated at 50°C and those heated at 70°C could explain why their textural properties were different in spite of the similar MHC degradation extent. It was likely that gel formation of hydrolyzed MHC at 75°C would be strengthened by actin. Thus, textural degradation at this temperature was not evident. Since a decrease in actin did not show any particular trend, degradation kinetic of actin was not determined. It should be noted that hydrolytic reaction of MHC and actin

occurred simultaneously. Proteolysis in whiting surimi involved hydrolytic reactions with at least two substrates and presumably one proteinase. This represented a complicated system in which the true mechanism of the reaction was unknown. A knowledge of hydrolytic mechanism could lead one to gain more understanding about the effect of MHC and actin on gelation of whiting surimi.

Conclusions

Kinetic models of textural and MHC degradation of whiting surimi gel were developed using the Arrhenius equation to account for thermal lag during heat-up periods. Kinetic models for textural properties were also readily obtained using isothermal principle. Changes of rate constant with temperature consisting of activation and inactivation range resembled the reported temperature profiles of the cathepsin L purified from Pacific whiting flesh. The models can be used to determine the optimum heating conditions that provide the desired textural properties and MHC content to whiting surimi gel.

Chapter 5: CONCLUSIONS

Ohmic heating was an effective method to maximize gel functionality of Pacific whiting surimi. Textural properties of whiting surimi gel without proteinase inhibitors heated in a 90°C water bath for 15 min were unacceptably low. However, those of surimi gels heated using ohmic heating were more than twofold. The textural improvement was accompanied by a reduced degradation of myosin heavy chain (MHC) and actin shown on SDS-PAGE, and continuous gel network structure observed by scanning electron microscopy.

Electrical conductivity significantly increased with temperature and added NaCl, and slightly increased with moisture content. Linear relationship between electrical conductivity and temperature was evident (r^2 = ~0.99). Deviation from Ohm's law was observed in the samples containing high added NaCl content (4%), resulting in variations of electrical conductivity with voltage gradients. This was due to electrochemical reactions at the electrodes, which was induced by exceeding current density (> 3,500 A/m^2). Empirical model of electrical conductivity as a function of moisture and NaCl content was developed and satisfactorily predicted the electrical conductivity with an error range of 0-15.6%.

Isothermal and nonisothermal procedures provided the similar first order kinetic models for textural degradation. Kinetic of MHC degradation derived from nonisothermal method was well described by n=1.4. The model estimated MHC content of the sample heated ohmically at 13.3 V/cm with the errors of 10.8%. Rate of textural and MHC degradation increased with temperature until 55, 57°C, then decreased to minimum at 70

and 75°C, respectively. At the range of 40-55°C, MHC content was a predominant factor controlling surimi gel texture as it was evident by the similar E_a values of both reactions.

BIBLIOGRAPHY

AbuDagga, Y. and Kolbe, E. 1996. Analysis of heat transfer in surimi paste heated by conventional and ohmic means. J. Food Aquat. Food Prod. Technol. Submitted.

Ajibola, O.O. 1985. Moisture dependence of some electrical and dielectric properties of cassava. J. Sci. Food Agric. 36: 359-366.

Akazawa, H., Miyauchi, Y., Sakurada, Wasson, D.H., and Reppond, K.D. 1993. Evaluation of protease inhibitors in Pacific whiting surimi. J. Food Aquat. Food Prod. Technol. 2 (3): 79-95

An, H., Peter, M.Y., Seymour, T.A., and Morrissey, M.T. 1995. Isolation and activation of cathepsin L-inhibitor complex from Pacific whiting (*Merluccius productus)*. J. Agric. Food Chem. 43: 327-330.

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

An, H., Wu, J.W., Fan, X. B., Morrissey, M.T., and Seymour, T.A. 1996. Molecular and kinetic properties of cathepsin L from Pacific whiting (*Merluccius productus*). Biochem. J. Submitted.

AOAC. 1990. *Official Methods of Analysis*, 14th ed. Association of Official Analytical Chemists. Washington, DC.

Arabshahi, A. and Lund, D. 1985. Considerations in calculating kinetic parameters from experimental data. J. Food Proc. Eng. 7: 239-251.

Arntfield, S.D. and Murray, E.D. 1992. Heating rate affects thermal properties and network formation for vicilin and ovalumin at various pH values. J. Food Sci. 57: 640-646.

Asghar, A. and Bhatti, A.R. 1987. Endogenous proteolytic enzymes in skeletal muscle: their significance in muscle physiology and during postmortem aging events in carcasses. Adv. Food Res. 31: 343-451.

Asghar, A. and Pearson, A.M. 1980. Influence of ante- and postmortem treatments upon muscle composition and meat quality. Adv. Food Res. 26: 53-213.

Awuah, G.B., Ramaswamy, H.S., Simpson, B.K., and Smith, J.P. 1993. Thermal inactivation kinetics of trypsin at aseptic processing temperatures. J. Food Proc. Eng. 16: 315-328.

Barbut, S. and Mittal, G.S. 1990. Effect of heating rate on meat batter stability texture and gelation. J. Food Sci. 57: 640-646.

Bechtel, P.J. 1986. Muscle development and contractile proteins. In *Muscle as Food*, P.J. Bechtel (Ed.), pp. 2-31. Academic press, Inc., Orlando, FL.

Biss, C.H., Coombes, S.A., and Skudder, P.J., 1989. The development and application of ohmic heating for the continuous processing of particulate food stuffs. In *Processing Engineering in the Food Industry*, R.W. Field and J.A. Howell (Ed.), p. 17-27. Elsevier Applied Science Publishers, Essex, England.

Box, G.E.P., Hunter, W.G., and Hunter, J.S. 1978. *Statistics for Experimenters.* John Wiley & Sons, Inc., New York.

Boye, S.M. and Lanier, T.C. 1988. Effect of heat-stable alkaline protease activity of Atlantic menhaden (*Brevoorti tyrannus*) on surimi gels. J. Food Sci. 53: 1340-1342.

Busconi, L., Folco, E.J., Martone, C., Trucco, R.E., and Sanchez, J.J. 1984. Identification of two alkaline proteases and a trypsin inhibitor from muscle of white croaker *(Micropogon opercularis*). FEBS Lett. 176: 211-214.

Camou, J.P., Sebranek, J.G., and Olson, D.G. 1989. Effect of heating rate and protein concentration on gel strength and water loss of muscle protein gels. J. Food Sci. 54: 850-854.

Chan, J.K., Gill, T.A., and Paulson, A.T. 1992. The dynamics of thermal denaturation of fish myosins. Food Research International. 25: 117-123.

Chan, J.K., Gill, T.A., and Paulson, A.T. 1993. Thermal aggregation of myosin subfragments from cod and herring. J. Food Sci. 58: 1057-1061, 1069

Chang-Lee, M.V., Lampila, L.E., and Crawford, D.L. 1990. Yield and composition of surimi from Pacific whiting (*Merluccius productus*) and the effect of various protein additives on gel strength. J. Food Sci. 55: 83-86.

Chang-Lee, M.V., Pacheco-Aguilar, R., Crawford, D.L., and Lampila, L.E. 1989. Proteolytic activity of surimi from Pacific whiting (*Merluccius productus*) and heat-set gel texture. J. Food Sci. 54: 1116-1119, 1124.

Chung, Y.C., Richardson, L., and Morrissey, M.T. 1993. Effect of pH and NaCl on gel strength of Pacific whiting surimi. J. Aquatic Food Prod. Technol. 2(3): 19-35.

Cohen, E., Birk, Y., Mannheim, C.H., and Saguy, I.S. 1994. Kinetic parameter estimation for quality change during continuous thermal processing of grapefruit juice. J. Food Sci. 59: 155-158.

Dannenberg, F., and Kessler, H.G. 1988. Reaction kinetics of the denaturation of whey proteins in milk. J. Food Sci. 53: 258-263.

Datta, A.K. and Hu, W. 1992. Optimization of quality in microwave heating. Food Technol. 46(12): 53-56.

David, J. R.D., Merson, R.L. 1990. Kinetic parameters for inactivation of *Bacillus stearothermophilus* at high temperatures. J. Food Sci. 55: 488-493, 515.

Davies, F.L., Underwood, H.M., Perkin, A.G., and Burton, H. 1977. Thermal death kinetics of *Bacillus stearothermophilus* spores at ultra high temperatures I. Laboratory determination of temperature determination of temperature coefficients. J. Food Technol. 12: 115-129.

de Alwis, A.A.P. and Fryer P.J. 1990. The use of direct resistance heating in the food industry. J. Food Eng. 11: 3-27.

de Alwis, A.A.P. and Fryer P.J. 1992. Operability of the ohmic heating process: electrical conductivity effects: J. Food Eng. 15: 21-48.

Decareau, R.V. 1985. *Microwave in the Food Processing Industry*. Academic Press, Inc., Orlando, FL.

Dunn, O.J. and Clark, V.A. *Applied Statistics: Analysis of Variance and Regression.* John Wiley & Sons, New York.

Erickson, M.C., Gordon, D.T., and Anglemier, A.F. 1983. Proteolytic activity in the sarcoplasmic fluids of parasitized Pacific whiting (*Merluccius productus*) and unparasitized true cod (*Gadus marcocephalus*). J. Food Sci. 48: 1315-1319.

Ferry, J.D. 1948. Protein gels. Adv. Protein Chem. 4: 1-78.

Foegeding, E.A., Dayton, W.R., and Allen, C.E. 1986a. Effect of heating rate on thermally formed myosin, fibrinogen, and albumin gels. J. Food Sci. 51: 104-108, 112.

Foegeding, E.A., Dayton, W.R., and Allen, C.E. 1986b. Interaction of myosin-albumin and myosin-fibrinogen to form protein gels. J. Food Sci. 51: 109-112.

Folco, E.J., Busconi, L., Martone, C., Trucco, R.E., and Sanchez, J.J. 1984. Action of two alkaline proteases and a trypsin inhibitor from white croaker skeletal muscle (*Micropogon percularis*) in the degradation of myofibrillar proteins. FEBS Lett. 176: 215-219.

Fogler, S.H. 1992. *Elements of Chemical Reaction Engineering*, 2nd Ed. PTR Prentice-Hall Inc., Englewood Cliffs, NJ.

Folk, J.E. 1980. Transglutaminases. Ann. Rev. Biochem. 49: 517-531.

Fontana, A.J., Howard, L., Criddle, R.S., Hansen, L.D., and Wilhelmsen, E. 1993. Kinetics of deterioration of pineapple concentrate. J. Food Sci. 58: 1411-1417.

Forsythe, G.E., Malcolm, M.A., and Moler, C.B. 1977. Computer Methods for Mathematical Computations. Prentice-Hall, Inc., Englewood Cliffs, NJ.

Fryer, P.J. and Li, Z. 1993. Electrical resistance heating of foods. Trends in Food Sci. Technol. 4: 364-369.

Gill, T.A. and Conway, J.T. 1989. Thermal aggregation of cod muscle proteins using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide as a zero-length cross-linker. Agric. Biol. Chem. 53: 2553-2562.

Goodno, C.C. and Swenson, C.A. 1975. Thermal transition of myosin and its helical fragments. II. Solvent-induced variations in conformational stability. Biochem. 14: 873-877.

Greene, D.H. and Babbitt, J. 1990. Control of muscle softening and protease-parasite interactions in arrowtooth flounder (*Atheresthes stomias*). J. Food Sci. 55: 579-580.

Halden, K., de Alwis, A.A.P., and Fryer, P.J. 1990. Changes in electrical conductivity of foods during ohmic heating. Int. J. Food Sci. Technol. 25: 9-25.

Hamann, D.D. 1983. Structural failure in solid foods. In *Physical Properties of Foods.* M. Peleg and E.B. Bagely (Eds.), pp. 351-383. AVI Publishing Co., Inc., Westport, CT.

Hamann, D.D. and MacDonald, G.A. 1992. Rheology and texture properties of surimi and surimi-based foods. In *Surimi Technology*. T.C. Lanier and C.M. Lee (Eds.), pp.429-501. Marcel Dekker, Inc., New York.

Hamann, D.D., Amato, P.M., Wu, M.C., and Foegeding, E.A. 1990. Inhibition of modori (gel weakening) in surimi by plasma hydrolysate and egg white. J. Food Sci. 55: 665-669.

Hermansson, A.M. 1979. Aggregation and denaturation involved in gel formation. In *Functionality and Protein Structure*, A. Pour-El (Ed.), pp. 81-103. American Chemical Society, Washington, DC

Hill, Jr., C.G. and Grieger-Block, R.A. 1980. Kinetic data: generation, interpretation and use. Food Technol. 34(2): 56-66.

Howe, J.R., Hamann, D.D., Lanier, T.C., and Park, J.W. 1994. Fracture of Alaska pollock gels in water: effect of minced muscle processing and test temperature. J. Food Sci. 59: 777-780.

Hultin, H.O. 1985. Characterization of muscle tissue. In *Food Chemistry*, O.W. Fennema (Ed.), p. 726-788. Marcel Dekker, Inc., New York.

Ishioroshi, M., Samejima, K., Arie, Y., and Yasui, T. 1980. Effect of blocking the myosin-actin interaction in heat induced gelation of myosin in the presence of actin. Agric. Biol. Chem. 44: 2185.

Iwata, K., Kobashi, K., and Hase, J. 1974. Studies of muscle alkaline protease - II. Some enzymatic properties of carp muscular alkaline protease. Bull. Japan. Soc. Sci. Fish. 40: 189-200.

Johnson, J.R., Braddock, R.J., and Chen, C.S. 1995. Kinetics of ascorbic acid loss and nonenzymatic browning in orange juice serum: experimental rate constants. J. Food Sci. 60: 502-505.

Joseph, D., Lanier, T.C, and Hamann, D.D. 1994. Temperature and pH affect transglutaminase-catalyzed "setting" of crude fish actomyosin. J. Food Sci. 59: 1018-1036.

Kamath, G.G., Lanier, T.C., Foegeding, E.A., and Hamann, D.D. 1992. Nondisulfide covalent cross-linking of myosin heavy chain in "setting" of Alaska pollock and Atlantic croaker surimi. J. Food Biochem. 16: 151-172.

Kim, B.Y. 1987. Rheological investigation of gel structure formation by fish proteins during setting and heat processing. Ph.D. thesis. North Carolina State University, Raleigh, NC.

Kim, S.H., Carpenter, J.A., Lanier, T.C., and Wicker, L., 1993. Setting response of Alaska pollock surimi compared with beef myofibrils. J. Food Sci. 58: 531-534.

Kimura, I., Sugimoto, M., Toyoda, K., Seki, N., Arai, K., and Fujita, T. 1991. A study on the cross-linking reaction of myosin in kamaboko "suwari" gels. Nippon Suisan Gakkaishi 57: 1389-1396.

Kinoshita, M., Toyohara, H., Shimizu, Y., and Sakaguchi, M. 1991. Induction of modori-phenomenon (thermal gel degradation) by latent serine proteinase. Nippon Suisan Gakkaishi 57: 1935-1938.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685.

Lanier, T.C. 1992. Measurement of surimi composition and functional properties. In *Surimi Technology*, T.C. Lanier and C.M. Lee (Ed.), p. 429-501. Marcel Dekker, Inc., New York.

Lenz, M.K. and Lund, D.B. 1977a. The lethality-Fourier number method. Experimental verification of a model for calculating average quality factor retention in conduction-heating canned foods. J. Food Sci. 42: 997-1001.

Lenz, M.K. and Lund, D.B. 1977b. The lethality-Fourier number method. Experimental verification of a model for calculating temperature profiles and lethality in conduction-heating canned foods. J. Food Sci. 42: 989-996.

Lenz, M.K. and Lund, D.B. 1980. Experimental procedures for determining destruction kinetics of food components. Food Technol. 34(2): 51-55.

Lin, T.M. 1992. Characteristics and storage stability of unwashed and washed butterfish (*Peprillus buri*) mince. MS thesis. Louisiana State University, Baton Rouge, LA.

Liu, Y.M., Lin, T.S., and Lanier, T.C. 1982. Thermal denaturation and aggregation of actomyosin and surimi prepared from Atlantic croaker. J. Food Sci. 47: 1916-1920.

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

Luijerink, J.H. 1962. Process for the preservation of non-homogenous meat. US Patent 3 053 667.

MacDonald, G.A., Stevens, J., and Lanier, T.C. 1994. Characterization of New Zealand hoki and Southern blue whiting surimi compared to Alaska pollock surimi. J. Food Aquat. Food Prod. Technol. 3 (1): 19-38.

Makinoden, Y., Toyohara, H., and Niwa, E. 1985. Implication of muscle alkaline proteinase in the textural degradation of fish meat gel. J. Food Sci. 50: 1351-1355.

Makinoden, Y., Yokiyama, Y., Kinoshita, M., and Toyohara, H. 1987. Characterization of an alkaline protease of fish muscle. Comp. Biochem. Physiol. 87B: 1041-1046.

Mishkin, M., Saguy, I., and Karel, M. 1984. A dynamic test for kinetic models of chemical changes during processing: ascorbic acid degradation in dehydration of potatoes. J. Food Sci. 49: 1267-1270, 1274.

Mizrahi, S., Kopelman, I.J., and Perlman, J. 1975. Blanching by electroconductive heating. J. Food Technol. 10: 281-288.

Montejano, J.G., Hamann, D.D., and Lanier, T.C. 1984. Thermally induced gelation of selected comminuted muscle systems: Rheological changes during processing, final strengths and microstructure. J. Food Sci. 49: 1496-1505.

Morrissey, M.T., Wu, J.W., Lin, D.D., and An, H. 1993. Effect of food grade protease inhibitors on autolysis and gel strength of surimi. J. Food Sci. 58: 1050-1054.

Mulvihill, D.M. and Kinsella, J.E. 1987. Gelation characteristics of whey proteins and β-lactoglobulin. Food Technol. 41(9): 102-111.

Nagahisa, E., Nishimuro, S., and Fujita, T. 1981. Kamaboko-forming ability of the jellied meat of Pacific hake. Bull. Jap. Soc. Sci. Fish. 49: 901-906.

Naveh, D., Kopelman, I., J., and Mizrahi, S. 1983. Electroconductive thawing by liquid contact. J. Food Technol. 18: 171-176.

Nishimoto, S., Hashimoto, A., Seki, N., and Arai, K. 1988. Setting of mixed salt paste of two fish species in relation to cross-linking reaction of myosin heavy chain. Nippon Suisan Gakkaishi 54: 1227-1235.

Niwa, E. 1992. Chemistry of surimi gelation. In *Surimi Technology*. T.C. Lanier and C.M. Lee (Eds.), p. 429-501. Marcel Dekker, Inc., New York.

NFI 1991. *A Manual of standard methods for measuring and specifying the properties of surimi*. T.C. Lanier, K. Hart, and R.E. Martin (Eds), University of North Carolina Sea Grant College Program, Raleigh, NC.

NMFS 1993. Progress Report: Pacific whiting Fishery. National Marine Fisheries Service. Seattle, WA.

Nowsad, A., Kanoh, S., and Niwa, E. 1993. Electrophoretic behavior of cross-linked myosin heavy chain in suwari gel. Nippon Suisan Gakkaishi 59: 667-671.

Numakura, T., Seki, N., Kimura, I., Toyoda, K., Fujita, T., Takama, K., and Arai, K. 1985. Cross-linking reaction of myosin in the fish paste during setting (suwari). Nippon Suisan Gakkaishi 53: 1559-1565.

Numakura, T., Seki, N., Kimura, T., Toyoda, K., Fujita, T., Takama, K., and Arai, K. 1987. Effect of quality surimi on cross-linking reaction of myosin heavy chain during setting. Nippon Suisan Gakkaishi 53: 633-639.

Nunes, R.V., Swartzel, K.R. 1990. Modeling thermal processes using the equivalent point method. J. Food Eng. 11: 103-117.

OCZMA, 1994. Development plan for a quality assurance program for Pacific whiting. Oregon Coastal Zone Management Association. Newport, OR.

Okada, M. 1992. History of surimi technology in Japan. In *Surimi Technology*, T.C. Lanier and C.M. Lee (Ed.), p. 3-21. Marcel Dekker, Inc., New York.

Okitani, A., Matsukura, U., Kato, H., and Fujimaki, M. 1980. Purification and some properties of a myofibrillar protein-degrading protease, cathepsin L, from rabbit skeletal muscle. J. Biochem. 87:1133-1143.

Oldham, K.B. and Myland J.C. 1994. *Fundamentals of Electrochemical Science*. Academic Press, Inc., San Diego, CA.

Pacheco-Aguilar, R., Crowford, D.L., and Lampila, L.E. 1989. Procedures for the efficient washing of minced whiting (Merluccius productus) flesh for surimi production. J. Food Sci. 54: 248-252.

Palaniappan, S. and Sastry, S.K. 1991a. Electrical conductivity of selected solid foods during ohmic heating. J. Food Proc. Eng. 14: 221-236.

Palaniappan, S. and Sastry, S.K. 1991b. Electrical conductivity of selected juices: influence of temperature, solids content, applied voltage, and particle size. J. Food Proc. Eng. 14: 247-260.

Palaniappan, S. and Sastry, S.K., and Richter, E.R. 1992. Effect of electroconductive heat treatment and electrical pretreatment on thermal death kinetics of selected microorganisms. Biotech. Bioeng. 39: 225-232.

Park, J.W. 1995. Surimi gel colors as affected by moisture content and physical conditions. J. Food Sci. 60: 15-18.

Park, J.W. and Lanier, T.C. 1990. Effects of salt and sucrose addition on thermal denaturation and aggregation of water-leached fish muscle. J. Food Biochem. 14: 395-404.

Park, J.W, Yongsawatdigul, J., and Lin, T.M. 1994. Rheological behavior and potential cross-linking of Pacific whiting (*Merluccius productus*) surimi gel. J. Food Sci. 59: 773-776.

Park, K.H., Kim, Y.M., and Lee, C.W. 1988. Thermal inactivation kinetics of potato tuber lipoxygenase. J. Agric. Food Chem. 12: 1012-1014.

Parrot, D.L. 1992. Use of ohmic heating for aseptic processing of food particulates. Food Technol. 46(12): 68-72.

Patashnik, M., Groninger, H.S., Barnett, H., Kudo, G., and Koury, B. 1982. Pacific whiting, *Merluccius productus*: I. Abnormal muscle texture caused by Myxosporidian-induced proteolysis. Marine Fish. Rev. 44(5): 1-12.

Perkin, A.G., Burton, H., Underwood, H.M., and Davies, F.L. 1977. Thermal death kinetics of *Bacillus stearothermophilus* spores at ultra high temperatures II. Effect of heating period on experimental results. J. Food Technol. 12: 131-148.

Petersen, R. G. 1985. *Design and Analysis of Experiments.* Marcel Dekker, Inc., New York, NY.

Peterson, B.I., Tong, C.H., Ho, C.T., and Welt, B.A. Effect of moisture content on maillard browning kinetics of a model system during microwave heating. J. Agric. Food Chem. 42: 1884-1887.

Pethig, R. 1979. *Dielectric and Electronic Properties of Biological Materials.* John Wiley, New York, NY.

Piyachomkwan, K. 1994. Apparent inhibition of Pacific whiting surimi-associated protease by whey protein concentrate. MS thesis. Dept. of Food Science and Technology. Oregon State University, Corvallis, OR.

Porter, R.W., Koury, B.J., and Kudo, G. 1990. Method for treating fish meat contaminated with sporozoa and potato product for improving heat gelation of fish muscle. U.S. Patent 4,935,192.

Porter, R.W., Koury, B., and Kudo, G. 1993. Inhibition of protease activity in muscle extracts and surimi from Pacific whiting, *Merluccius productus*, and arrowtooth flounder, *Atheresthes stomias.* Marine Fish. Rev. 55(3): 10-15.

Ramaswamy, H. and Abdelrahim, K. 1992. Thermal processing and computer modeling. In *Encyclopedia of Food Science and Technology Volume 4.* Y.H. Hui (Ed.), p. 2538-2561. John Wiley & Sons., Inc., New York.

Rhim, J.W., Nunes, R.V., Jones, V.A., and Swartzel, K.R. 1989. Determination of kinetic parameters using linearly increasing temperature. J. Food Sci. 54: 446-450.

Roa, V., DeStefano, M.V., Perez, C.R., and Barreiro., J.A. 1989. Kinetic of thermal inactivation of proteinase (trypsin and chymotrypsin) inhibitors in black bean (*Phaseolus vulgaris*) flours. J. Food Eng. 1989: 35-46.

Roussel, H and Cheftel, J.C. 1988. Characteristics of surimi and kamaboko from sardines. Int. J. Food. Sci. Technol. 23: 607-623.

Saeki, H., Iseya, Z., Sugiura, S., and Seki, N. 1995. Gel forming characterization of frozen surimi from chum salmon in the presence of protease inhibitors. J. Food Sci. 60: 917-928.

Saguy, I., and Karel, M. 1980. Modeling of quality deterioration during food processing and storage. Food Technol. 34(2): 78-85.

Sakamoto, H., Kumazawa, Y., Toiguchi, S., Seguro, K., Soeda, T., and Motoki, M. 1995. Gel strength enhancement by addition of microbial transglutaminase during onshore surimi manufacture. J. Food Sci. 60: 300-304.

Samejima, K., Hashimoto, Y., Yasui, T., and Fukazawa, T. 1969. Heat gelling properties of myosin, actin, actomyosin and myosin-subunits in a saline model system. J. Food Sci. 34: 242.

Samejima, K., Ishioroshi, M., and Yasui, T. 1981. Relative role of the head and the tail portions of the molecule in heat-induced gelation of myosin. J. Food Sci. 46: 1412-1418.

Samejima, K., Ishioroshi, M., and Yasui, T. 1982. Heat induced gelling properties of actomyosin: effect of tropomyosin and troponin. Agric. Biol. Chem. 46: 535-540.

Sano, T., Noguchi, S.F., Matsumoto, J.J., and Tsuchiya, T. 1990a. Effect of ionic strength on dynamic viscoelastic behavior of myosin during thermal gelation. J. Food Sci. 55: 51-54, 70.

Sano, T., Noguchi, S.F., Matsumoto, J.J., and Tsuchiya, T. 1990b. Thermal gelation characteristics of myosin subfragments. J. Food Sci. 55: 55-58, 70.

SAS Institute, Inc., 1990. *SAS User's Guide Version 6, Fourth edition*, Vol. 2, Ch. 36, p. 1351-1456. SAS Institute Inc., Cary, NC.

Sastry, S.K. and Palaniappan, S. 1992a. Ohmic heating of liquid-particle mixtures. Food Technol. 46(12): 64-67.

Sastry, S.K. and Palaniappan, S. 1992b. Influence of particle orientation on the effective electrical resistance and ohmic heating rate of a liquid-particle mixture. J. Food Proc. Eng. 15: 213-227.

Schreier, P.J.R., Reid, D.G., and Fryer, P.J. 1993. Enhanced diffusion during the electrical heating of foods. Int. J. Food Sci. Technol. 28: 249-260.

Seguro, K., Kumazawa, Y., Ohtsuka, T., Toiguchi, S., and Motoki, M. 1995. Microbial transglutaminase and ϵ-(γ-glutamyl) lysine crosslink effects on elastic properties of kamaboko gels. J. Food Sci. 60: 305-311.

Seki, N., Uno, H., Lee, N.H., Kimura, I., Toyoda, K., Fujita, T., and Arai., K. 1990. Transglutaminase activity in Alaska pollock muscle and surimi, and its reaction with myosin B. Nippon Suisan Gakkaishi 56: 125-132.

Seymour, T.A., Morrissey, M.T., Peters, M.Y., and An, H. 1994. Purification and characterization of Pacific whiting proteases. J. Agric. Food Chem. 42: 2421-2427.

Shiba, M. 1992. Properties of kamaboko gels prepared by using a new heating apparatus. Nippon Suisan Gakkaishi 58: 895-901.

Shiba, M. 1993. Quality of kamaboko from vacuum-treated salt ground meat from several fish by applying joule heat. Nippon Suisan Gakkaishi 59: 1007-1011.

Shiba, M. and Numakura, T. 1992. Quality of heated gel from walleye pollock surimi by applying joule heat. Nippon Suisan Gakkaishi 58: 903-907.

Skudder, P. and Biss, C. 1987. Aseptic processing of food products using ohmic heating. The Chemical Engineer February: 26-28.

Stauffer, C.E. 1989. *Enzyme Assays for Food Scientists* p. 79-85. Van Nostrand Reinhold, New York.

Stirling, R. 1987. Ohmic heating - a new process for the food industry. Power Eng. J. 6: 365-371.

Suwansakornkul, P., Itoh, Y., Hara, S., Obatake, A., 1993. Identification of proteolytic activities of gel-degrading factors in three lizardfish species. Nippon Suisan Gakkaishi 59: 1039-1045.

Swartzel, K.R. 1982. Arrhenius kinetics as applied to product constituent losses in ultra high temperature processing. J. Food Sci. 47: 1886-1891.

Swartzel, K.R. 1984. A continuous flow procedure for reaction kinetic data generation. J. Food Sci. 49: 803-806

Taguchi, T., Ishizaka, M., Tanaka, M., Nagashima, Y., and Amano, K. 1987. Protein-protein interaction of fish myosin fragment . J. Food Sci. 52: 1103-1104.

Theimer, E.T., Heinze, G.E. 1977. Electrically conductible gellable composition with ionized salt. US Patent 4 016 301

Toyohara, H. and Shimizu, Y. 1988. Relation between the modori phenomenon and myosin heavy chain breakdown in threadfin-bream gel. Agric. Biol. Chem. 52: 255-257.

Toyohara, H., Kinoshita, M., and Shimizu, Y. 1990. Proteolytic degradation of threadfin-bream meat gel. J. Food Sci. 55: 259-260.

Toyohara, H., Sakata, T., Yamashita, K., Kinoshita, M., and Shimizu, Y. 1992. Degradation of oval-filefish meat gel caused by myofibrillar proteinase(s). J. Food Sci. 55: 364-368.

Toyohara, H., Kinoshita, M., Kimura, I., Satake, M., and Sakaguchi, M. 1993. Cathepsin L-like protease in Pacific hake muscle infected by myxosporidian parasites. Nippon Suisan Gakkaishi 59: 1101.

Vigerstrom, K.B. 1976. Passing an electric current of 50-60 CPS through potato pieces during blanching. US Patent 3 997 678.

Wang, S.S. 1992. Kinetics. In *Encyclopedia of Food Science and Technology Vol. 3.* Y.H. Hui. (Ed.), p. 1573-1585. John Wiley & Son, Inc., New York.

Wasson, D.H., Babbitt, J.K., and French, J.S. 1992a. Characterization of heat stable protease from arrowtooth flounder, *Atheres stomias*. J. Aquat. Food Prod. Technol. 1(3/4): 167-182.

Wasson, D.H., Reppond, K.D., Babbitt, J.K., and French, J.S. 1992b. Effects of additives on proteolytic and functional properties of arrowtooth flounder surimi. J. Aquat. Food Prod. Technol. 1(3/4): 147-165.

Weber, K. and Osborn, M. 1969. The reliability of molecular weight determination by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. 224: 4406-4412.

Wicker, L. and Temelli, F. 1988. Heat inactivation of pectinesterase in orange juice pulp. J. Food Sci. 53: 162-164.

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

Yamashita, M. and Konagaya, S. 1990a. Purification and characterization of cathepsin L from the white muscle of chum salmon, *Oncorhynchu keta*. Comp. Biochem. Physiol. 96B: 247-252.

Yamashita, M. and Konagaya, S. 1990b. Purification and characterization of cathepsin B from the white muscle of chum salmon, *Oncorhynchu keta*. Comp. Biochem. Physiol. 96B: 733-737.

Yamashita, M. and Konagaya, S. 1990c. Participation of cathepsin L into extensive softening of the muscle of chum salmon caught during spawning migration. Nippon Suisan Gakkaishi 56: 1271-1277.

Yamashita, M. and Konagaya, S. 1991. Hydrolytic action of salmon cathepsin B and L to muscle structural proteins in respect to muscle softening. Nippon Suisan Gakkaishi 57: 1917-1922.

Yasui, T., Ishioroshi, M., and Samejima, K. 1980. Heat-induced gelation of myosin in the presence of actin. J. Food Biochem. 4: 61-78

Yasui, T., Ishioroshi, M., and Samejima, K. 1982. Effect of actomyosin on heat-induced gelation of myosin. Agric. Biol. Chem. 46: 1049-1059.

Yongsawatdigul, J., Park, J.W. 1996. Linear heating rate affects gelation of Alaska pollock and Pacific whiting surimi. J. Food Sci. In press.

Yongsawatdigul, J., Park, J.W., Kolbe, E., AbuDagga, Y., and Morrissey, M.T. 1995. Ohmic heating maximizes gel functionality of Pacific whiting surimi. J. Food Sci. 60: 1-5

Zeece, M.G., Woods, T.L., Keen, M.A., and Reville, W.J. 1992. Role of proteinases and inhibitors in postmortem muscle protein degradation. Reciprocal Meat Conference Proceedings, Vol 45: 51-61.

Ziegler, G.R. and Acton, J.C. 1984. Mechanisms of gel formation by proteins of muscle tissue. Food Technol. 38(5):77-82.

APPENDICES

APPENDIX A

LINEAR HEATING RATE AFFECTS GELATION OF ALASKA POLLOCK AND PACIFIC WHITING SURIMI

Jirawat Yongsawatdigul and Jae W. Park

Submitted to *Journal of Food Science*,
Institute of Food Technologists, Chicago, IL,
September 1995, in press.

Abstract

Shear stress at failure of Alaska pollock surimi gels with and without beef plasma protein (BPP) increased as heating rate decreased, but shear strain at failure was unaffected. An increase in shear stress was accompanied by an increase of cross-linked myosin heavy chain. Slow heating rates increased proteolysis in Pacific whiting surimi as shown by degradation of myosin heavy chain and low shear stress and shear strain. Proteolysis of whiting surimi was lessened by BPP to a greater extent at rapid heating rates (20 and 30°C/min) than at slow heating rates (1 and 5°C/min).

Key words: Heating rate, whiting, pollock, surimi, gelation

Introduction

The formation of gel networks upon heating contributes to textural characteristics of food proteins. According to a classical mechanism for protein gelation (Ferry,1948), denaturation and aggregation are critical steps in heat-induced gelation. If aggregation is slow with respect to denaturation, heat-denatured proteins align in an ordered fashion to form a fine gel network. Resulting gels exhibit more elasticity (Hermansson, 1979). Based on this mechanism, gel networks are greatly influenced by heating time and temperature. Foegeding et al.(1986a) demonstrated that stronger myosin and fibrinogen gels were produced at 12°C/hr than at 50°C/hr. Gel strength of the mixture of myofibrillar and sarcoplasmic proteins increased when heating rate decreased from 85°C/hr to 17°C/hr (Camou et al., 1989). Arntfield and Murray (1992) reported that heating rate had no effect on gel strength of vicilin and ovalbumin at pH near the isoelectric points, when random aggregation, rather than ordered alignment of proteins, was favored. However, at a higher pH, gel strength of vicilin and ovalbumin decreased as heating rates increased from 0.3°C/min to 3°C/min. Such results indicated that textural properties of protein gels could be improved through slow heating treatments.

Surimi is stabilized fish myofibrillar proteins which provide important textural characteristics. Heating modes influence gelation of surimi. Yongsawatdigul et al. (1995) demonstrated that Pacific whiting surimi exhibited high shear stress and shear strain when heated ohmically from 5°C to 90°C within 1 min. Rheological properties of surimi heated in a 90°C water bath were unacceptably low. This was attributed to a rapid inactivation of endogenous proteinase with ohmic heating; consequently, degradation of myosin was greatly reduced. However, a 2-step heating process improved textural

properties of surimi made from a variety of fish species; such as, Alaska pollock (Montejano et al., 1984; Numakura et al., 1985), sardine (Roussel and Cheftel, 1988), Atlantic croaker (Kim, 1987; Kamath et al., 1992), Southern blue whiting, hoki (MacDonald et al., 1994), and Pacific whiting with beef plasma protein (Park et al., 1994). Gel elasticity increased when surimi pastes were subjected to setting at 5-40°C prior to heating to 90°C. In pollock surimi, improved gel quality was associated with increased ε-(γ-glutamyl)-lysine contents, resulting from polymerization of myosin heavy chains. Endogenous transglutaminase was reported to catalyze such reactions (Seki et al., 1990; Kimura et al., 1991).

The effect of linear heating rate on surimi gelation has not been reported. Since water bath heating provides non-linear temperature profiles, the influence of such heating rates on gelation cannot be validated. Linear heating rates may be achieved using a programmable water bath only when very slow heating rates are applied (Foegeding et al., 1987a, b; Camou et al., 1989; Arntfield and Murray, 1992). In ohmic heating, heat is internally generated due to electrical resistance of food products (de Alwis and Fryer, 1990). A wide range of linear heating rates can be provided using appropriate voltage gradients and temperature controllers.

Our objective was to investigate the effects of linear heating rates on textural properties and myofibrillar proteins of Pacific whiting and Alaska pollock surimi. These two species were selected to determine the influence of inherent properties of fish myofibrillar proteins on heat-induced gelation. Pacific whiting surimi has been known to undergo proteolysis, resulting in undesired gel quality (Chang-Lee et al., 1989;

Morrissey et al., 1993), while Alaska pollock surimi exhibits no proteolysis and results in desirable textures upon heating.

Materials and Methods

High grade (FA) frozen surimi from Alaska pollock (*Theragra chalcogramma*), containing 4% sucrose, 5% sorbitol and 0.3% mixture (1:1) of sodium tripolyphosphate and tetrasodium pyrophosphate, was purchased from Alaska Ocean Seafood Co. (Anacortes, WA). Pollock surimi was ≈6 mo old at purchase. Surimi blocks (10kg) were cut into ≈1,200g portions, vacuum-packed, and kept at -30°C. Pacific whiting (*Merluccius productus*) surimi without food grade enzyme inhibitors was obtained from Point Adams Packing Co. (Hammond, OR). The whiting surimi was mixed with 4% sucrose, 4% sorbitol and 0.3% sodium tripolyphosphate. Surimi was divided into ≈500g portions and vacuum-packed in plastic trays before freezing using a -30°C plate freezer (APV Crepaco, Inc., Rosemont, IL). All samples were stored at -30°C throughout the experiment.

Moisture and total nitrogen contents of pollock and whiting surimi were determined using standard oven and Kjeldahl procedures (AOAC, 1990). Moisture and protein contents (% total nitrogen × 6.25) of pollock surimi were 74.85 ± 0.04% (mean ± standard deviation) and 17.16 ± 0.12%, whereas, those of whiting surimi were 76.25 ± 0.35% and 15.06 ± 0.59%, respectively.

Surimi gel preparation

Surimi pastes of pollock (P), whiting (W), pollock with beef plasma protein (BPP) (P+BPP), and whiting with BPP (W+BPP) were prepared. BPP (AMP 600N, AMPC, Inc., Ames, IA) was used as an enzyme inhibitor to control proteolytic activity caused by endogenous proteinase(s) during heating (Hamann et al., 1990; Morrissey et al., 1993). The protein content of BPP analyzed using AOAC (1990) methods was 70.15%. Final protein of all surimi pastes was adjusted to 12.2% (w/w). Partially-thawed surimi (≈1.2kg) was chopped for 1 min in a Stephan vertical vacuum cutter (model UM 5 Universal, Stephan Machinery Co., Columbus, OH). Salt (2% w/w) was added and mixed with surimi for another 1.5 min. Ice water was added and chopping continued for 30 sec. For P+BPP and W+BPP samples, 1% protein of surimi was replaced by 1% protein of BPP. The amount of BPP added was ≈1.4% (w/w). The paste was further chopped at high speed under vacuum of 50 kPa for 3 min. The final moisture content of P, W, P+BPP, and W+BPP were 79.7, 78.6, 80.2, and 78.8%, respectively.

The paste was stuffed into chlorinated polyvinyl chloride (CPVC) tubes (1.9 cm i.d. × 18 cm long) and heated using the ohmic heating apparatus as described by Yongsawatdigul et al. (1995). Temperatures at the geometric center of the sample were measured by a 30-gauge teflon-sealed thermocouple (Type-T) and were recorded on a datalogger (model 21x, Campbell Scientific, Inc., Logan, UT). The heating device was connected to a temperature controller (OMEGA Engineering Inc., Stamford, CT) and solid state relay, which automatically turned the power supply on and off to provide a linear heating rate. The samples were heated from 10 to 90°C with 5 different heating rates: 1, 5, 10, 20, or 30°C/min (Fig. A.1). The approximate applied voltage for each heating

rate was 50 (1°C/min), 80 (5°C/min), 100 (10°C/min), 120 (20°C/min), and 150 V (30°C/min). Three sample tubes were individually heated for each heating rate to obtain enough gel samples for torsion testing. Surimi gels were cooled in ice water and kept at 4°C for torsion tests and gel electrophoresis. Gel preparation was repeated.

Torsion test

Torsion failure tests were performed at room temperature, 18-24 hr after gel preparation as described by NFI (1991). Ten hourglass-shaped samples were measured for each treatment. Shear stress and shear strain at failure were calculated from equations described by Hamann (1983).

SDS-PAGE

Protein patterns of all samples were initially studied using SDS-PAGE described by Laemmli (1970). Solubilization of samples with 5% sodium dodecyl sulfate solution (95°C) was carried out according to Morrissey et al. (1993). Stacking and separating gels were 4% (w/v) and 10% (w/v) acrylamide, respectively. The amount of protein applied on the polyacrylamide gel was 60 μg. High molecular weight standard markers (Sigma Chemical Co., St. Louis, MO) contained rabbit myosin (205 kD), β-galactosidase (116 kD), phosphorylase b (97.4 kD), bovine albumin (66 kD), egg albumin (45 kD), and carbonic anhydrase (29 kD). Gels were fixed and stained with 0.125% Coomassie brilliant blue R-250 (Bio-Rad, Richmond, CA), and destained in a solution containing 25% ethanol and 10% acetic acid.

The continuous SDS-PAGE system described by Weber and Osborn (1969) was used to investigate polymerization of myosin heavy chain in surimi gels. Samples for electrophoresis were prepared according to Kamath et al. (1992). The surimi samples were cut into small pieces and 0.4g were solubilized in 7.5 mL of 2% SDS-8 M urea-2% β-mercaptoethanol-20 mM Tris-HCl (pH 8.0) buffer. The samples were heated for 2 min at 100°C and stirred continuously for 24 hr at room temperature (≈23°C). Homogenates were centrifuged at 10,000 × g (Sorvall, DuPont Co., Newton, CT) for 20 min at room temperature. The protein concentration of supernatants was measured by the Lowry method (Lowry et al., 1951), using bovine serum albumin (Sigma Chemical Co., St. Louis, MO) as a standard. Gels were prepared of 3% (w/v) polyacrylamide and contained 6 M urea. GelBond PAG film (FMC Bioproducts, Rockland, ME) was used to support the gel. The phosphate buffer (0.06 M NaH_2PO_4, 0.14 M Na_2HPO_4, 0.002% (w/v) SDS, pH 7.0) was diluted with water (1:2) and used as a running buffer. A constant current of 80 mA was applied. Staining and destaining were carried out as described.

Statistical analysis

Whiting samples without BPP heated at 1 and 5°C/min were too mushy to be measured by torsion test, therefore, only data from gels heated at 10, 20, and 30°C/min were evaluated. A split plot design (Petersen, 1985) was used to evaluate effects of type of surimi, addition of BPP, and heating rate on textural properties. Whole plots were assigned to types of surimi (pollock and whiting) and BPP (with and without BPP). Five levels of heating rates were assigned as a subplot factor. Since interactions of surimi vs addition of BPP, and interactions of surimi vs heating rate were significant ($P<0.05$),

data were independently analyzed for each type of surimi. Additions of BPP were whole plots and heating rates were subplots. Test averages were subjected to analysis of variance using the General Linear Models (GLM) procedure of the Statistical Analysis System (SAS Institute, Inc., 1990). Significant interaction means (addition of BPP*heating rate) were separated using the PDIFF procedure of SAS (SAS Institute, Inc., 1990).

Results and Discussion

Rheological properties at various heating rates

Heating rates were referred to as linear since temperature increased equally in each time interval (Fig. A.1). Temperature was assumed to be uniformly distributed throughout the sample because of the homogeneity of the surimi paste. The effects of voltage gradient on myofibrillar protein structure were also assumed to be insignificant. This was because voltage gradients were relatively small, 2.7-8.1 V/cm, and were applied intermittently to the sample through temperature controller. Changes in textural properties and myofibrillar proteins were primarily due to heating rates.

Shear stress (Fig. A.2.1) and shear strain (Fig. A.2.2) of whiting gels with and without BPP were compared. The W samples heated at 1 and 5°C/min were very soft and mushy. At 10°C/min, the whiting gel was strong enough to measure its rheological properties. Shear stress and shear strain of the whiting gels increased when heating rates were raised. However, shear stress of samples heated at 30°C/min was not different from those heated at 20°C/min ($P>0.05$). Textural properties of whiting surimi were increased

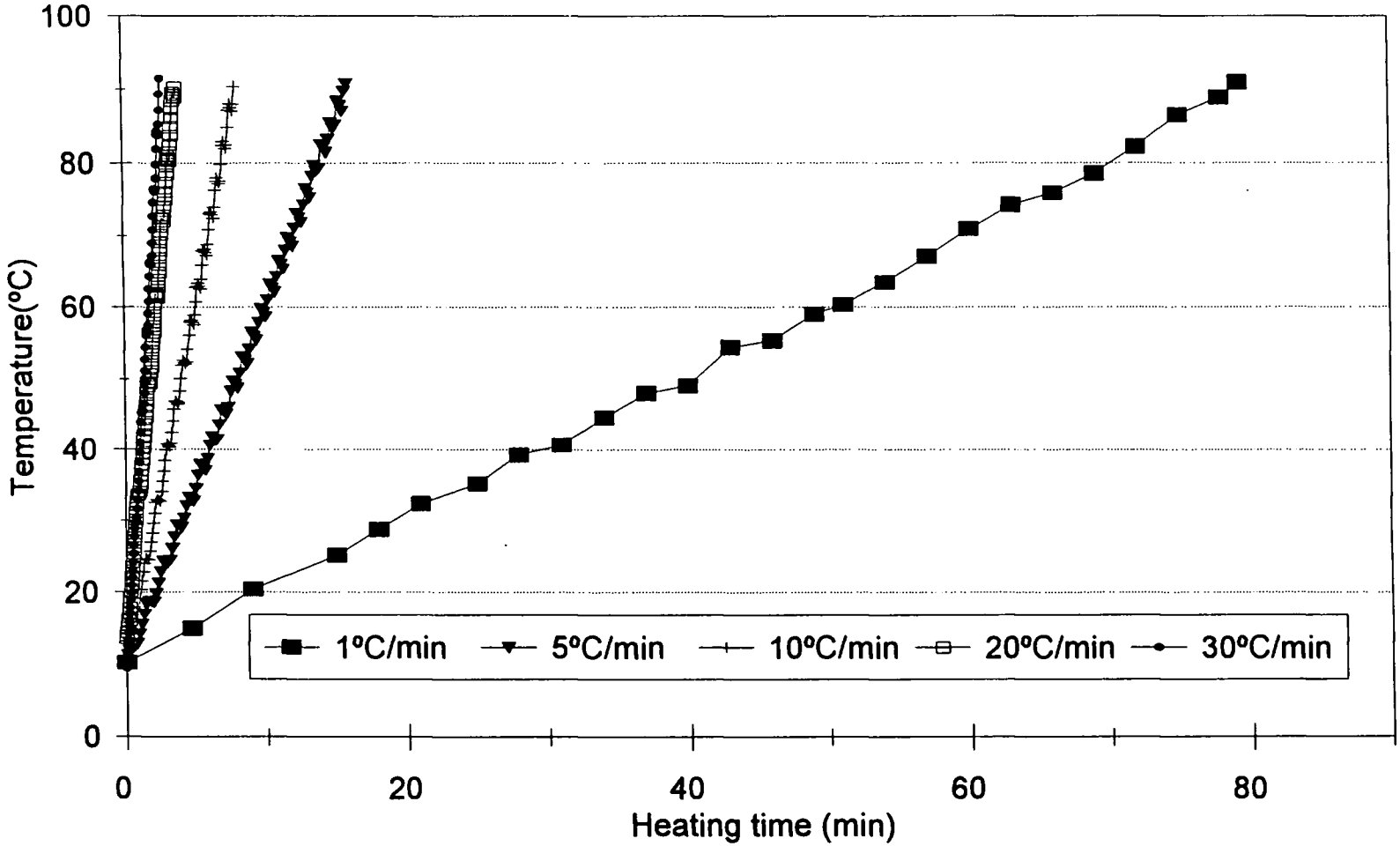


Figure A.1. Temperature profiles of various heating treatments.

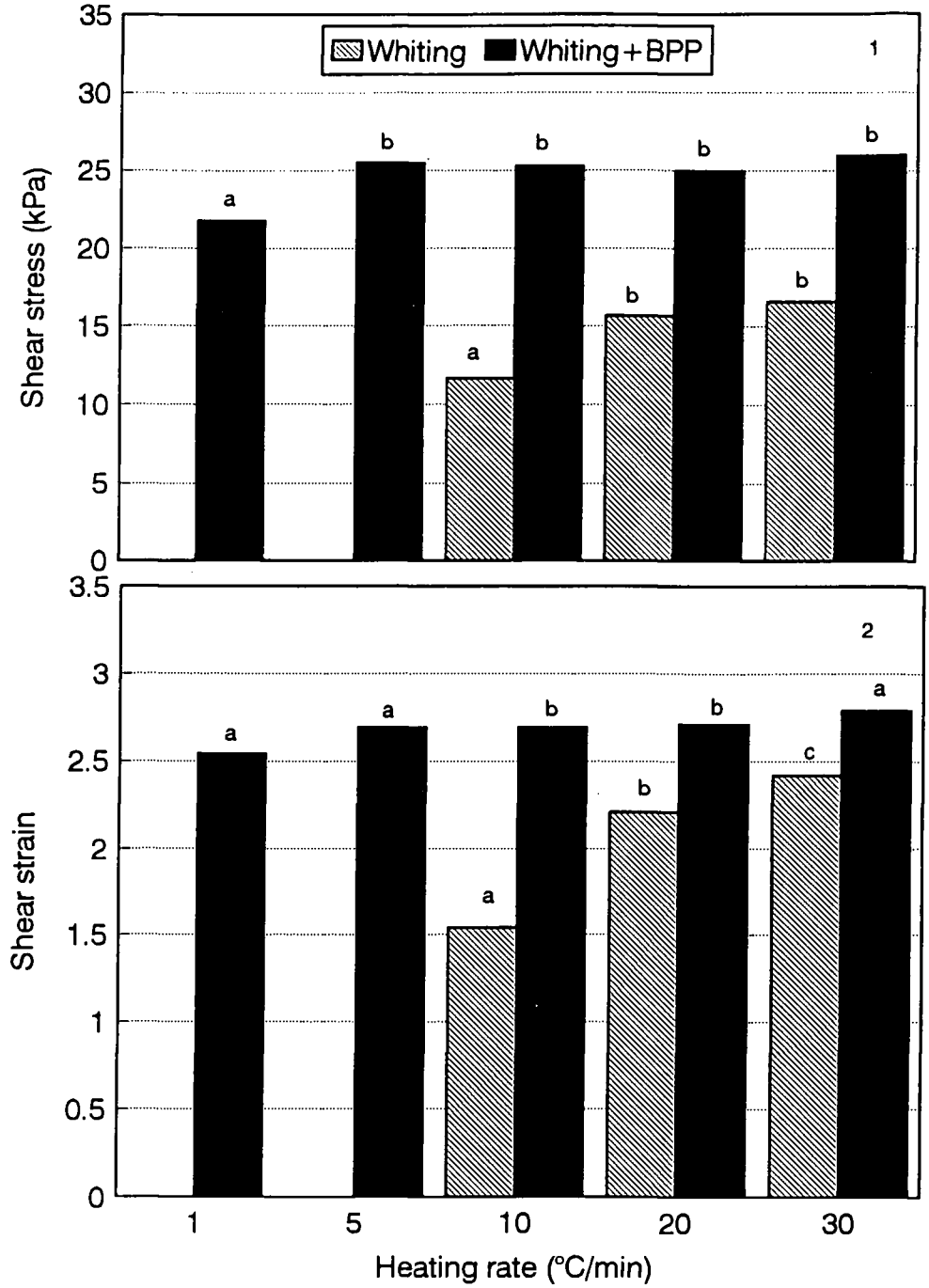


Figure A.2. Shear stress (1) and shear strain (2) of whiting surimi gels heated at various heating rates. Statistically significant differences among heating rates within whiting surimi and whiting surimi with addition of BPP samples were denoted as a, b, c.

at all heating rates when BPP was added ($P<0.05$). This implied that textural degradation due to proteolysis also occurred in the W samples heated at 20 and 30°C/min. Yongsawatdigul et al. (1995) reported that the gel-weakening of whiting surimi was effectively minimized when the surimi was heated ohmically at ≈80°C/min. Note that shear stress and shear strain of the W+BPP gels heated at 1°C/min were lower ($P<0.05$) than those heated at 5, 10, 20, and 30°C/min.

Shear stress of pollock gels without BPP heated at 1°C/min was higher ($P<0.05$) than those heated at faster rates (Fig. A.3.1). Lowest shear stress was observed in the sample heated at 30°C/min. Heating rates did not appear to affect shear strain of these samples (Fig. A.3.2). Changes of shear stress and shear strain of P+BPP showed similar trends to those of P samples (Figs. A.3.1 and A.3.2). The heating rate of 1°C/min provided the highest shear stress, and the effect of heating rate on shear strain was insignificant ($P>0.05$). There was no statistical difference in shear stress and shear strain between the pooled data of pollock surimi with and without BPP at that level (1.4% w/w). This indicated that proteolysis of pollock myosin did not occur or was minimal during heating. Moreover, BPP at that level did not significantly enhance gel-forming ability of pollock surimi. Montejano et al. (1984) and Kamath et al. (1992) reported setting treatments had a greater effect on shear stress than on shear strain of pollock surimi, which was similar to the effect of heating rate we observed. Textural properties of P and P+BPP improved with slow heating, corresponding to studies reported in porcine myosin (Foegeding et al., 1986 a,b; Camou et al., 1989), beef batter (Barbut and Mittal, 1990),

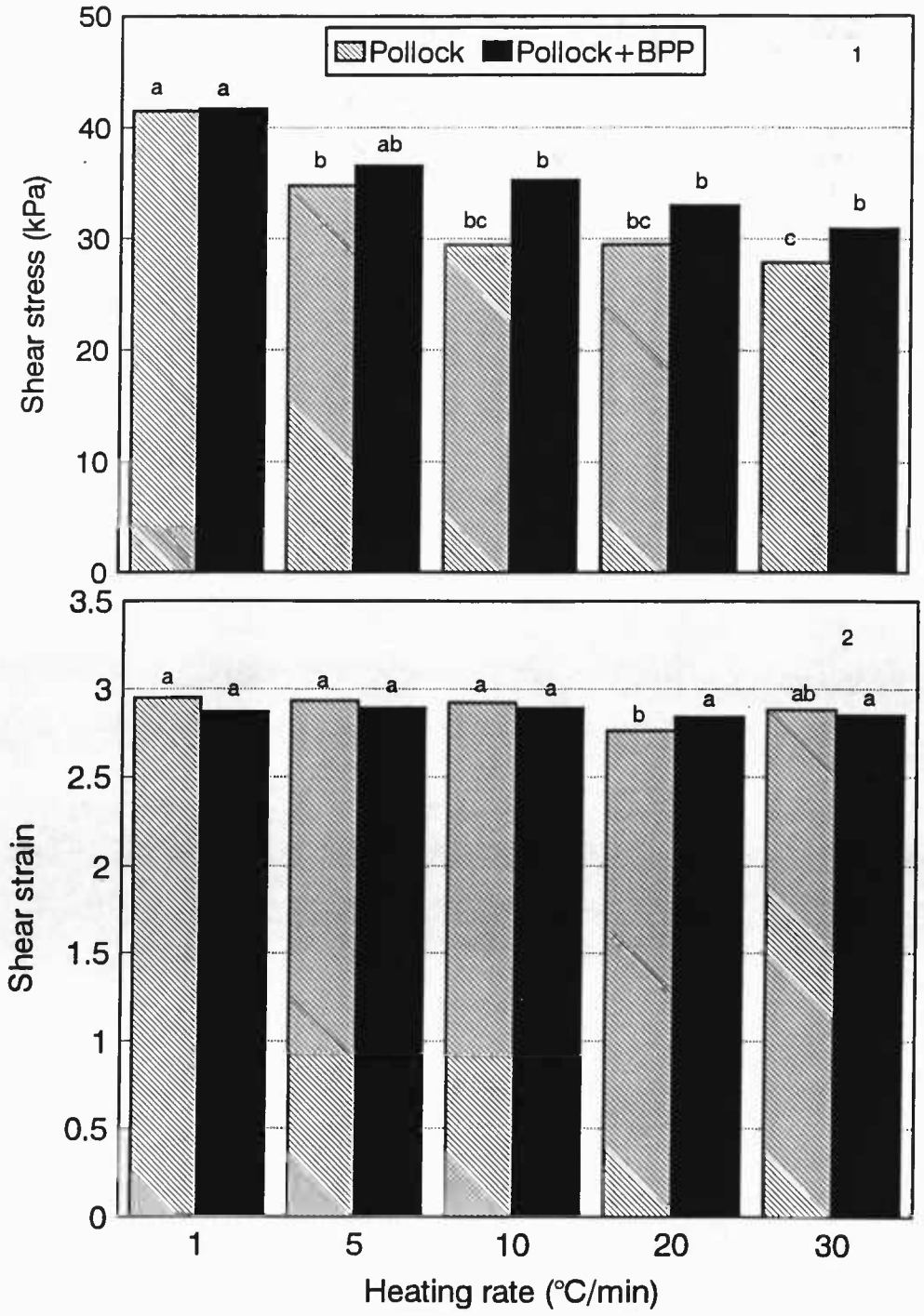


Figure A.3. Shear stress (1) and shear strain (2) of pollock surimi gels heated at various heating rates. Statistically significant differences among heating rates within pollock surimi without or with BPP were denoted as a, b, c.

vicilin, and ovalbumin (Arntfield and Murray, 1992). These reports suggested that slower heating rates allowed more time for proteins to unfold and interact with each other, thus a stronger gel matrix could be formed.

Changes of myosin heavy chain on SDS-PAGE

Myosin heavy chain of W samples decreased as heating rate decreased and primary hydrolysis products ($66,000 < M_r < 116,000$) appeared in samples heated at 5, 10, 20, and 30°C/min (Fig. A.4.1). Disappearance of myosin heavy chain and the extensive loss of actin were observed in the sample heated at 1°C/min. Consequently, the number of smaller molecular weight proteins ($29,000 < M_r < 45,000$) increased. A decreased myosin heavy chain at lower heating rates coincided with changes in shear stress and shear strain (Figs. A.2.1 and A.2.2). This emphasized the importance of myosin as a major component responsible for gel-forming ability of surimi (Niwa, 1992). Although a considerable amount of actin remained in the surimi gel heated at 5°C/min, the sample was very soft and mushy. This indicated that actin affected gel functionality to a lesser extent. The endogenous proteinase in Pacific whiting surimi was purified and characterized as cathepsin L, exhibiting its maximum activity at 55°C with no activity at >70°C (Seymour et al., 1994). An et al. (1994) illustrated that the enzyme was responsible for degradation of myosin heavy chain and actin at 55°C. Proteolysis could occur in surimi heated at 1°C/min for ≈1 hr (Fig. A.1) before the enzyme was thermally inactivated at >70°C. This was about 30 times longer than the samples heated at 30°C/min (2 min). Therefore, the extent of proteolysis was greater in the slow heating treatment than in the rapid heating treatment.

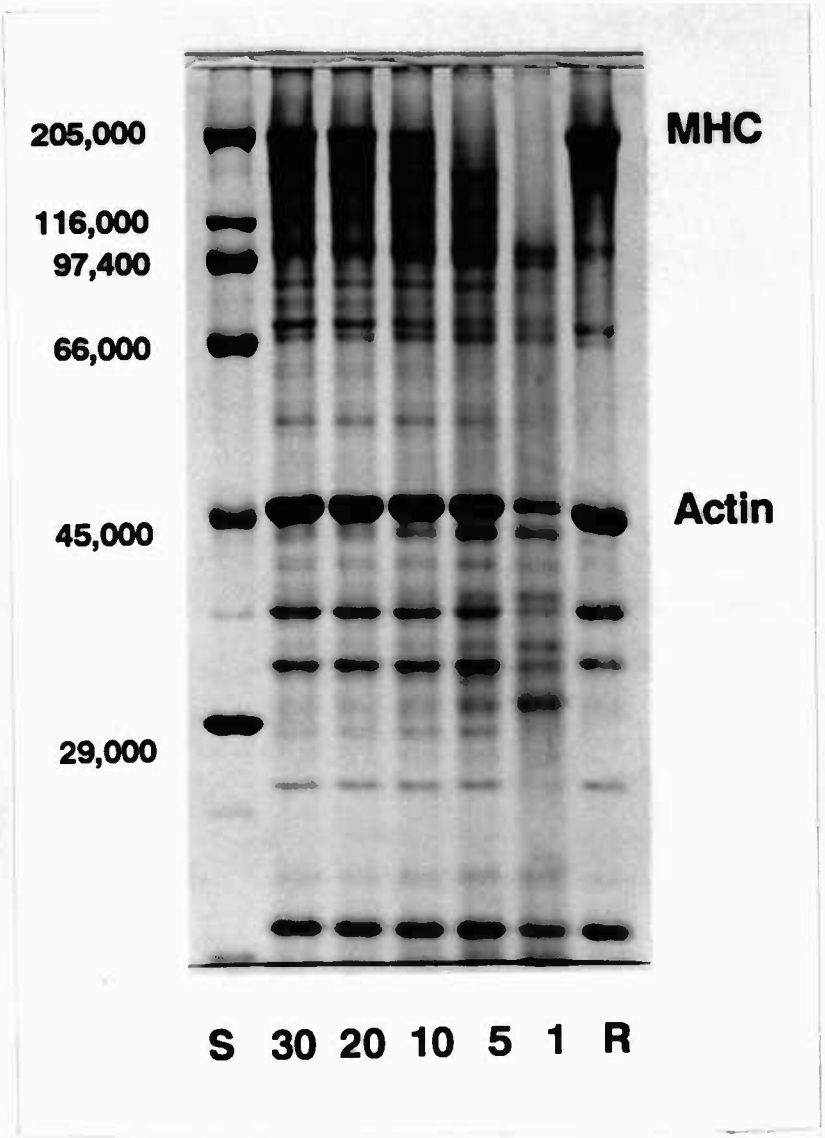


Figure A.4.1. SDS-PAGE pattern of whiting surimi on 10% polyacrylamide gel. Samples were applied at 60 μg protein/lane. Numbers designate heating rate (°C/min). (R) unheated surimi paste; (S) high molecular weight standard; MHC: myosin heavy chain.

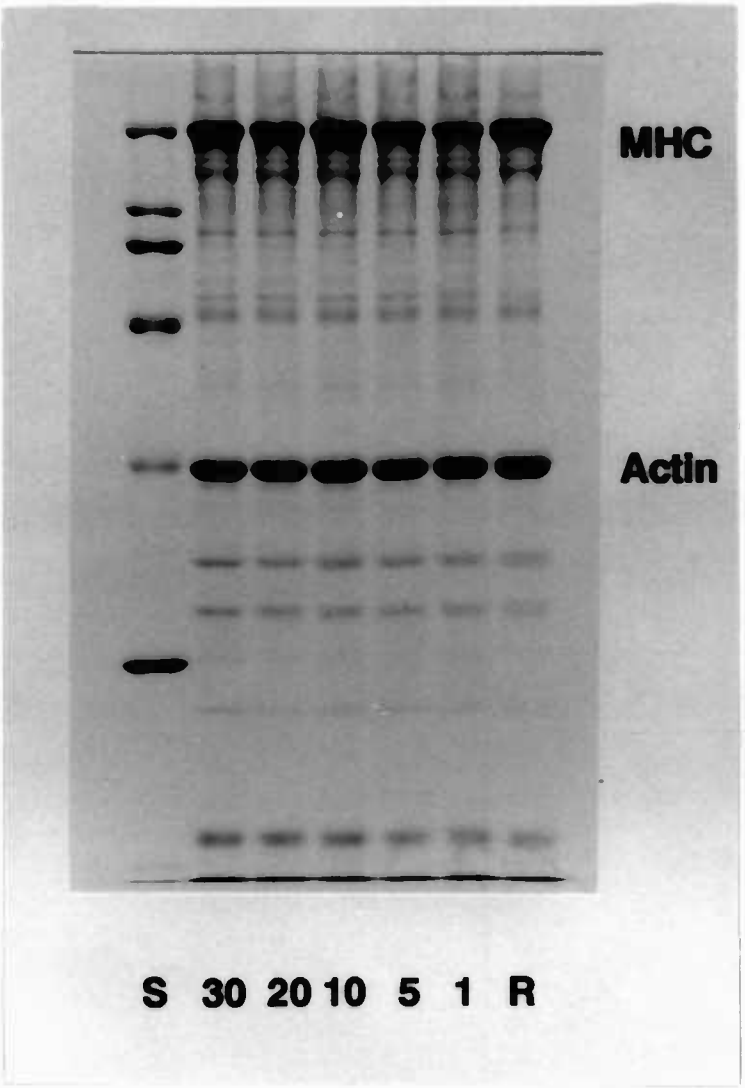


Figure A.4.2. SDS-PAGE pattern of whiting with addition of BPP on 10% polyacrylamide gel. Samples were applied at 60 μg protein/lane. Abbreviations are the same as shown in Fig. A.4.1.

Protein patterns of W+BPP on SDS-PAGE (Fig. A.4.1) showed decreased myosin heavy chain as heating rates decreased. Degradation of myosin heavy chain at all heating rates was not as extensive as Fig. A.4.1. For this reason, textural properties of W+BPP samples were improved (Figs. A.2.1 and A.2.2). This confirmed inhibitory effects of BPP as reported (Hamann et al., 1990; Morrissey et al., 1993; Park et al., 1994). However, the loss of myosin heavy chain accompanied by decreased shear stress and shear strain at 1°C/min indicated proteolysis. Note that the inhibitory function of BPP decreased as the activation period of the enzyme was prolonged at slow heating rates (1 and 5°C/min). Morrissey et al. (1993) reported that 1% BPP inhibited 90% of proteolytic activity in whiting surimi as determined by autolysis assay. Minimal improvement in proteinase inhibition was noticed at concentrations > 1%. Since BPP did not completely inhibit proteolysis, a decrease in myosin heavy chain in the sample heated at 1 and 5°C/min was due to residual proteolytic activity. The effect of such activity was not pronounced in the samples heated at rapid heating rates because of rapid thermal inactivation of residual enzyme.

Based on the 10% polyacrylamide gel of P samples (Fig. A.4.3), myosin heavy chain markedly decreased at the heating rate of 1°C/min. However, small fragments of degraded myosin heavy chain were not noted. The same trend was also observed on SDS-PAGE of P+BPP samples (not shown). Results indicated that decreased myosin heavy chain was unlikely caused by proteolysis. To investigate the loss of myosin heavy chain, the protein patterns of P and P+BPP samples were further analyzed on 3% polyacrylamide (Figs. A.5.1 and A.5.2). Proteins with higher molecular weight than myosin heavy chain were observed; the highest intensity appeared on the gel heated at

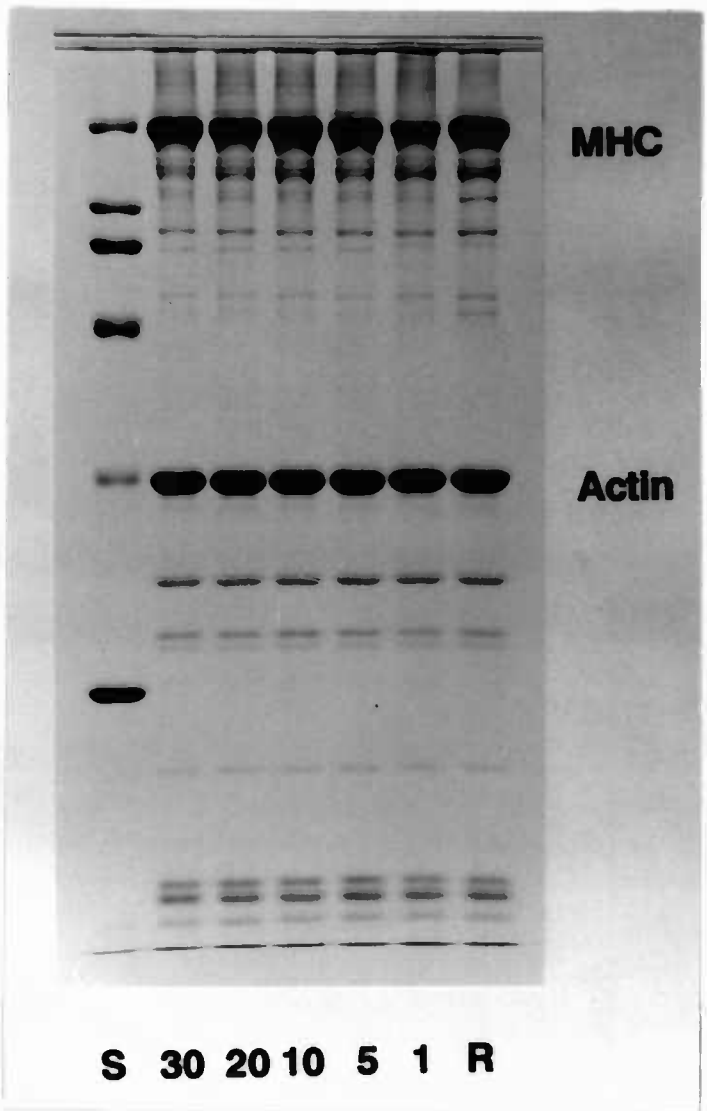


Figure A.4.3. SDS-PAGE pattern of pollock surimi on 10% polyacrylamide gel. Samples were applied at 60 μg protein/lane. Abbreviations are the same as shown in Fig. A.4.1.

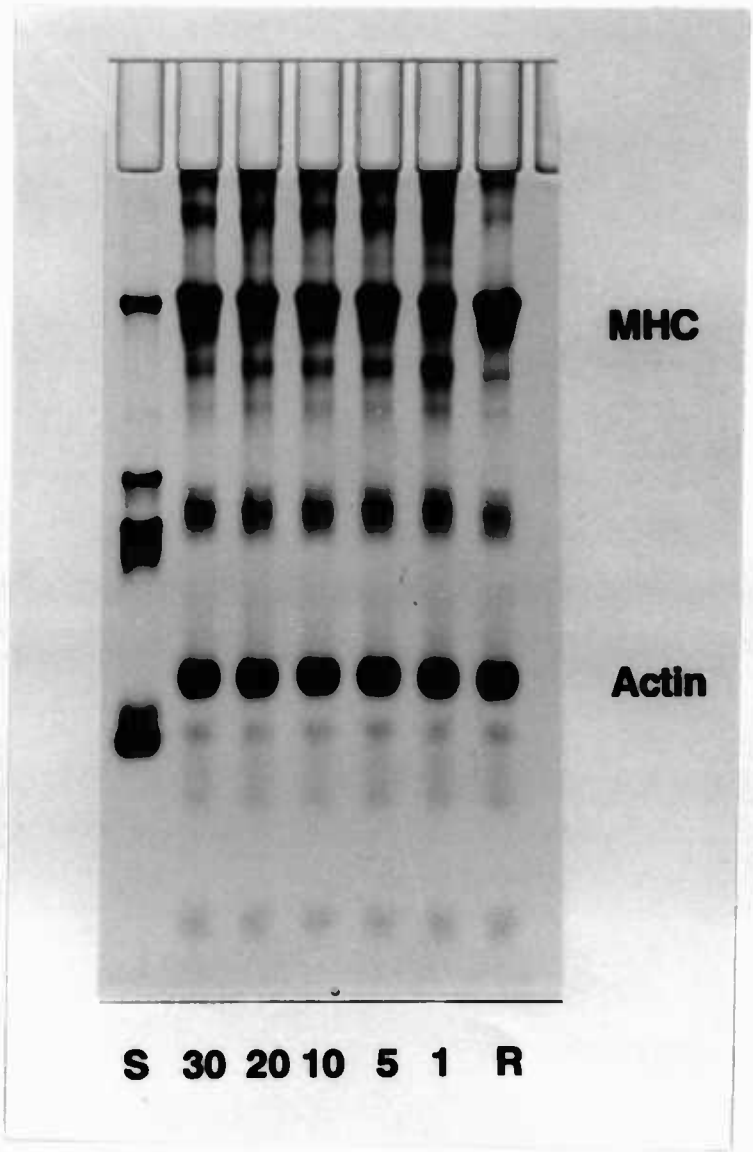


Figure A.5.1. SDS-PAGE pattern of pollock surimi on 3% polyacrylamide gel. Samples were applied at 60 μg protein/lane. Numbers designate heating rate (°C/min). (R) unheated surimi paste; (S) high molecular weight standard; MHC: myosin heavy chain.

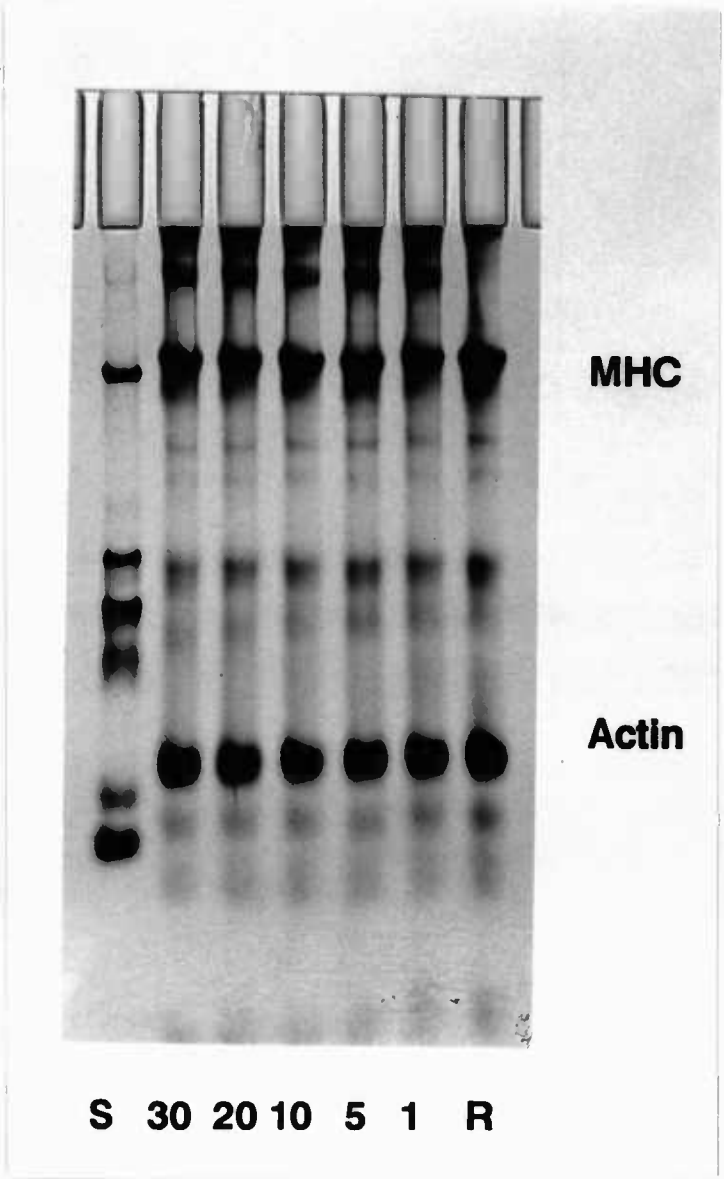


Figure A.5.2. SDS-PAGE pattern of pollock surimi with BPP on 3% polyacrylamide gel. Samples were applied at 60 μg protein/lane. Abbreviations are the same as shown in Fig. A.5.1

1°C/min. Although myosin heavy chains decreased as high molecular weight proteins increased, the changes in actin band were not pronounced. This suggested that high molecular weight proteins were formed by cross-linking of myosin heavy chain. Since an increase in cross-linked myosin was accompanied by an increase in shear stress, myosin cross-linking could be responsible for a change in rheological properties of pollock surimi gels heated at slow heating rates. Cross-linking of pollock myosin has been well elucidated during gel setting, 'suwari' (Numakura et al., 1985; Seki et al., 1990; Kimura et al., 1991; Kamath et al., 1992; Kim et al., 1993; Nowsad et al., 1993; Joseph et al., 1994). Preincubation of pollock surimi at 25°C for 1-18 hr induced cross-linking of myosin heavy chain. The reaction was possibly catalyzed by endogenous transglutaminase (Seki et al., 1990; Kimura et al., 1991). The enzyme catalyzes the cross-linking through covalent bonds between γ-carboxamide group of glutamine and ϵ-amino group of lysine (Folk, 1980). Microbial transglutaminase was also reported to increase formation of ϵ-(γ-glutamyl)lysine crosslinks in pollock surimi (Sakamoto et al., 1995; Seguro et al., 1995).

The surimi gels were solubilized using SDS, urea, and β-mercaptoethanol buffer prior to electrophoresis. Thus, polymerization of myosin heavy chain (Figs. A.5.1 and A.5.2) involved nondisulfide covalent bonds. This suggested that cross-linked myosin that appeared during slow heating could be the same type as that formed during setting. Probably slow heating enhanced enzymatic cross-linking of pollock myosin by prolonging the reaction period.

Cross-linking of myosin heavy chain of W (Fig. A.5.3) and W+BPP (not shown) were also investigated on 3% SDS-PAGE. No proteins with M_r > 205 kDa were found

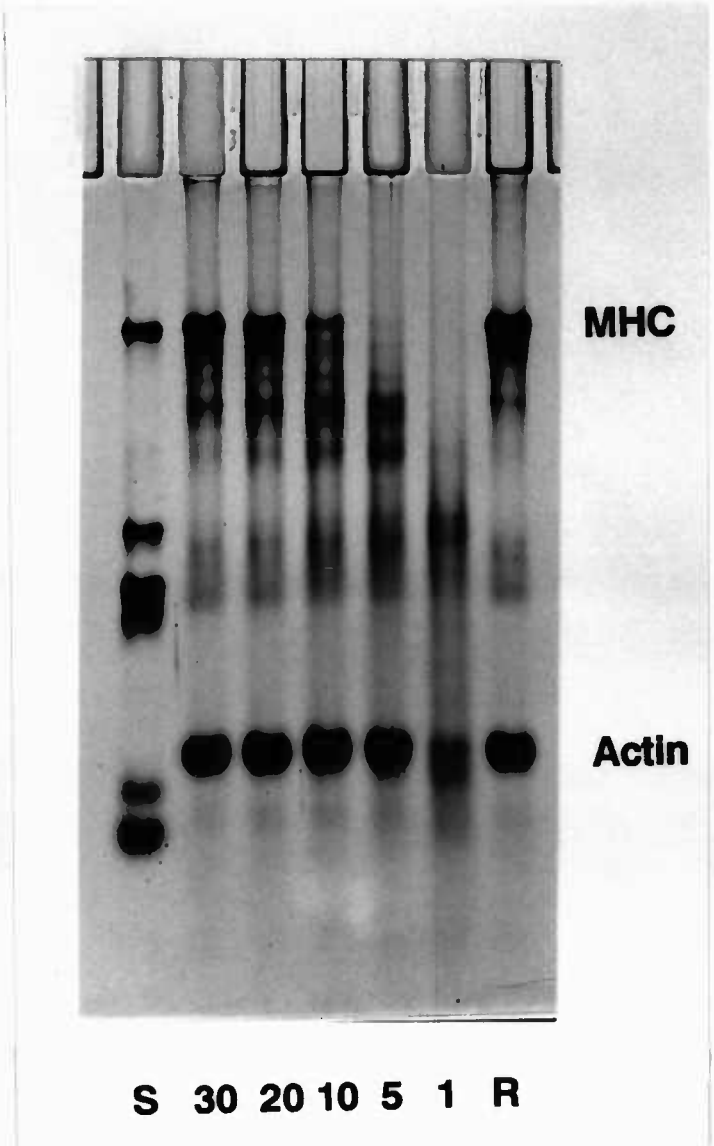


Figure A.5.3. SDS-PAGE pattern of whiting surimi on 3% polyacrylamide gel. Samples were applied at 60 μg protein/lane. Abbreviations are the same as shown in Fig. A.5.1

in either sample heated at all rates, indicating that nondisulfide covalent bonds were not involved in gelation of whiting surimi. Chan et al. (1992) also reported that polymerization of silver hake (*Merluccius bilinearis*) myosin during thermal gelation was noncovalent. Thus, low gel-forming ability of whiting surimi could be the result of both the presence of an endogenous proteinase and lack of covalent cross-linking of whiting myosin.

Conclusions

Linear heating rates were achieved using an ohmic heating device. The effect of heating rate on gelation was different, depending on inherent characteristics of the fish. Textural properties of pollock surimi could be improved through slow heating regimes. At rapid heating rates, degradation of whiting myosin heavy chain was greatly reduced. Rapid inactivation of endogenous proteinase resulted in increased shear stress and shear strain of whiting surimi gels.

Acknowledgement

This work was supported by grant No. NA36RG0451 (project no. R-SF-2) from the National Oceanic and Atmospheric Administration to the Oregon State University Sea Grant College Program and by appropriations made by the Oregon State Legislature. The views expressed herein are those of the authors and do not necessarily reflect the view of NOAA or any of its subagencies.

APPENDIX B

STATISTICAL ANALYSIS OF ESTIMATED COEFFICIENT (a_0 and b_0)

General Linear Models Procedure
Class Level Information

Class	Levels	Values
SALT	4	1 2 3 4
MOIS	4	75 78 81 84
VOLT	3	50 100 200
REP	2	1 2

Number of observations in data set = 96

General Linear Models Procedure

Dependent Variable: INT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	29	57.8829624	1.9959642	181.31	0.0001
Error	66	0.7265533	0.0110084		
Corrected Total	95	58.6095157			

R-Square	C.V.	Root MSE	INT Mean
0.987603	6.246972	0.10492	1.67955

Dependent Variable: INT

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SALT	3	52.1451186	17.3817062	1578.95	0.0001
MOIS	3	3.2846727	1.0948909	99.46	0.0001
VOLT	2	0.4769732	0.2384866	21.66	0.0001
SALT*MOIS	9	1.5183873	0.1687097	15.33	0.0001
SALT*VOLT	6	0.3626627	0.0604438	5.49	0.0001
MOIS*VOLT	6	0.0951479	0.0158580	1.44	0.2127

Tests of Hypotheses using the Type III MS for SALT*MOIS as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
MOIS	3	3.28467266	1.09489089	6.49	0.0125
SALT	3	52.1451186	17.3817062	103.03	0.0001

Dependent Variable: SLOPE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	29	0.07215027	0.00248794	200.31	0.0001
Error	66	0.00081974	0.00001242		
Corrected Total	95	0.07297001			

R-Square	C.V.	Root MSE	SLOPE Mean
0.988766	5.293727	0.00352	0.06657

Dependent Variable: SLOPE

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SALT	3	0.06853761	0.02284587	1839.39	0.0001
MOIS	3	0.00133386	0.00044462	35.80	0.0001
VOLT	2	0.00024220	0.00012110	9.75	0.0002
SALT*MOIS	9	0.00119522	0.00013280	10.69	0.0001
SALT*VOLT	6	0.00062948	0.00010491	8.45	0.0001
MOIS*VOLT	6	0.00021190	0.00003532	2.84	0.0159

Tests of Hypotheses using the Type III MS for SALT*MOIS as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
MOIS	3	0.00133386	0.00044462	3.35	0.0694
SALT	3	0.06853761	0.02284587	172.03	0.0001

Estimated mean values

Level of		INT		SLOPE	
MOIS	N	Mean	SD	Mean	SD
75	24	1.48763250	0.67392327	0.06132292	0.02506801
78	24	1.58721933	0.71671498	0.06506950	0.02657784
81	24	1.66181083	0.70564544	0.07116000	0.03300725
84	24	1.98152583	0.96935006	0.06874396	0.02627450

Level of salt	N	INT Mean	INT SD	SLOPE Mean	SLOPE SD
1	24	0.67463417	0.08152608	0.03141125	0.00235655
2	24	1.38090808	0.17105544	0.05404971	0.00353209
3	24	1.99661875	0.25797689	0.07766208	0.00687600
4	24	2.66602750	0.42261353	0.10317333	0.01128737

Level of VOLT	N	INT Mean	INT SD	SLOPE Mean	SLOPE SD
50	32	1.58150197	0.78313989	0.06617813	0.02543261
100	32	1.74416031	0.84994144	0.06485719	0.02657455
200	32	1.71297909	0.73453031	0.06868697	0.03151244

Level of SALT	Level of VOLT	N	INT Mean	INT SD	SLOPE Mean	SLOPE SD
1	50	8	0.61505875	0.05446053	0.03291500	0.00253607
1	100	8	0.67278375	0.06543637	0.03159750	0.00179529
1	200	8	0.73606000	0.07885626	0.02972125	0.00164432
2	50	8	1.27890788	0.13125035	0.05560250	0.00370860
2	100	8	1.38346250	0.16397337	0.05296750	0.00310297
2	200	8	1.48035388	0.16978199	0.05357913	0.00364172
3	50	8	1.82392375	0.16255456	0.07753250	0.00434510
3	100	8	2.11155125	0.27059397	0.07400000	0.00595208
3	200	8	2.05438125	0.25696743	0.08145375	0.00832244
4	50	8	2.60811750	0.47508900	0.09866250	0.00893149
4	100	8	2.80884375	0.42959469	0.10086375	0.00872908
4	200	8	2.58112125	0.37575627	0.10999375	0.01342138

Level of MOIS	Level of VOLT	N	INT Mean	INT SD	SLOPE Mean	SLOPE SD
75	50	8	1.40178000	0.69079211	0.06194875	0.02491196
75	100	8	1.56207875	0.77526170	0.05920125	0.02371176
75	200	8	1.49903875	0.63178366	0.06281875	0.02955939
78	50	8	1.45201663	0.65346750	0.06605125	0.02716594
78	100	8	1.62267000	0.78651116	0.06253750	0.02578879
78	200	8	1.68697138	0.78005635	0.06661975	0.03011219
81	50	8	1.60736625	0.75819931	0.07122500	0.03143404
81	100	8	1.71442500	0.79230587	0.06768750	0.03117660
81	200	8	1.66364125	0.65337872	0.07456750	0.03990715
84	50	8	1.86484500	1.04326708	0.06548750	0.02162565
84	100	8	2.07746750	1.08124455	0.07000250	0.02908740
84	200	8	2.00226500	0.89628143	0.07074188	0.03059500