AN ABSTRACT OF THE THESIS OF

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Jae W. Park

The effects of freeze-drying and flake freezing of surimi on biochemical and physical properties as affected by various storage conditions were examined. Using three cooking parameters shear stress and strain values were evaluated to measure gelation properties of surimi gels. Stress values increased up to 3 months and then decreased substantially as storage time was extended. In general, strain values decreased substantially over time, however, freeze-dried surimi kept in the freezer at -18° C did not change during 9 months of storage. Overall, color decreased during storage and b* values of the freeze-dried surimi kept at room temperature increased significantly. Salt extractable proteins decreased while dimethylamine increased. Electrophoretic patterns, however, did not show any apparent damage to the MHC due to frozen storage and/or proteolysis (with 60°C incubation) until the 9th month of storage. At 9 months, a reduction of the MHC was observed and the lower molecular weight bands were more intense. Freeze-dried samples stored in the freezer maintained the

highest quality for the duration of the 9 month study. Low temperature storage is important for retaining good functionality in freeze-dried surimi.

The effects of cross-section diameter on shear stress and strain and effects of individual variation in measuring diameter were studied. Gelation properties of surimi using milled and molded gels were compared. The possibility of skin formation using various cook times was also evaluated. Shear stress values were significantly affected by diameter accuracy, whereas the effect was not as significant for shear strain values. Individual variation in measurement was also greatly noted. Molded gels resulted in significantly lower strain values than milled samples, whereas stress values were significantly higher in molded gels than in milled gels. Using a lechitin-based spray appeared to eliminate skin formation on all samples.

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PHYSIO-CHEMICAL PROPERTIES OF PACIFIC WHITING SURIMI AS AFFECTED BY VARIOUS FREEZING/STORAGE CONDITIONS AND GEL PREPARATION METHODS

by

Justine D. Hoffman

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APPROVED:

Major Professor, representing Food Science and Technology

Chair of 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 authorizes release of my thesis to any reader upon request.

Justine D. Hoffman, author

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CONTRIBUTION OF AUTHORS

Dr. Jae W. Park was involved in the experimental design, analysis, and writing of each manuscript. Dr. Yeung J. Choi guided the biochemical experimentation.

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Chapter 1

Introduction

The Pacific Northwest coast is the largest single Pacific Whiting (*Merluccius productus)* resource with an annual allowable catch in excess of 230,000 metric tons (Anonymous 1999a). This small, 1-1 1/2 pound fish migrates back and forth between Baja Mexico, California and British Columbia and is landed in a high volume fishery when the fish school. Utilization of Pacific whiting, by the US, was limited until new developments of surimi in 1991-1992 due to its strong proteolytic enzymes. The protease present in Pacific whiting is problematic during post-mortem. An unacceptable softness of the flesh may develop in as little as 4 hours of iced storage, as evidenced by a rapid decline in texture shear values (Tsuyuki and others 1982). At present, the majority of the Pacific whiting harvest is utilized for surimi production because of its bland taste, white color, low cost, and large availability.

Surimi is a Japanese term for mechanically deboned fish flesh that has been washed with water and mixed with cryoprotectants to enhance frozen shelf life (Lee 1984). It is used as an intermediate product for a variety of engineered seafood, such as crab legs and flakes. Surimi is graded based on such factors as moisture, whiteness,

impurities, and gel strength. High quality surimi is naturally colorless and odorless. Surimi has a long shelf life and is a highly functional protein ingredient of good nutritional quality and current technology permits mass production with consistent quality (Lee 1986).

All commercially produced surimi is kept frozen and cryoprotectants are required to minimize protein denaturation during frozen storage. Sucrose (4%) and sorbitol (4-5%) are commonly used in addition to 0.2-0.3% mixture (50:50) of sodium tripolyphosphate and tetrasodiumpyrophosphate. In addition, Pacific whiting surimi requires that a minimum of 1% beef plasma protein (BPP) be added in order to inhibit proteolysis when slow heating is applied to cook the surimi (Morrissey and others 1993). Rapid freezing of the surimi product is desirable to prevent the formation of large ice crystals, which can damage cells, allow moisture loss and subsequent loss of texture upon thawing (Mermelstein 1998). Currently, in the commercial industry, plate freezers are used to freeze surimi, where the product is frozen between plates on top and bottom and using either ammonia or freon as a chilling agent. This system takes approximately 2-2 1/2 hours to bring the product from 10° C to -20° C. The product is then stored in a freezer and recommended to be used within 2 years.

During frozen storage, several changes occur in fish muscle proteins. These include denaturation, ice crystallization, dehydration, and changes in intramolecular conformation. Common tests used to measure these changes are salt-soluble protein extraction, pH, dimethylamine formation and ionic strength (Park 1994). Many proteins exhibit instability as measured by the partial loss of functionality at subfreezing

temperatures. The deterioration of proteins during frozen storage is reflected by a sharp decrease in their gel forming ability, water holding capacity, and fat emulsifying capacity (Iwata and Okada 1971; Chang and others 1989; Holmquist and others 1984; LeBlanc and others 1988; Park and others 1988; Hsu 1990; Yoon and Lee, 1990; Sych and others 1991). Quality changes due to freeze-denaturation can be minimized during storage with the proper use of cryoprotectants. However, Park and others (1988) and Sych and others (1991) reported a loss of salt soluble proteins in cryoprotected surimi during frozen storage indicating that cryoprotectants can not prevent the occurrence of freeze-induced denaturation. Furthermore, gel functionalities, as measured by stress and strain, of surimi mixed with cryoprotectants decreased notably due to freezing (Hsu 1990; Sych and others 1991; Simpson and others 1994) and continued to decrease over prolonged storage (Park and others 1988; Sych and others 1990).

Storage is an important factor that influences product quality, therefore it has become a major area of interest. The conventional methods of freezing to produce frozen blocks has exhibited a few disadvantages with the primary concern being the chemical deterioration that occurs with relatively slow freezing processes and long term frozen storage. In addition to quality concerns, there are logistical parameters involved. Frozen blocks take up a large amount of space and storage. There is also the added risk of temperature abuse during transport. For the proper use of frozen blocks at surimi seafood manufacturers, either thawing or breaking is required. Abusive thawing often causes protein denaturation resulting in lower gel properties, while inappropriately

broken surimi blocks can cause damage to the blades of the silent cutter. They lead us to look into alternative forms of surimi and their storage conditions.

The overall objective of this study was to determine the effects of various freezing and storage conditions on the biochemical and physical properties of Pacific whiting surimi. Comparisons were made to determine if different forms of freezing could prevent the loss of functional properties in Pacific whiting surimi during frozen storage. The specific objectives of this research included: (1) determining if freeze-drying greatly reduced the rate of denaturation and changes in intramolecular conformation, and (2) determining if a faster rate of freezing (flake) increased the shelf-life stability of the product.

The ultimate goal was to aid the seafood industry in finding a way to lower the costs of distribution and storage of a frozen product and to find new applications for fish proteins, which could lead to a wider acceptance of using them as food ingredients.

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Chapter 2

Literature Review

J. Hoffman

Surimi production

Surimi can be described as stabilized and refined myofibrillar protein, which has been produced in frozen block form. It is produced by continuous processing steps of heading, deboning, mincing, washing, and dewatering, until it becomes a flavorless protein paste (Lee 1984; Park and Morrissey 2000) (Fig. 2.1). The washing and dewatering isolate fish myofibrillar proteins from sarcoplasmic proteins and other undesirable materials such as blood, fat, soluble pigments, skin and fine bones (Lee 1984). Minced fish on the other hand, is mechanically separated flesh that has not been washed and does not have good frozen shelf life. The myofibrillar protein is known to be the most active in performing the functions of texture formation or particle cohesion and binding of fat and water in many processed muscle food systems (Acton and others 1983). The myofibrillar proteins of most fish species are more susceptible to heat denaturation than the same proteins of mammalian and avian species (Montejano and others 1985; Matsumoto 1980; Lanier 2000). This property, although undesirable from the standpoint of storage stability, contributes much to the functionality of the material. Therefore, it is imperative that protective compounds be added prior to freezing or drying if the surimi is to be held in long-term frozen storage (more than two-three weeks in the frozen state) or dried (Lanier 1986).

Freezing of surimi became commercially possible after the discovery of the cryoprotective role of sucrose, which prevents muscle protein, particularly actomyosin, from denaturation during frozen storage (Matsumoto 1978). The cryoprotective effect of sugar is greatly enhanced by addition of polyphosphates. It is not entirely clear as to

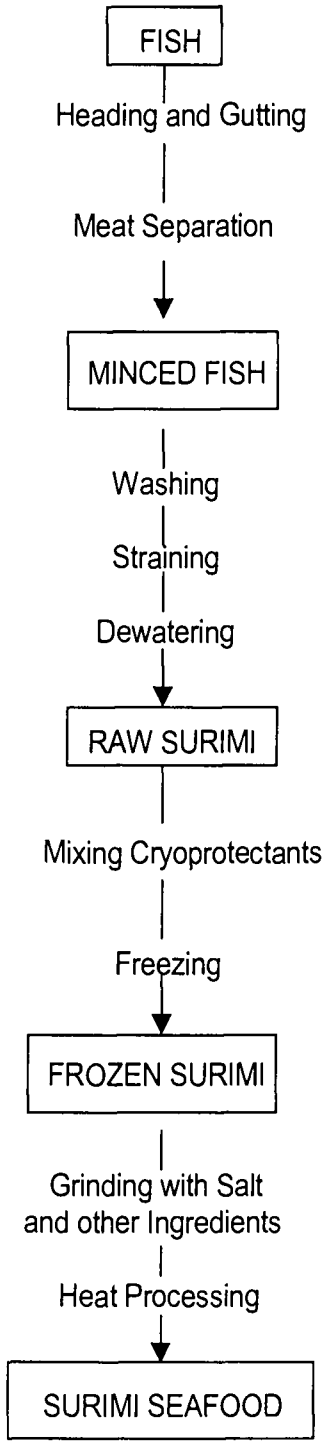


Fig. 2.1 – Flow chart of surimi and surimi seafood production

how the phosphates work as a cryoprotectant. They may serve to chelate calcium and other metal ions that can induce protein aggregation (Saeki 1996). It has also been proposed that they may act as antioxidants of lipids, which protect proteins from denaturation induced by hydrolysis or auto-oxidation of phospholipids (Wessels and others 1981). Additionally, because of the strength of the phosphate in raising pH, the water holding/binding of the gel improves and better salt solubilization of myofibrillar proteins results (Park 2000a).

Prevention of protein denaturation, by sugars, can be explained by their ability to increase the surface tension of the water (Arakawa and Timasheff 1982), as well as, the amount of bound water, which prevents withdrawal of water molecules from the protein, thus stabilizing the protein (Buttkus 1970; Sun and Wang 1984). Surimi of initially good quality can quickly deteriorate as a result of poor frozen storage and temperature fluctuations. Therefore, it is important to closely monitor these processes.

Freezing methods / freezing rates

A commercial freezer is used to extract heat from the product and converts most of the free moisture to a solid. This heat removal must be rapid enough that the product experiences only a minimum amount of quality degradation. Additionally, the rate of heat removal must keep pace with the production schedule and the final product temperature, once frozen, should roughly match the subsequent storage temperature (Kolbe 2000). It is a basic property of aqueous solutions that increasing the concentration of dissolved solids will lower the freezing points. Therefore, the more salt,

sugar, minerals, or proteins in a solution the lower the freezing point will be (Potter 1986a). The rate of temperature decrease in the product, at constant outer temperature, changes in time. This is due to the fact that most of the latent heat of crystallization is removed in the range -1° to -5° C and that the thermal diffusivity of the material increases due to formation of ice crystals (Sikorski and Kolakowska 1990) (Fig. 2.2).

The rate of freezing (the movement of the ice front) effects the histological changes in the frozen tissue, as it controls the size and distribution of the ice crystals (Sikorski and Kolakowska 1990). During slow freezing, (0.2 cm/hr), the rate of nucleation is lower than that of water migration from within the cells, into the intracellular spaces (Potter 1986a). Therefore, in slowly frozen muscle, large ice crystals are formed in the intercellular spaces, while at high freezing rates (5 cm/hr) a large number of small crystals are evenly distributed throughout the whole cross-section of the tissue. Fast freezing minimizes concentration effects by decreasing the time concentrated solutes are in contact with food tissues, colloids, and individual constituents during the transition from the unfrozen state to the fully frozen state (Potter 1986a). Large ice crystals in the intercellular spaces may cause some damage to the histological structure of the muscle (Sikorski and Kolakowska 1990).

Several considerations are involved in determining which freezing system to use: location (whether on a vessel or on shore), product type, quality of the final product, capital requirements and cost of operation, as well as, convenience, maintenance problems, and space limitations. Surimi is frozen primarily in plate

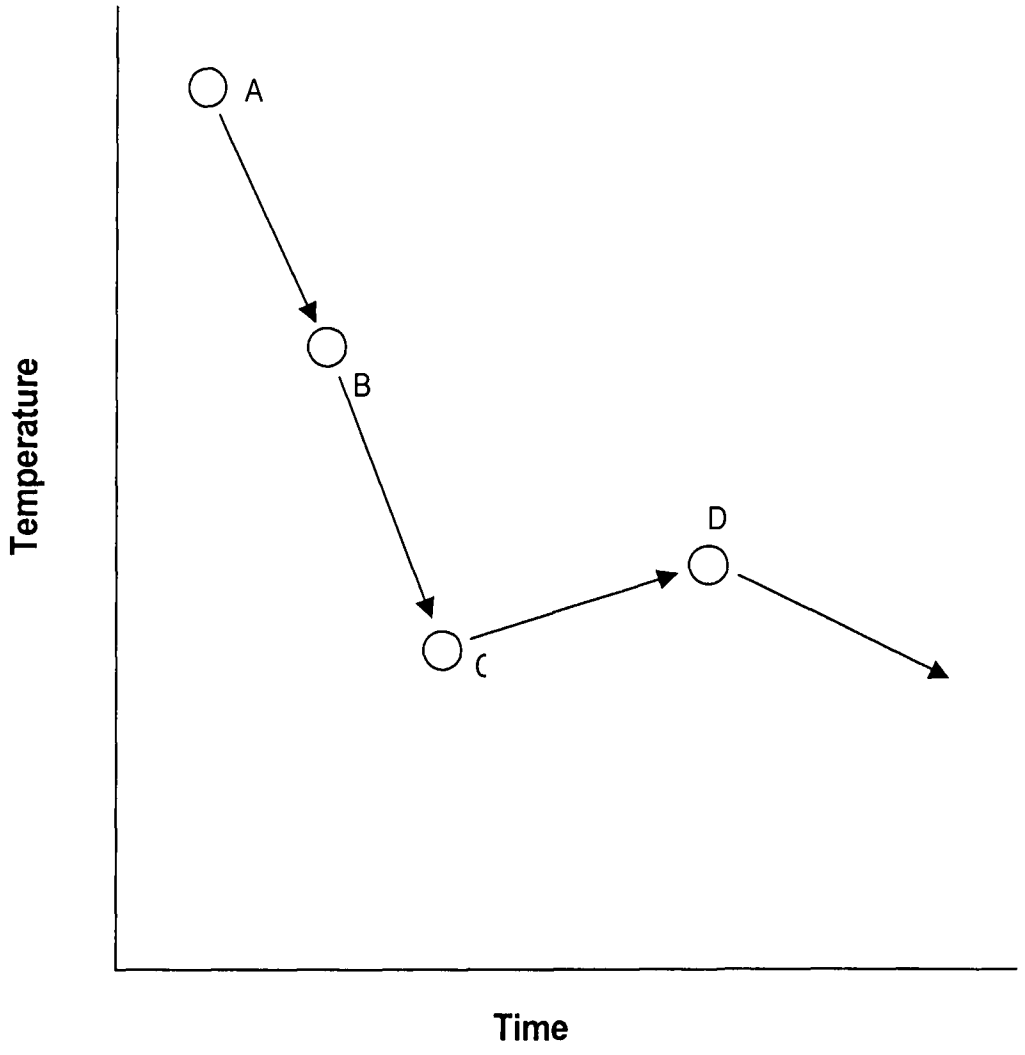


Fig. 2.2 – Typical Freezing Curve.

A→ B Removal of heat: B is the initial freezing point of the sample
B→ C Further removal of heat

C Nucleation begins

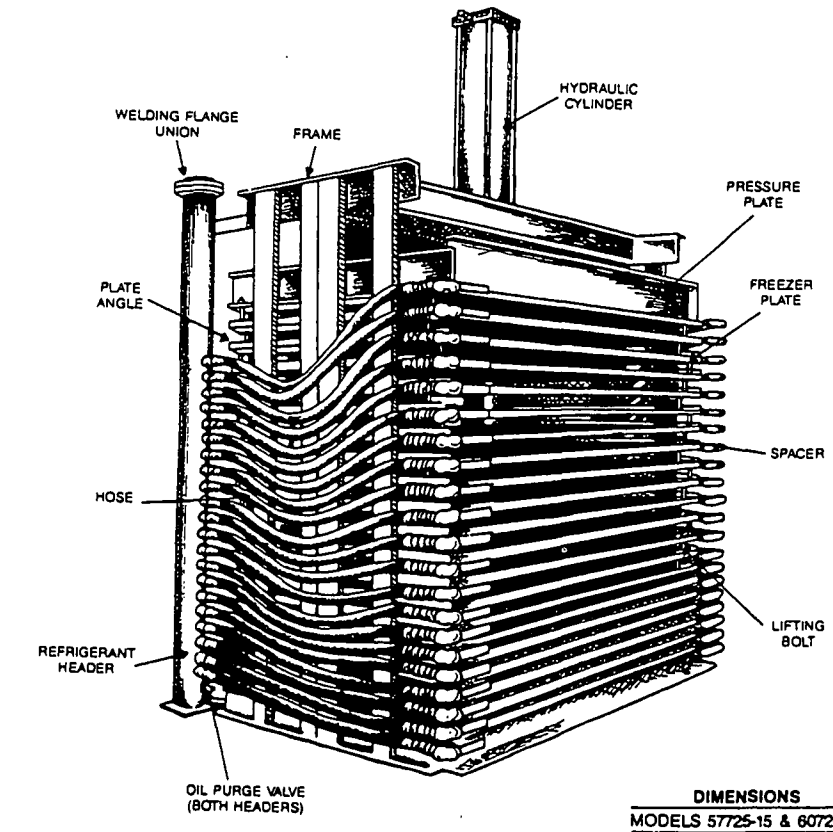
C→ D Nucleation is immediately followed by crystal growth, release of latent heat of crystallization and a rise in temperature (D)

Adapted from Fennema (1996)

freezers (Fig. 2.3). Plate freezers are mainly employed for freezing fillets in consumer packs or marine products in blocks. Tight contact with the frozen material is required for efficient utilization of the high rate of heat transfer by conduction to the plates at –40° C. A common characteristic of plate freezers is minimum dehydration of the product and a high rate of freezing. A plate freezer generally needs less space and energy than an air-blast installation with the same output (Sikorski and Kolakowska 1990).

Different types of air-blast freezers (liquid nitrogen cabinet freezers) have had widespread application because of their high versatility (Fig. 2.4). The term 'blast freezer' commonly refers to a batch freezing operation during which product is wheeled into a room or large cabinet, where it remains until frozen (Kolbe 2000). The air temperature is –30° to –40° C and air is re-circulated in a blast freezer at a velocity of 4 to 6 m/s, which causes fairly rapid freezing. Assuming proper design, a major impediment to performance involves product loading and uniform airflow, which can seriously affect freezing time (Kolbe 2000).

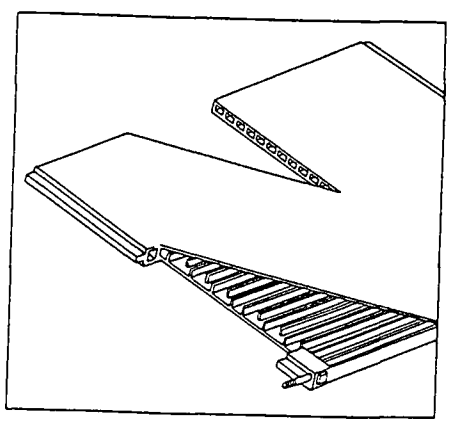
Food dehydration refers to the nearly complete removal of water from foods under controlled conditions that cause minimal or ideally no other changes in the food properties. The food is usually dried to final moistures within the range of 1-5% and such products are expected to have storage stability at room temperature of a year or longer (Potter 1986b). A major criterion of the quality of dehydrated foods is that when reconstituted by the addition of water they are very close to, or virtually indistinguishable from, the original food material used in their preparation. In food dehydration, the technical challenge is especially difficult since very low moisture levels, with minimum



DIMENSIONS

MODELS 57725-15 & 60725-15		
Height	Overall*	123 1/2"
	Frame	81 1/2"
Width Overall		87 1/8"
Depth Overall		71 1/2"

*Includes 1" clearance required for mounting cylinder.



ALUMINUM PLATE CUT-AWAY VIEW

Fig. 2.3- A horizontal plate freezer shown without the enclosure housing. Inset shows cutaway of an extruded aluminum plate (Kolbe 2000).

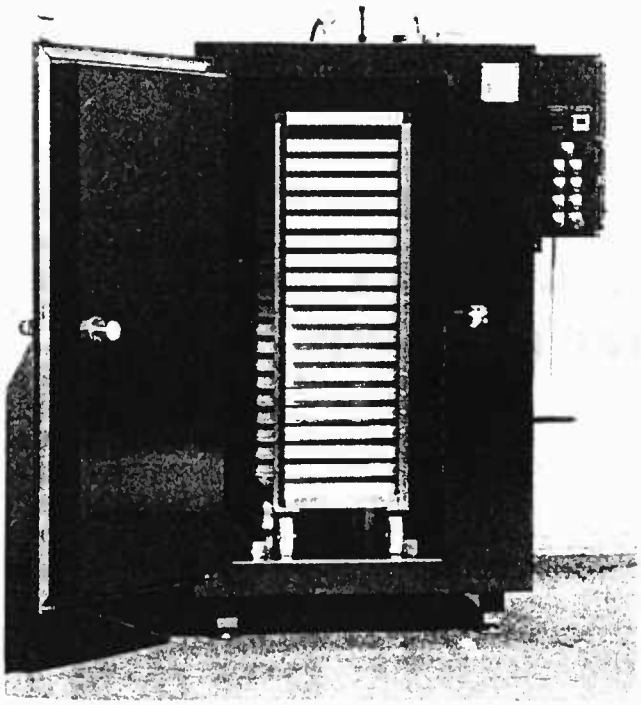


Fig. 2.4- Liquid nitrogen cabinet freezer (Kolbe 2000).

change to food materials, is not easily obtained. Such optimization frequently can be approached only at the expense of increased drying costs (Potter 1986b).

Freeze-drying can be used to dehydrate heat sensitive foods. With this method the product is dried directly from the frozen state, and under such conditions there is little deterioration. The principle behind freeze-drying is that under certain conditions of low vapor pressure, water can evaporate from ice, without the ice melting (Potter 1986b). A material is said to be sublime when it can exist as a solid, a liquid, and a gas, but can go directly from a solid to a gas without passing through the liquid phase. Frozen water will sublime if the temperature is 0° C or below and the frozen water is placed in a vacuum chamber at a pressure of 4.7 mm or less (Potter 1986b). Under these conditions the water will remain frozen and water molecules will leave the ice block at a faster rate than water molecules from the surrounding atmosphere re-enter the frozen block (Potter 1986b). Within the vacuum chamber, heat is applied to the frozen food to speed sublimation. If the vacuum is maintained sufficiently high (0.1-2 mm Hg) and the heat is controlled, just short of melting the ice, moisture vapor will sublime at a near maximum rate (Potter 1986b). The food dries from the surface inward and when the freeze-drying process is complete, the food is below 5% moisture. Since the frozen food remains rigid during the process, escaping water molecules leave voids behind them, resulting in a porous sponge-like dried structure (left Fig. 2.5). This causes freeze-dried foods to reconstitute rapidly so they must be protected from ready absorption of atmospheric moisture and oxygen with proper packaging (Potter 1986b).

Fig. 2.5- Picture of freeze-dried surimi; rice cake form (left) powder (right).

Methods of drying fish cover the spectrum from traditional sun-drying to high technology computer- controlled processes (Doe and Olley 1990). The degree of technology of a drying process greatly affects the relative costs, with freeze-drying being the most expensive. The method preferred by a producer depends on many factors, with the most important being the profit margin to the fish processor. Food companies desiring to install freeze-drying equipment on a major scale must consider the process from an overall systems approach. This includes material handling, the freezing operation, loading of drier trays, the drying operation, high vacuum and condenser requirements, unloading of trays, packaging requirements, equipment, labor and utility costs. The assessment of the cost/benefit of using this technology is not a simple one. The benefit to a community through the introduction of a new freeze-drying plant, which could supply a more stable product and create employment in other industries, might outweigh the additional cost of the process.

Chemical aspects

Denaturation during frozen storage

Proteins in their natural form are called native proteins and if they undergo any change in their structure it is called denaturation. Denaturation occurs in the secondary, tertiary, and quaternary structures of proteins. Freeze denaturation of fish proteins has been reviewed by several authors, including Connell (1959), Sikorski (1977), Matsumoto (1979), Shenouda (1980), Park (1994), and Park and others (1988). The factors involved, with the denaturation of proteins include the denaturing and catalytic

effect of ice and inorganic salts, the binding of fatty acids and lipid oxidation products, crosslinking induced by formaldehyde, and other possible reactions that lead to the formation of new covalent bonds which denatures proteins (Fig. 2.6). The proteins lose at least a part of their solubility and may have lower enzyme activity due to denaturation (Sikorski and Kolakowska1990). As a result of these changes, significant deterioration of the functional properties of the fish myofibrillar protein may also occur. Loss of functional properties is manifested by decreases in water retention, gel-forming ability, and lipid emulsifying capacity. Textural deterioration and increased dryness of the fish meat also occurs as a result of freeze-induced denaturation (Sikorski and Kolakowska 1990).

Moisture plays a significant role in the denaturation of myofibrillar proteins. Ice crystallization may disturb the water structures surrounding the areas of hydrophobic interactions in proteins. It may also disrupt the water-mediated hydrophobic-hydrophilic interactions, which participate in buttressing the native conformation of protein molecules (Lewin 1974). Increasing the concentration of salts in the unfrozen pools of water, however, may decrease the hydration of proteins, as the concentrated inorganic ions compete for the water molecules (Powrie 1973). Inorganic salts may also play a role in freeze denaturation by catalyzing the hydrolysis and autooxidation of lipids (Kolodziejska and others 1980).

The deteriorative protein changes in frozen fish are caused by several factors including interactions with other tissue components. The importance of these factors depends in each case upon the species characteristics of the fish, i.e., the composition

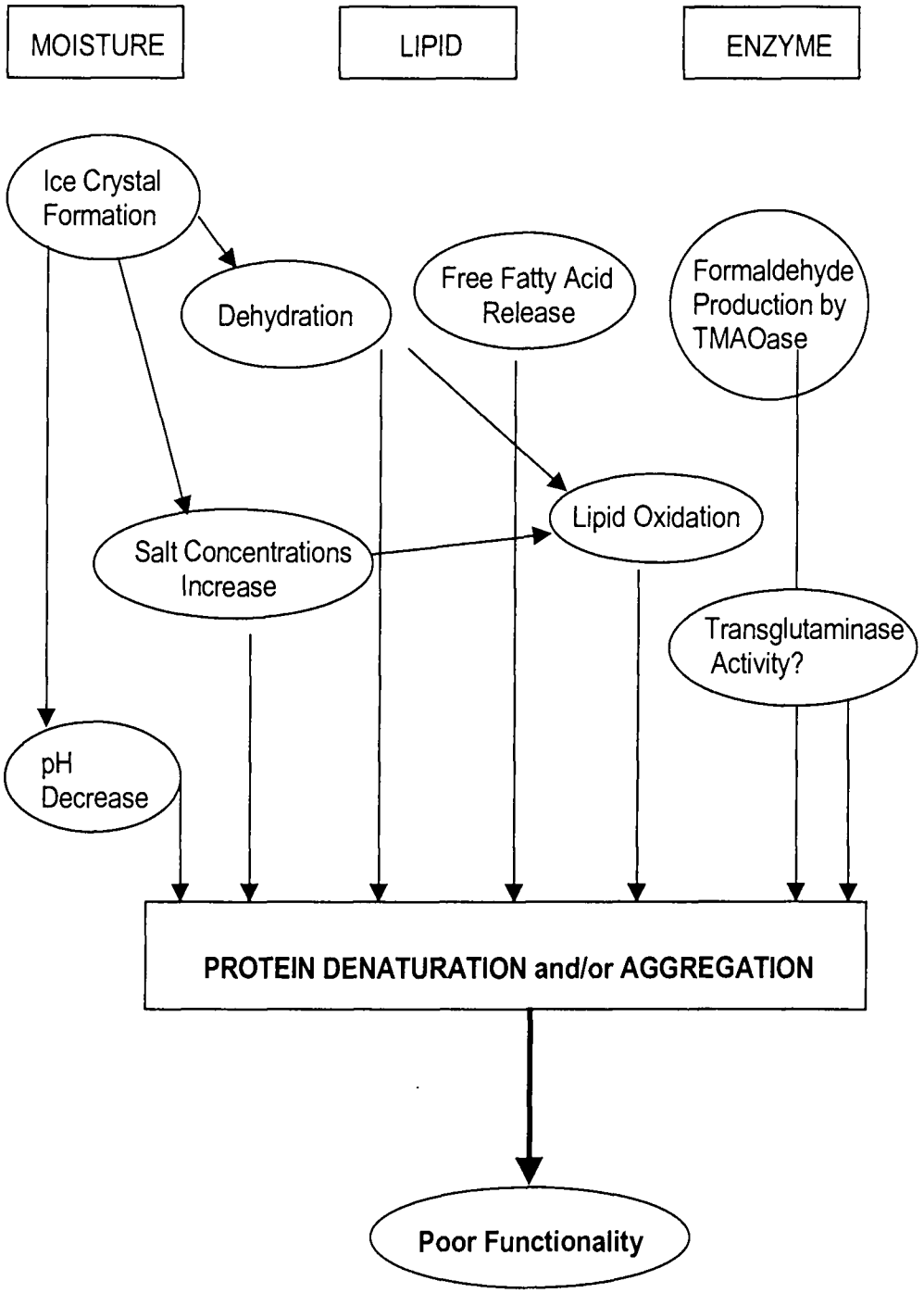


Fig. 2.6- Factors that affect, directly or indirectly, fish muscle protein denaturation and consequent loss of protein functionality during frozen storage. Adapted from Shenouda (1980).

of the flesh and on the parameters of processing and storage. Mincing of the meat before freezing promotes oxidation of the tissue lipids. High frozen storage temperature and long storage times lead to extensive deteriorative reactions. On the other hand, removing the water-soluble components from the minced meat, protecting against oxidation, and adding cryoprotectants is effective in suppressing freeze denaturation although they will not eliminate it (Sikorski and Kolakowska 1990; Park and others 1988; Sych and others 1990).

Salt extractable protein (SEP)

Gel-forming properties of surimi may be affected by factors other than protein denaturation (Table 2.1), therefore a more accurate indication of the cryoprotective action of an ingredient against protein denaturation is the maintenance of the extractability of salt-soluble proteins during frozen storage (Park and others 1988). It is the salt extractable proteins (SEP) that are the functional proteins in surimi gel formation (Niwa 1992). Some researchers have shown that SEP decreases in Alaska pollock after 8 months at higher storage temperatures, such as –18° C (Babbitt and others 1984) while others have found no change (Umemoto and Kanna 1969). Tokunaga (1965, 1974) showed that a decrease in SEP corresponded to increases in formaldehyde and dimethylamine.

Rapid texture deterioration occurs during frozen storage and these changes are connected to protein changes, especially of the myofibrillar proteins, myosin and actin, which are responsible for the two-step process of gel formation. In this process,

Table 2.1- Degree of influence of several factors on failure stress and strain of surimi gels.

Factor	Stress	Strain
Protein denaturation	Medium	Strong
Proteolysis	Strong	Strong
Protein concentration	Strong	Weak
Filler Ingredients	Strong	Weak
Low temperature setting	Strong	Weak
Chemical Oxidants	Strong	Weak
Fish Age	Strong	Strong
pH	Strong	Strong
Refrigerated Storage	Strong	Strong
Test (Gel Measurement) Temp	Strong	Strong
Hydrogen Bonds	Strong	Weak
Hydrophobic Bonds	Weak	Strong
Moisture Content	Strong	Weak
Freeze-thaw Abuse	Strong	Strong

Adapted from Kim and Park (2000)

proteins are partially unfolded and become more reactive in the first step by addition of salt during chopping and/or heating. The main function of the salt is to solubilize the myofibrillar proteins. In the second step, the proteins aggregate and form a continual three-dimensional network or they aggregate randomly and form lumps, which precipitate (Madsen 1984). Actomyosin in monomeric form is soluble, but the solubility is lost when a dimer or higher polymers are formed (Rodger and others 1979). A decrease in the amount of salt soluble actomyosin is generally regarded as a primary criterion of freeze denaturation and/or aggregation (Matsumoto 1980; Park and others 1988).

Dimethylamine (DMA)

Until about 1960, the only relation demonstrated between free amines and the condition or quality of fish was that between free trimethylamine (TMA) and fish spoilage (Spinelli and Dassow 1982). The origin of TMA was trimethylamine oxide (TMAO). TMAO was shown to be degraded by enzymes produced by certain spoilage microorganisms such as the pseudomonas. Little attention was given to DMA as a spoilage index because very little was found in spoiling fish and because its formation was species related, forming primarily in the gadoids (Regenstein and others 1982). Spinelli and Koury (1979) have shown that DMA is produced in very significant quantities in both drum- and freeze-dried fish, and that its formation is neither species-related nor enzymically induced.

TMAO is found in large amounts in gadoid species (cod, haddock, pollock, and hake). The TMAO chemistry of all gadoid fish in both fresh and frozen storage seems to be similar (Regenstein and others 1982). Amano and Yamada (1965) studied the formation of DMA in cod. They stated that TMAO was degraded to dimethylamine (DMA) and formaldehyde by an enzyme in equimolar concentrations. They also proceeded to state that the liberated formaldehyde reacted with the structural proteins causing textural alterations. Subsequent work has shown evidence that formaldehyde can react with the structural proteins to reduce protein extractability and cause related textural changes (Tokunaga 1964, 1965; Castell and others 1973). Hoogland (1958) and Castell and others (1970) have suggested that DMA be used as a measure of frozen storage deterioration for the gadoid species in the same way TMA has been used as a measure of microbial spoilage in unfrozen fish. This is based on their findings that during frozen storage, production of DMA is much larger than production of TMA, which is minimal and negligible in most cases.

Aldehydes are very reactive compounds known to interact with different functional groups in proteins. The aldehyde accumulation that occurs during frozen storage may bind to some groups of protein side chains and form intra- and intermolecular methylene bridges. These reactions may be involved in the formation of protein aggregates and may decrease protein solubility (Sikorski and Kolakowska 1990). Sikorski continues by stating that by removing the water-soluble substrate and the enzyme system responsible for the degradation of TMAO, the undesirable protein changes can be effectively reduced. On the other hand, the decrease in the

concentration of TMAO in fish meat may by itself contribute to freeze denaturation, as the TMAO may serve in the fish muscle as a protective osmolyte.

Formaldehyde is believed to cause the toughness of fish flesh through the methylene crosslinking of muscle proteins (Regenstein and others 1982; Sikorski and Kolakowska 1990). The chemical changes of the proteins lead to a decrease in drip loss but an increase in the expressible moisture of a system. This will lead to a dry and spongy texture. A fishy odor is also associated with TMA and is produced when TMA is split from phospholipids, by bacteria and natural fish enzymes (Potter 1986c). Fish that is taken fresh from the water has little or no odor, yet virtually all fish products that consumers encounter have a fishy odor, which is indicative of some deterioration. Since formaldehyde is an unstable compound and difficult to extract from fish (Ota 1958), DMA can be measured instead to determine the degree of deterioration. A decrease in extractable protein coupled with a proportional increase in DMA and formaldehyde has been observed by Tokunaga (1974). At present, there is nothing that can be done to eliminate deterioration of fish, but there are some ways to decrease its rate. The removal of dark muscle, kidney and other internal organs, and blood before deboning and mincing help to reduce the rate of DMA and formaldehyde formation (Castell and others 1971). It is also very beneficial to maintain lower storage temperatures, such as -18°C.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-gel electrophoresis systems are used to resolve and characterize the number and size of protein chains or protein subunit chains in a protein preparation. SDS-gel systems are a simple and useful qualitative and quantitative gel-electrophoresis tool. Purified protein preparations can be readily analyzed for their homogeneity. Alternately, 'pure' proteins can be analyzed for the number and the size of the molecular subunits (Clark and Switzer 1964).

The formation of formaldehyde from TMA is believed to react with the fish proteins to accelerate the undesirable texture changes (Hebard and others 1982; Regenstein and others 1982). Most electrophoresis studies of fish muscles after frozen storage have not shown any major differences among the muscle proteins, except for a general loss in solubility (Ragnarsson and Regenstein, 1989).

The effects of different heating parameters can also be analyzed using SDS-PAGE to determine the extent of denaturation, as seen by the thickness of the myosin heavy chain.

Physical aspects

Texture / rheology

The use of surimi was limited only to oriental diets. Consequently, surimi was manufactured on a kitchen-type processing scale until the success in developing the theory and technology for preparing frozen fish mince (surimi) using cryoprotectants by Japanese scientists, Nishiya, Takeda, Tamato, and collaborators in 1961 (Matsumoto

1978). Today, minced fish products have gained favor in international markets and have evolved into continuous mass production of versatile products (Sikorski and Kolakowska1990; Park 2000b, Anonymous 1999b).

Surimi is highly concentrated myofibrillar protein, primarily actomyosin, which is solubilized by salt during comminution. The solubilized protein paste forms an irreversible gel upon heating. Several factors determine the gel-forming ability of fish muscle proteins. These include freshness, fishing methods, season, size, and species (Shimizu 1985). It is the gel structure that accounts for the textural strength and elasticity of the food, as well as, the entrapment of water, fat, and other food constituents (Lanier 1986). Studies have indicated that myosin is the most important protein affecting the gel-forming ability of fish during low temperature setting (Gill and Conway 1989; Niwa and others 1980; Numakura and others 1987). A three-dimensional network is formed during the setting process through linkages in the tail portion of the myosin molecule via hydrophobic interactions (Stone and Stanley 1992). The continuous intermeshing system of protein molecules holds or traps water. The water contains soluble materials such as enzymes, salt, and fat droplets (Madsen 1984).

Pacific whiting is known to have a soft texture, which is associated with proteolytic activity (Morrissey and others 1995; Porter and others 1993). Cathepsin L is the most predominant protease present and causes a loss of surimi gel strength (An and others 1994). Proteolytic degradation is highest at ~55° C whereas the sol is stable at ~ 30° C (Tsuyuki and others 1982; An and others 1994; Chang-Lee and others 1989;

Yongsawatdigul and others 1997). Rapid heating of the flesh to a minimum of 70° C for 10 minutes completely inactivates the proteases and preserves the textural properties of the surimi gels (Patashnik and others 1982; Yongsawatdigul and others 1997).

Yasui and others (1979) made the following conclusions concerning the gelation of rabbit myosin: (1) the thermal transition from sol to gel begins at 30° C and reaches a maximum at 60°-70° C; (2) on the basis of nuclear magnetic resonance spin-spin relaxation times, the mobility of water within the gel network is more restricted than that of free water; and (3) the properties of the gel network are dependent upon pH. The optimal pH for the development of rigidity of myosin and actomyosin gels is between pH 5.0 and 6.3 (Trautman 1966; Ishioroshi and others1979; Itoh and others 1979; Acton and others 1981).

Surimi gels exhibit a high water-binding capacity and very strong elastic qualities, depending on the quantity of the myofibrillar protein (Ishioroshi and others 1979). A setting at <40° C or as low as 5-10° C prior to cooking at 90° C results in a stronger gel than cooking alone (Okada 1959; Hashimoto and others 1986; Stone and Stanley 1992). The setting process allows more protein-protein interactions to occur and form a more ordered and stronger 3-dimensional gel (Foegeding and others 1986), which induces greater elasticity and higher water holding capacity in surimi gels (Kimura and others 1991).

The hydration of protein prior to heat-induced gelation is an important step in forming gels capable of immobilizing or trapping large amounts of water (Oakenfull 1987). Adding salt during the comminution of fish mince increases the water-binding

capacity of myofibrillar proteins by increasing the negative charges, which attracts water molecules (Chung and Lee 1991). Phosphates with their anionic groups also add to the water binding capacity of proteins. This is essential because grinding and freezing processes rupture cells and can result in the loss of fluid (Pigott 1986).

The quality of surimi is graded on the basis of the chemical and visual conditions of raw surimi and its gel-forming ability. The texture of gels prepared from a given surimi system is affected by the moisture content of the surimi, the levels of added salt and polyphosphates, the extent of actomyosin solubilization (chopping time), the pH, and the heating parameters. Generally, the gel weakens as the moisture content increases (Lee and Toledo 1976; Lippincott and Lee 1983). The salt concentration required for gel formation ranges from 2-3% of the weight of the surimi. Gel strength reaches a maximum at 1M NaCl (4.4%) and gradually decreases with increases in salt concentration as salting-out occurs (Shimizu and Simidu 1955). Solubilization of actomyosin increases with extended comminution of surimi and reaches a maximum within 15-20 min of chopping. Beyond this chopping period, the temperature of the meat rises, resulting in a protein-protein interaction, which causes a decrease in gel-forming ability (Lee and Toledo 1976). The final paste temperature at the completion of chopping, in industrial practices, is generally near 8° C. It has been suggested that the temperature of the paste must be kept at or less than the temperature above which fish actomyosin becomes unstable (Lee 1984). Park (1997) found that maintaining chopping temperatures between 0-5° C provides maximum gelling functionality.

It was previously thought that elasticity and resilience of surimi gels increase with an increase in the concentration of actomyosin, but decreased with an increase in the concentration of water-soluble sarcoplasmic protein. The presence of water-soluble sarcoplasmic proteins retards gel setting by interfering with the actomyosin cross-linking process (Okada 1964). Shimizu and Nishioka (1974) proposed a mechanism whereby water-soluble sarcoplasmic protein binds actomyosin, making it less available for the cross-linking process.

More recently, there is new information that sarcoplasmic proteins may not be as detrimental as once believed. However, researchers have shown that sarcoplasmic proteins do not have an enhancing effect on gelation (Morioka and Shimizu 1990; Ko and Hwang 1995). Hultin and Kelleher (2000) have demonstrated in their lab that the presence, or absence, of sarcoplasmic proteins contributes, at most, only a minor effect to gelation ability. Good solubilization of the sarcoplasmic proteins occurs under the same conditions, which allow the water solubility of the myofibrillar proteins to be expressed. Additionally, Nishioka and others (1990) concluded that the function of washing was to improve the quality of the myofibrils and this improved gel formation, not the removal of the sarcoplasmic proteins.

There are a number of important considerations in making surimi-based products. Use of top-quality surimi is essential in making surimi seafoods that require a highly elastic and resilient texture. Surimi of good quality can be readily recognizable as its paste becomes tacky, glossy, and translucent upon chopping with salt and is extruded smoothly. Surimi of poor quality produces a dull opaque, and less tacky paste

that breaks easily when extruded (Lee 1984). Consistency in product quality is required for successful industrial production.

Color

Surimi processing requires extensive washing to remove fat and other undesirable materials, such as pigments. Therefore, raw surimi is naturally mild in odor and translucent in appearance. Myoglobin and hemoglobin, responsible for the red hue of fish meat, is thoroughly removed during the dewatering step of surimi production (Park 1995).

Color of surimi gels is typically evaluated using the CIE Lab color scale (Commission Internationale de l'Eclairage). Commercial surimi has four to five different grades based on the L* and b* values of cooked gels (Park and Morrissey 1994). Surimi gels are generally opaque to translucent. Color, like texture and flavor, is another important quality factor of surimi and its related products (Park 1995). In addition to affecting gel strength, moisture will also affect the color of the product. The addition of water affects both the L* and b* values. The higher the moisture, the lighter and less yellow the color. Therefore, gels with higher moisture contents look whiter (Park 1995). Park (1995) has also shown that freezing results in darker colored gels.

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Chapter 3

Physio-Chemical Properties of Pacific Whiting Surimi as Affected by Various Freezing and Storage Conditions

J. Hoffman, J.W. Park, Y.J. Choi

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Abstract

The effects of freeze-drying and flake freezing of surimi on biochemical and physical properties as affected by various storage conditions were examined. Using three cooking parameters, shear stress and strain were evaluated to measure gelation properties of surimi gels. Stress values increased up to 3 months and then decreased substantially as storage time was extended. In general, strain values decreased substantially over time, however, freeze-dried surimi kept in the freezer at -18°C did not change during 9 months of storage. Overall, color decreased during storage and b* values of the freeze-dried surimi kept at room temperature increased significantly. Salt extractable proteins decreased while dimethylamine increased. Electrophoretic patterns, however, did not show any apparent damage to the Myosin Heavy Chain due to frozen storage and/or proteolysis (with 60° C incubation) until the 9^{th} month of storage. At 9 months, a reduction of the MHC was observed and the lower molecular weight bands were more intense. Freeze-dried samples stored in the freezer maintained the highest quality for the duration of the 9 month study. Low temperature storage is important for retaining good functionality in freeze-dried surimi.

Keywords: Freeze-dry, surimi, gelation, flake freezing, shelf-life

Introduction

Pacific whiting *(Merluccius productus)* is an important commercial species caught off the West Coast of North America. Pacific whiting has been a good source of surimi, producing strong and cohesive gels by adding protease inhibitors (Chang-Lee and others 1990; Morrissey and others 1992). Surimi is myofibrillar protein concentrate obtained from fish flesh, which has been mechanically deboned, washed, and mixed with cryoprotectants such as sugar and sorbitol. Surimi, depending upon fish species and grade, can be stored up to 24 months and still produce commercially acceptable gels for surimi-based analog products (Iwata and others 1971; Lee 1984; MacDonald 1992).

Due to seasonal availability of whiting, production is limited to approximately 4 months (May-Sept) per year. Therefore, frozen storage has proven to be an important long-term storage method. Frozen storage prevents microbial spoilage and minimizes the rate of biochemical reactions in muscle. Nevertheless, inevitably there is some deterioration of muscle protein functionality associated with frozen storage (Powrie 1973; Matsumoto 1979, 1980; Park and others 1987). Extended frozen storage can produce profound effects on the structural and chemical properties of muscle proteins, which can, in turn, significantly influence the quality attributes of muscle food products (Park and Lanier 1987).

High quality surimi can only be made from fish with myofibrillar proteins that have not been denatured (Iwata and others 1971; Matsumoto 1979; Suzuki 1981; Acton and others 1983; MacDonald and others 1990). The loss of protein functionality and, in particular, the gel-forming ability in frozen fish is due to freeze denaturation and aggregation of the myofibrillar protein (Grabowska and Sikorski 1976; Sikorski and others 1976; Matsumoto 1980; Suzuki 1981). Factors influencing protein denaturation during freezing and frozen storage include salt concentration, pH, ionic strength, surface tension, and mechanical effects of ice and dehydration (Park 1994). Freezing and frozen storage also cause textural changes that often decrease the water retention of muscle proteins, particularly for fish of the *gadidae* family (gadoids). This is probably due to the enzymatic breakdown of trimethylamine (TMAO) to dimethylamine (DMA) and formaldehyde (FA). The FA is believed to react with the fish proteins to accelerate the undesirable texture changes (Hebard and others 1982; Regenstein and others 1982). Additionally, Childs (1973) reported that cross-linking caused texture toughening and loss of water holding capacity.

The mechanism of protein denaturation caused by drying can be considered the same as the mechanism of freeze denaturation because water molecules in the cell are removed (Park and Lanier 1987). Suzuki (1981) reported that denaturation induced by drying is considered more drastic than freeze denaturation. This is partly due to the higher

temperatures associated with the drying process. Spray-dried and freeze-dried fish protein powder with the incorporation of sucrose and/or sorbitol as anti-denaturation agents have been manufactured in Japan with little loss in functionality as compared to fresh fish meat (Niki and Igarashi 1982). However, a recent study indicates that protein functionality of dried fish protein can be well preserved even without sucrose and/or sorbitol as long as membrane lipid is removed (Hultin and Kelleher 2000a).

The discovery of cryoprotectants in 1960 to minimize changes of functional properties has revolutionized the world surimi industry. However, the conventional methods of plate-freezing to produce frozen blocks have exhibited a few disadvantages with the primary concern being the chemical deterioration that occurs with freezing and long term storage. Frozen blocks also take up a large amount of space and require a lot of energy to maintain the proper frozen storage parameters. In addition, there is the added risk of temperature abuse during transport. In order for the surimi seafood manufacturers to use the frozen blocks, either thawing or breaking is necessary. Abusive thawing often causes protein denaturation, resulting in lower gel properties (Kim and Park 2000). Breaking frozen blocks into small pieces also requires extra steps of handling. Consequently, alternative forms of surimi, as well as, storage conditions could result in improved surimi quality.

The overall objective of this study was to determine the effects of freeze-drying and flake freezing of surimi on the biochemical and physical properties as affected by various storage conditions. Comparisons were made to determine if different forms of freezing could prevent or minimize the loss of functional properties of Pacific whiting surimi during frozen storage. The specific objectives of this research included: (1) determining if freeze-drying greatly reduced the rate of denaturation and changes in intramolecular conformation, and (2) determining if a faster rate of freezing (flake), as compared to conventionally block-frozen surimi, increased the shelf-life stability of the product.

Materials and methods

Sample preparation

The experimental design is outlined in Fig. 3.1. Fresh whiting surimi blocks were obtained from Point Adams Packing (Hammond, Oregon). The cryoprotectant mix contained 5% sorbitol, 4% sugar, 0.24% phosphates, and 1.5% beef plasma protein (BPP). The surimi was extruded in 10 kg blocks directly from the processing line, put into totes and immediately transported to the OSU Seafood Laboratory where it was stored overnight in a cooler at 2° C. The following day the surimi was divided into three sample sets. Four blocks were frozen in a plate freezer (conventional) (APV Crepaco, Inc, Chicago, IL) (Fig. 3.2).

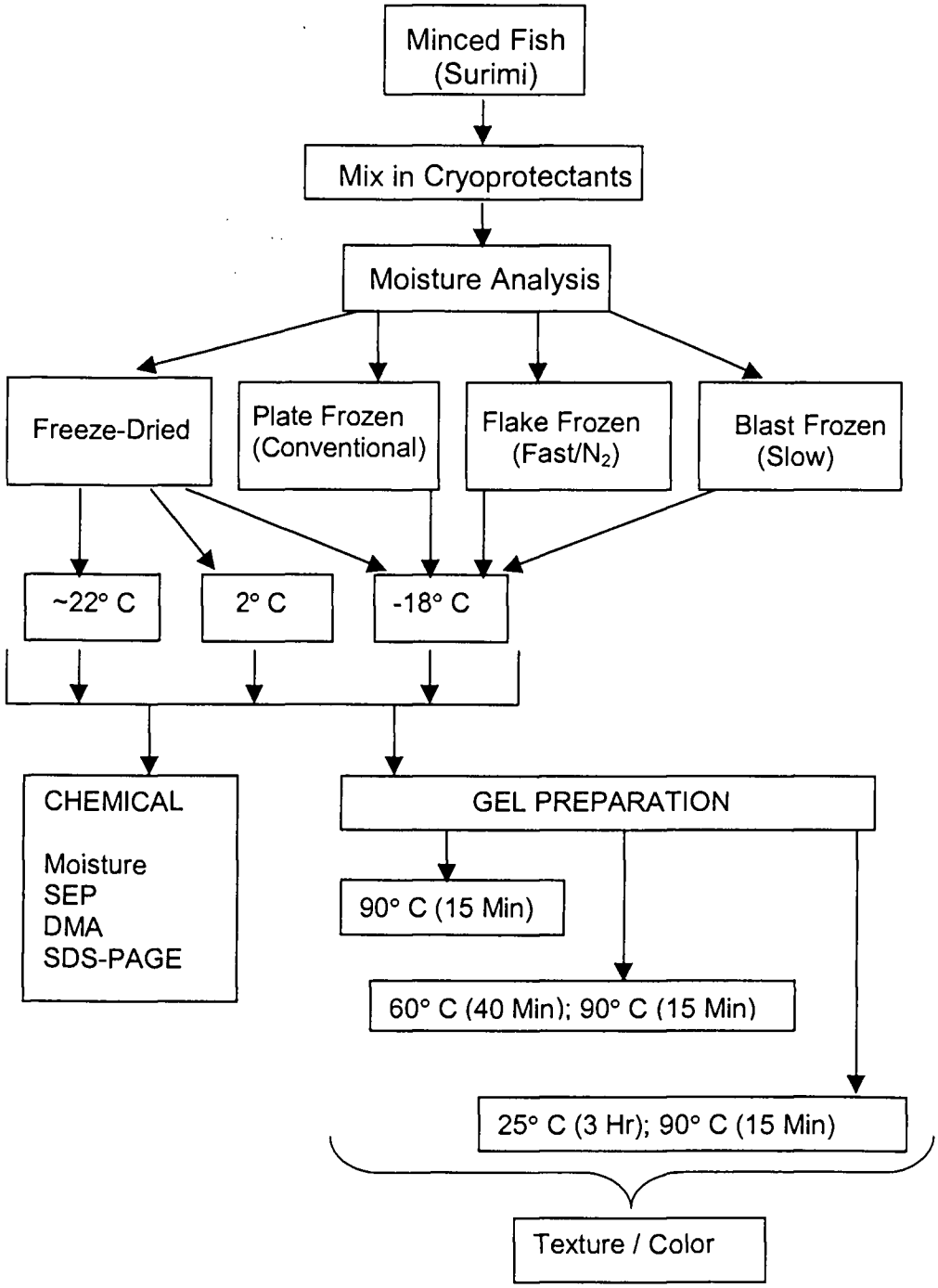

Fig. 3.1- Overview of experimental design, sample preparation, and storage

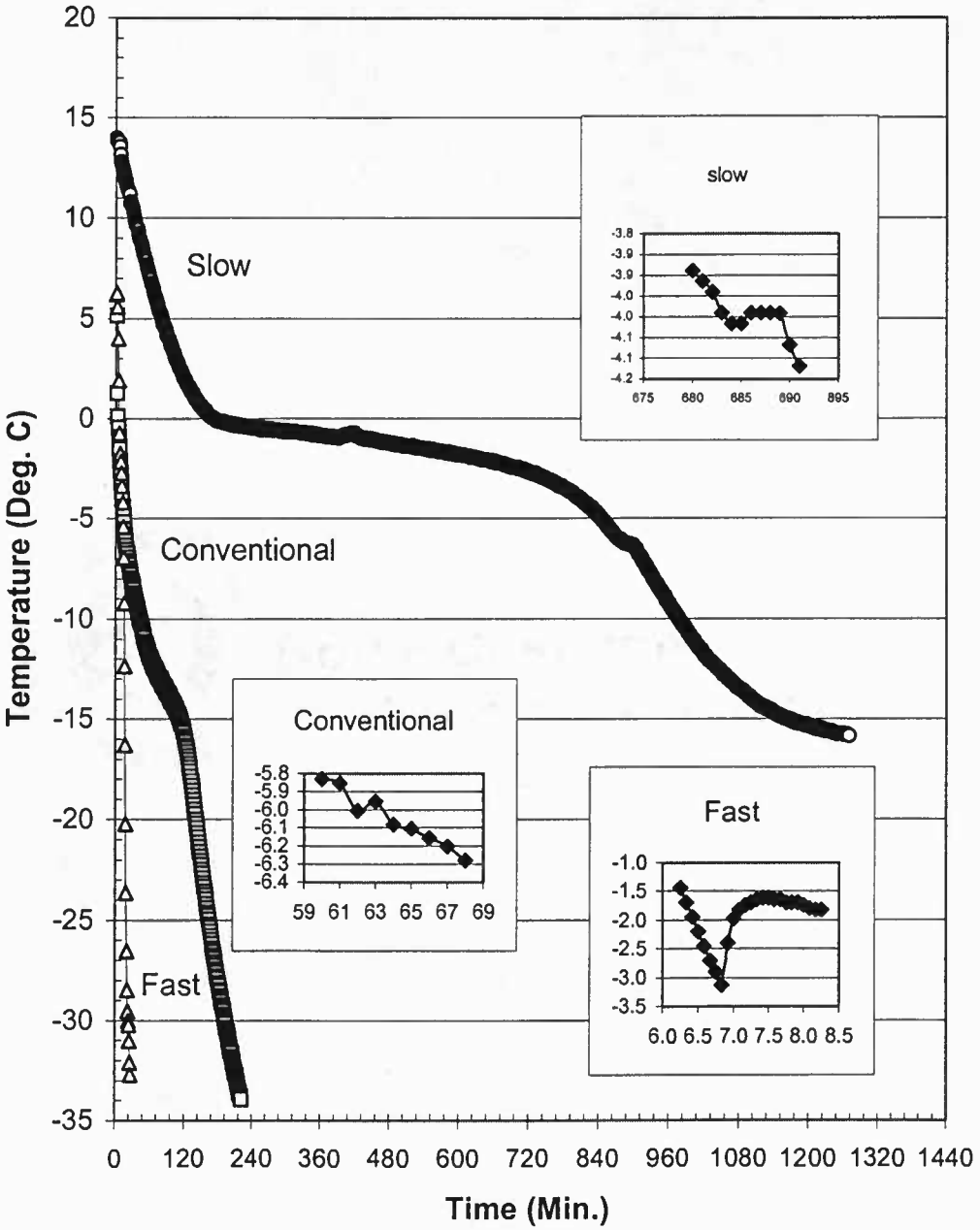

Fig. 3.2- Freezing temperature profiles measured at geometric center. The inserts illustrate the point of latent heat of ice crystallization

Twelve blocks were transported in coolers, on ice, to Oregon Freeze Dry (Albany, Oregon) where the product was freeze-dried in accordance with their guidelines (Fig. 3.3). The blocks maintained a temperature of ~1° C to the point of delivery. Prior to the drying operation, the surimi was frozen (-30° C) and no moisture was removed. The frozen product (-30° C) was de-pressurized in the chamber and the heating platen was heated. As drying progresses the ice sublimed. The food temperature rose due to contact with the heated platen, but the receding ice core remained frozen, cooled by the latent heat of sublimation (Potter 1986). Ultimately, all of the ice sublimed and the entire dried mass was heated and moisture removed below 3%.

Additionally, 4 blocks were kept in the cooler where the surimi was rolled into 2mm slabs using an aluminum frame and a rolling pin to produce a uniform size and then placed onto stainless steel pans. These pans were loaded onto a baker's rack, put into a blast freezer, and quickly frozen using liquid nitrogen (fast) (Airgas, Inc., Longview, WA) (Fig. 3.2). The frozen samples were vacuum-packed in oxygen/moisture impermeable bags and stored in the freezer at –18°C.

The final four blocks were frozen simply by putting the slabs wrapped in plastic bags into the -18° C freezer where they were allowed to freeze slowly until they equilibrated with the temperature of the freezer.

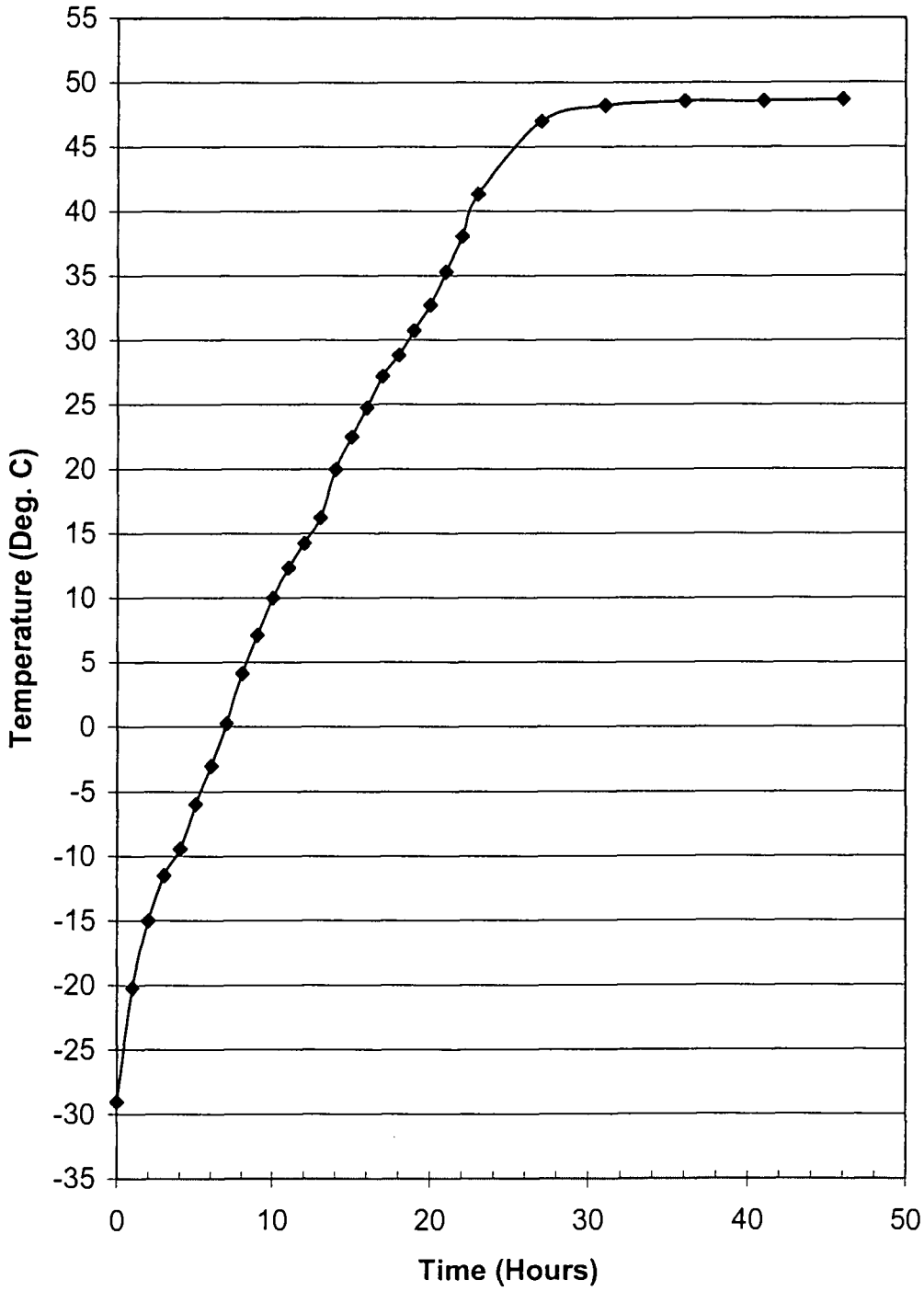

Fig. 3.3- Temperature profile of surimi during freeze-drying process

Before being put into storage, the freeze-dried samples were ground into fine particles using a Hobart mixer (Model VCM-40, Hobart Manufacturing Co., Troy, OH). The powder was then vacuum packed in oxygen/moisture impermeable bags and stored at three different storage parameters: room temperature (22° C), in the cooler (2° C), and in the freezer (-18° C). All samples were tested immediately after frozen preparation (0 month) and again at 1, 3, 6, and 9 months.

Salt extractable protein (SEP)

Salt soluble proteins were extracted at 0.6M KCl according to the method of Noguchi and Matsumoto (1970) with the following modification to sample size. Two and a half grams of surimi were homogenized with 50 mL 0.6 M chilled KCl solution (pH 7.0) in a blender (Kinematica CH-6010, Brinkman Instruments, Westbury, NY). The homogenized samples were centrifuged at 5000 x g for 30 min at 4°C. After centrifugation, 10 mL of the supernatant was transferred to a beaker and 10 mL 0.6 M KCl solution was added. Protein concentration of the extracted salt-soluble proteins was then determined using the procedure outlined by Lowry and others (1951). BSA (Bovine Serum Albumin, Sigma A 39120) was used as a standard. The concentration of the SEP was expressed as mg of proteins per g of sample.

Dimethylamine (DMA)

DMA content was determined according to the method of Dyer and Mounsey (1945) with the following modifications: Toluene was used instead of benzene as the solvent to extract the colored complex, 6% perchloric acid was used instead of 6.25% trichloroacetic acid and the amount of copper ammonia reagent and acetic acid was increased by 1 mL. The shaking period after heating was increased from 5 min to 30 min. A sample size of 3.5 mL was used throughout the test series. The concentration of DMA was expressed as ug of DMA per g of sample.

Protein extraction and sample preparation for gel electrophoresis

Sample preparation for protein extraction was done following the instructions of Choi (1999) using 0.5 g of surimi gel. This was added to 7.5 mL of an 8 M urea, 2% sodiumdodecyl sulfate, 2% ß-mercaptoethanol, and 20 mM sodium phosphate buffer, pH 7.2 solution then heated for 20 min at 90° C. The protein was extracted by shaking (Lab-Line Environ-Shaker, Lab Line Instruments, Inc, Melrose, Park, IL) for 20 hrs at 100 rpm. After shaking, the solution was centrifuged at 7800 x g for 20 min. The supernatant was then dialyzed against 0.1% SDS solution to remove the urea. From the dialysate, 0.5 mL was used to determine the protein concentration using the Lowry method (Lowry 1951). Another 1 mL sample was mixed with 250 ug of 5X sample buffer (1M Tris-hydrochloric acid- pH

6.8, 50% glycerol, 10% SDS, β-mercaptoethanol, 1% bromophenol blue, and water) and heated at 90° C for 3 min, then stored frozen at –20° C. The sample was thawed prior to running gel electrophoresis.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was run according to the procedure of Laemmli (1970) and outlined in Bollag and others (1996) using a FisherBiotech Protein Electrophoresis System (Model FB-VE16) vertical slab. Slab gels consisted of a separating gel (10%) and a stacking gel (5%) and 20 ug of protein was loaded onto the gel. The protein samples were run at a constant current of 200 volts. Following electrophoresis, the proteins were stained with 0.1% (w/v) Coomassie Brilliant Blue R-250 in 45% methanol, and 10% acetic acid. The protein was then destained in a 10% methanol and 10% acetic acid solution. Gels were scanned using a HP Desk Scan II program. Molecular weights of the protein bands were determined using a high molecular weight standard (Sigma M3788) with a protein concentration of 2-3.5 mg/mL.

Gel preparation

Moisture content of the samples was determined, in triplicate, with a gravity oven (VWR Scientific, 1330GM) set at 105° C for 24 hours. Samples (~5.0 g) were spread into a pre-weighed aluminum dish which

were then re-weighed after drying to calculate the percentage of moisture loss. All gel formulations were adjusted to equal moisture contents of 78% and a 2% salt content. The surimi was tempered (-5° C) then comminuted in a vacuum chopper (Model 5289, Stephan Machinery Corp. Columbus, Ohio). The samples were mixed to –1.5° C, salt was added, a vacuum was applied, then further mixed to a final temperature of 5° C. The paste was vacuum-packed in a plastic bag to eliminate air bubbles before it was put into a sausage stuffer (Model 14208, The Sausage Maker, Buffalo, NY), the paste was then extruded into stainless steel tubes (I.D. = 19mm) that were sprayed with a lechitin-based release agent (Pam™,butter flavor. International Home Food Products, Inc., Persnippany, NJ) and sealed with screwed caps. Three cooking parameters were used: A) direct cooking in a water bath at 90° C for 15 min; B) setting at 60° C for 40 minutes followed by cooking at 90° C for 15 minutes; C) setting at room temperature for 3 hours followed by cooking at 90° C for 15 minutes. After cooking, the samples were immediately chilled in ice water for 40 minutes. The gels were then removed from the tubes and stored in sealed plastic bags at 5° C overnight.

Rheological properties

Changes in textural properties were evaluated as shear stress and shear strain. To minimize the effects of various gel temperatures at

measurement the gels were kept at room temperature for >2 hours before analysis. The gels were cut into 2.9 cm long segments and both ends glued to plastic disks using super glue (Dupro, LocTite Corp, Rocky Hill, CT). Each segment was milled (Gel Consultants, Model 91, Raleigh, NC) into an hourglass shape with a minimum 1.0 cm diameter in the center as measured using calipers. Each gel segment was then placed in a Hamann Torsion Gelometer (Gel Consultants Inc, Raleigh, NC). After twisting the samples, shear stress and shear strain were calculated using equations developed by Hamann (1983). Shear stress indicates the strength and hardness, while shear strain denotes the cohesiveness.

Color measurements

Five samples cooked at 90° C for 15 min were subjected to a Minolta Chroma Meter (Minolta USA, Ramsey, NJ). CIE (Commission Internationale de l'Eclairage L* (lightness), a* (red to green), and b* (yellow to blue) were measured and whiteness was calculated as suggested by Park (1995) using the equation L* - 3b*. All samples were kept at room temperature in a plastic bag for >2 hours to eliminate the effects of varying gel temperatures at measurement.

Statistical analysis

Analysis of variance (ANOVA) (Statgraphics, version 3.0, Manugistics, Inc., Rockville, MD.) was conducted on torsion and color data to determine the significance of the different freezing and storage parameters. Fisher's least significant difference (LSD) at $p \leq 0.05$ was used to determine significant differences between mean values. This method can result in a 5.0% risk of calling each pair of means significantly different when the actual difference equals zero.

Results and discussion

Salt extractable protein (SEP)

After 1 month of storage SEP of both conventionally frozen and fast frozen samples decreased by approximately 10%, whereas the freeze-dried samples dropped by approximately 53% (Table 3.1). At 3 months, all SEP concentrations in all samples dropped drastically at an average of 48% with the freeze-dried samples retaining slightly higher concentrations, especially in the samples stored at the cooler temperatures of the refrigerator and freezer. SEP values decreased up to the 9^{th} month, but at a much slower rate. The overall decreases were: conventional frozen, 71%; fast frozen, 73%; freeze-dried room (22°C), 83%, refrigerator (2°), 80% and freezer (-18°C), 79%.

Table 3.1 - Salt extractable protein (SEP) concentration of Pacific whiting surimi at various freezing and storage conditions

Freezing Treatment (Storage Temp)	Month(s) of Frozen Storage	Protein Concentration mg/mL
Conventional (-18°C)	0	7.44
	1	6.49
	3	3.01
	6	2.95
	9	2.17
Fast (-18°C)	0	7.38
	1	6.68
	3	3.00
	6	2.67
	9	2.00
Freeze-Dried/ (22°C)	0	12.72
	1	5.68
	3	3.34
	6	2.84
	9	2.14
Freeze-Dried/ (2°C)	0	12.72
	1	6.03
	3	3.29
	6	3.25
	9	2.59
Freeze-Dried/ (-18°C)	0	12.72
	1	6.05
	3	3.39
	6	3.26
	9	2.66

0 (storage time) indicates surimi immediately after freezing.

While the SEP of freeze-dried samples decreased in greater proportions, in terms of percentages, the freeze-dried samples maintained the highest levels of SEP throughout the duration of the study. Again, freeze-dried samples stored in the freezer maintained the highest protein concentrations. This result is in agreement with shear strain values, which indicates the quality of the fish protein.

The function of salt-soluble proteins is not unanimously in agreement. Some researchers have reported that a truer indication of the cryoprotective action of an ingredient against protein denaturation is maintenance of the extractability of salt-soluble proteins during frozen storage (Park and others 1988; Scott and others 1988). This is because gel forming properties of surimi may be affected by factors other than protein denaturation. Others have reported that SEP is not a reliable test for measuring quality of Alaska pollock surimi (Iwata and Okada 1971; Iwata and others 1971; Kawashima and others 1973). Based on the results of this study SEP seems to be an appropriate test to indicate Pacific whiting surimi quality when analyzing shear strain data. As SEP concentrations decrease, strain decreased, and overall gel forming ability also decreased. With an initial rise in stress values for the first 3 months of the study, this would indicate that SEP is not a reliable test to measure surimi quality.

Dimethylamine (DMA)

All samples at 0 time contained the same concentration of DMA. The greatest changes were observed in the conventional frozen samples where DMA increased by 13-fold and fast frozen samples, which increased 12-fold (Table 3.2). Freeze-dried samples had a much slower reaction rate, particularly in the samples stored in the refrigerator and freezer. Freeze-dried samples stored at room temperature showed a 5-fold increase in DMA and those stored in the colder temperatures only increased 4-fold. DMA formation has been noted to be a reliable index of decomposition in gadoid fish in refrigerator or frozen storage (Hebard and others 1982). Textural change is more apparent and important than the change in taste or odor during frozen decomposition. Dyer and others (1964) reported that formaldehyde (FA), produced simultaneously with DMA, reduces extractable protein in fish muscle. Formaldehyde is a highly unstable compound that is difficult to extract from fish (Ota 1958), therefore, DMA can be measured instead to determine the deterioration of frozen gadoid fish (Castell and others 1971; Castell and others 1974).

Accordingly, as SEP levels decreased DMA levels increased (Tables 3.1 and 3.2). This supports the results of Tokunaga (1978) who found a decrease in extractable proteins coupled with a proportional increase in DMA and FA occurred in Alaska pollock. These lower levels of DMA in the

Table 3.2 - Dimethylamine (DMA) concentration of Pacific whiting surimi at various freezing and storage conditions

Freezing Treatment (Storage Temp)	Month(s) of Frozen Storage	DMA Concentration, ug/mL
Conventional (-18°C)	0	0.60
	1	1.33
	3	3.46
	6	3.74
	9	7.96
Fast (-18°C)	0	0.66
	1	1.88
	3	3.22
	6	3.56
	9	7.91
Freeze-Dried/ (22°C)	0	0.65
	1	0.55
	3	0.77
	6	1.55
	9	3.12
Freeze-Dried/ (2°C)	0	0.65
	1	1.10
	3	0.80
	6	1.40
	9	2.75
Freeze-Dried/ (-18°C)	0	0.65
	1	0.62
	3	0.59
	6	1.28
	9	2.59

0 (storage time) indicates surimi immediately after freezing.

freeze-dried samples may have been due to the lack of water present. Without water, movement within the substrate is significantly reduced, thereby effectively reducing reaction rates. The decrease in temperatures of the refrigerator and freezer also helped in further slowing down the reaction rates.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Myosin is the most important component contributing to the formation of surimi gels. The presence and concentration of intact myosin determine the gel strength of the surimi (Niwa 1992). Myosin heavy chain can easily be separated into a single band on SDS-PAGE, and its relative concentration could be roughly estimated by the intensity of the band on the gel. Figs. 3.4, 3.5, 3.6, 3.7, and 3.8 show the SDS gel electrophoretic pattern for all surimi samples from Cook A and B over the course of the 9 month study.

In this study, the results of the gel electrophoresis showed a good correlation with the strength of the heat-set surimi gels as measured by the torsion test. When the surimi was cooked at 60° C for 40 min followed by 90° C for 15 min there did not appear to be any damage to the proteins due to proteolysis. Changes, however, were observed at 6-9 months with the lower molecular weight bands (80-100k) appearing to be more intense. However, Ragnarsson and Regenstein (1989) noted that many

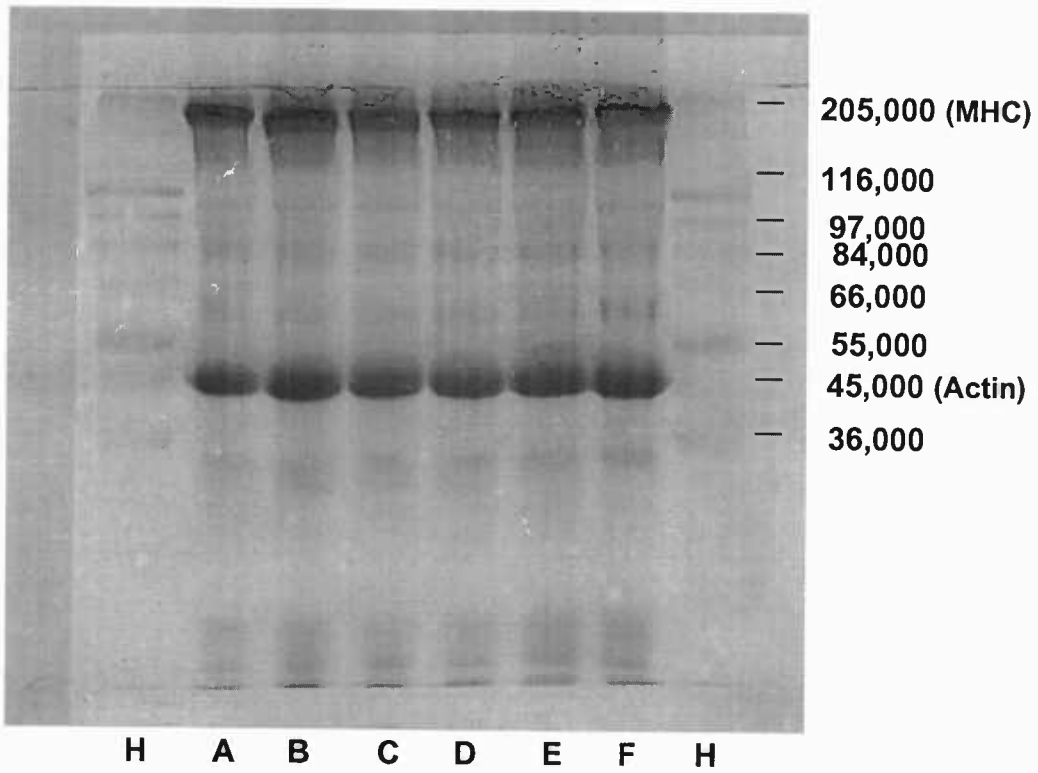

Fig. 3.4- SDS-PAGE pattern of Pacific whiting surimi stored at 0 month. Lanes A-C indicated conventional, fast, and freeze-dried samples from Cook A. Lanes D-F indicated conventional, fast and freeze-dried samples from Cook B. H designated high molecular weight protein standard.

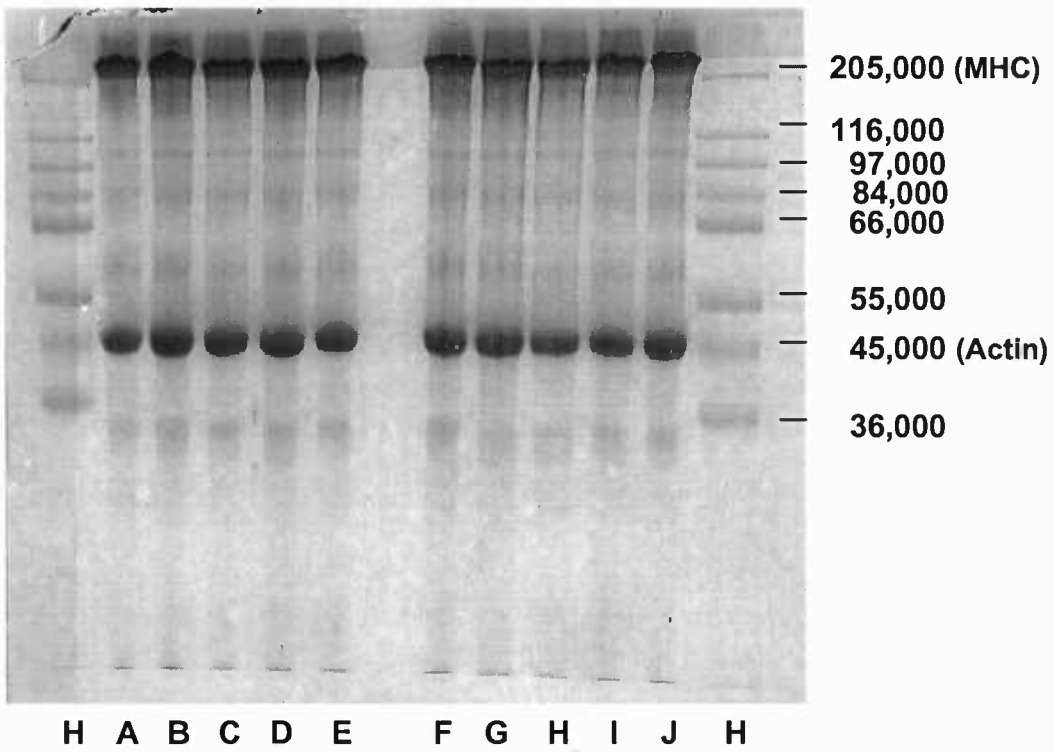

Fig. 3.5- SDS-PAGE pattern of Pacific whiting surimi stored at 1 month. Lanes A-E indicated conventional, fast, FD Room, FD Fridge, and FD Freezer samples from Cook A. Lanes F-J indicated conventional, fast, FD Room, FD Fridge, and FD Freezer samples from Cook B. H designated high molecular weight protein standard.

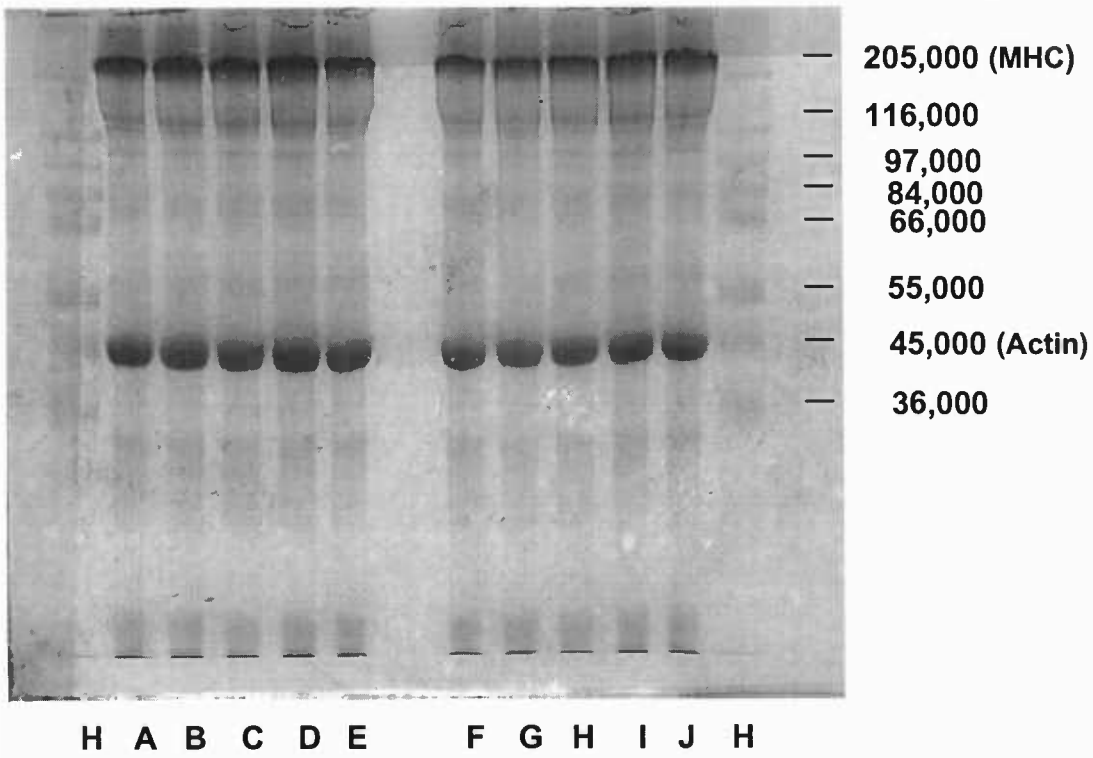

Fig. 3.6- SDS-PAGE pattern of Pacific whiting surimi stored at 3 months. Lanes A-E indicated conventional, fast, FD Room, FD Fridge, and FD Freezer samples from Cook A. Lanes F-J indicated conventional, fast, FD Room, FD Fridge, and FD Freezer samples from Cook B. H designated high molecular weight protein standard.

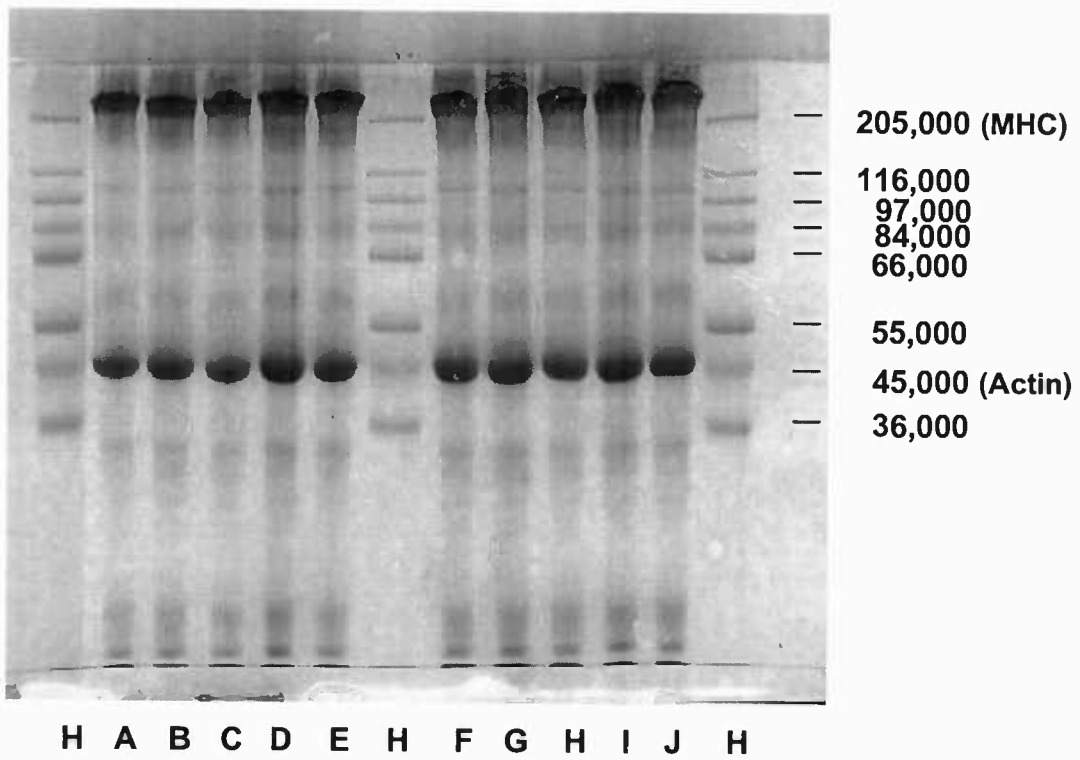

Fig. 3.7- SDS-PAGE pattern of Pacific whiting surimi stored at 6 months. Lanes A-E indicated conventional, fast, FD Room, FD Fridge, and FD Freezer samples from Cook A. Lanes F-J indicated conventional, fast, FD Room, FD Fridge, and FD Freezer samples from Cook B. H designated high molecular weight protein standard.

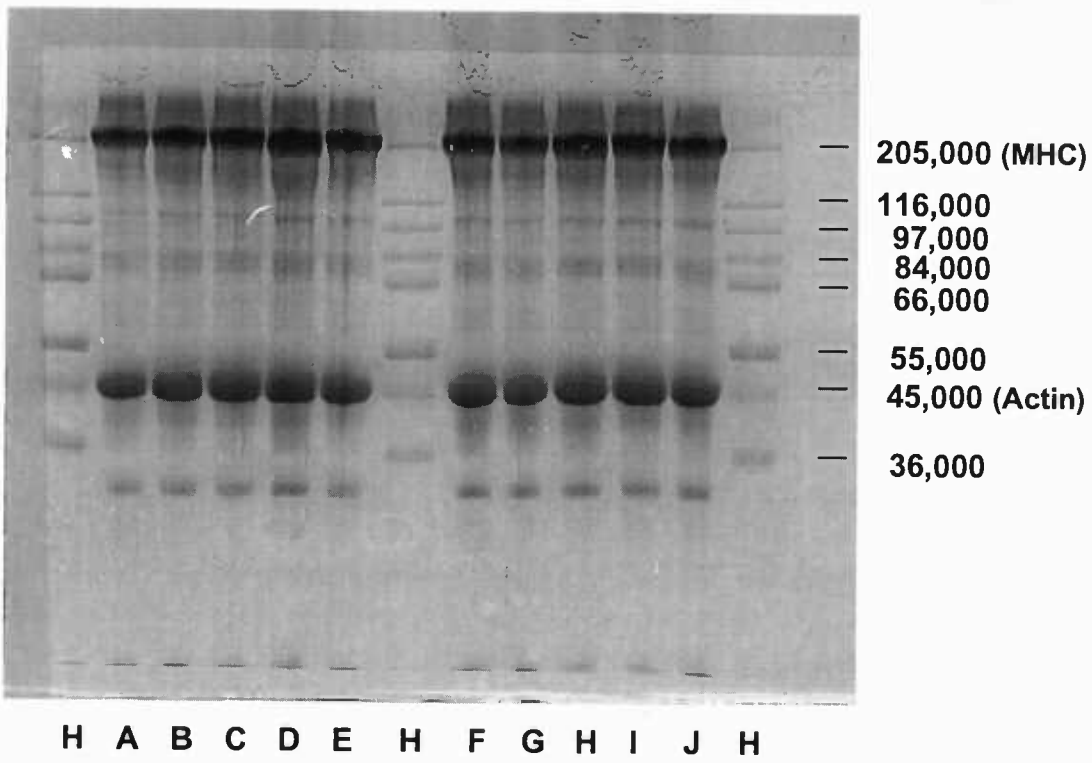

Fig. 3.8- SDS-PAGE pattern of Pacific whiting surimi stored at 9 months. Lanes A-E indicated conventional, fast, FD Room, FD Fridge, and FD Freezer samples from Cook A. Lanes F-J indicated conventional, fast, FD Room, FD Fridge, and FD Freezer samples from Cook B. H designated high molecular weight protein standard.

electrophoresis studies of fish muscles after frozen storage have not shown any major differences among the muscle proteins, except for a general loss in solubility. A new band (~30K) also appeared after 9 months of storage. This is an indication that the myosin had undergone some degradation. This correlates to the decrease in gel values observed at this time.

As mentioned previously, it had been anticipated that the gels cooked at 60° C for 40 min prior to a 90° C cook (Cook B) should have resulted in much lower gel values than Cook A due to anticipated proteolysis. If this had occurred, there would have been noticeable differences in the myosin heavy chain (MHC) bands on the electrophoresis gels. The MHC band would have been thinner and a lower intensity in the Cook B samples as compared to the bands from Cook A samples. From the Figs. 3.4-3.8 it can be seen that there is little, if any, variation in intensity between MHC of all samples. All appeared to have degraded equally, not by proteolysis, but by freeze denaturation. This supports the gelation data that states that very little differences resulted in gelation properties between Cooks A and B (Figs 3.9-3.12). This is supported by the work of Morrissey and others (1993) who found that the use of protease inhibitors, such as BPP, in surimi processing can provide substantial protection against proteolysis during cooking. They also found that at both 1% and 2% levels, the MHC band was well protected from proteolysis even at 60°, at which the proteases have high activity.

Gel functionality

Stress and strain values are illustrated, respectively, from Cook A samples (Figs. 3.9 and 3.10), Cook B (Figs. 3.11 and 3.12), and Cook C (Figs. 3.13 and 3.14). At 0 time, the freeze-dried samples had the highest stress values at cooking regimes A and C, but were significantly lower ($p<0.05$) with Cook B, where conventional and fast frozen gels were significantly higher ($p<0.05$). During the first three months of storage a slight increasing trend in shear stress values was observed. This is supported by Pipatsattayanuwong (1995) who noted an increasing trend, up to 2 months of frozen storage, in shear stress values of Pacific whiting surimi gels containing higher levels of cryoprotectants (9%). Many other investigators have noted a decrease in gel forming ability after 3 months resulting from frozen storage in other species (Nishimoto and Koreeda 1979; Kurokawa 1979; Jiang and others 1985; Holmquist and others 1984; Tanaka and others 1962). Several reports have indicated higher variation for shear stress values relative to that of true strain (Hamann 1988; Montejano and others 1984; MacDonald 1992). The strain-at-failure measurement indicates the cohesive nature of a gel, while the stress-at-failure measurement relates to the strength of the material (Park and others 1988). Stress value and rigidity are strongly influenced by the concentration of proteins or other solids in the surimi and processing conditions. Strain, however, is affected mainly by protein quality, such as

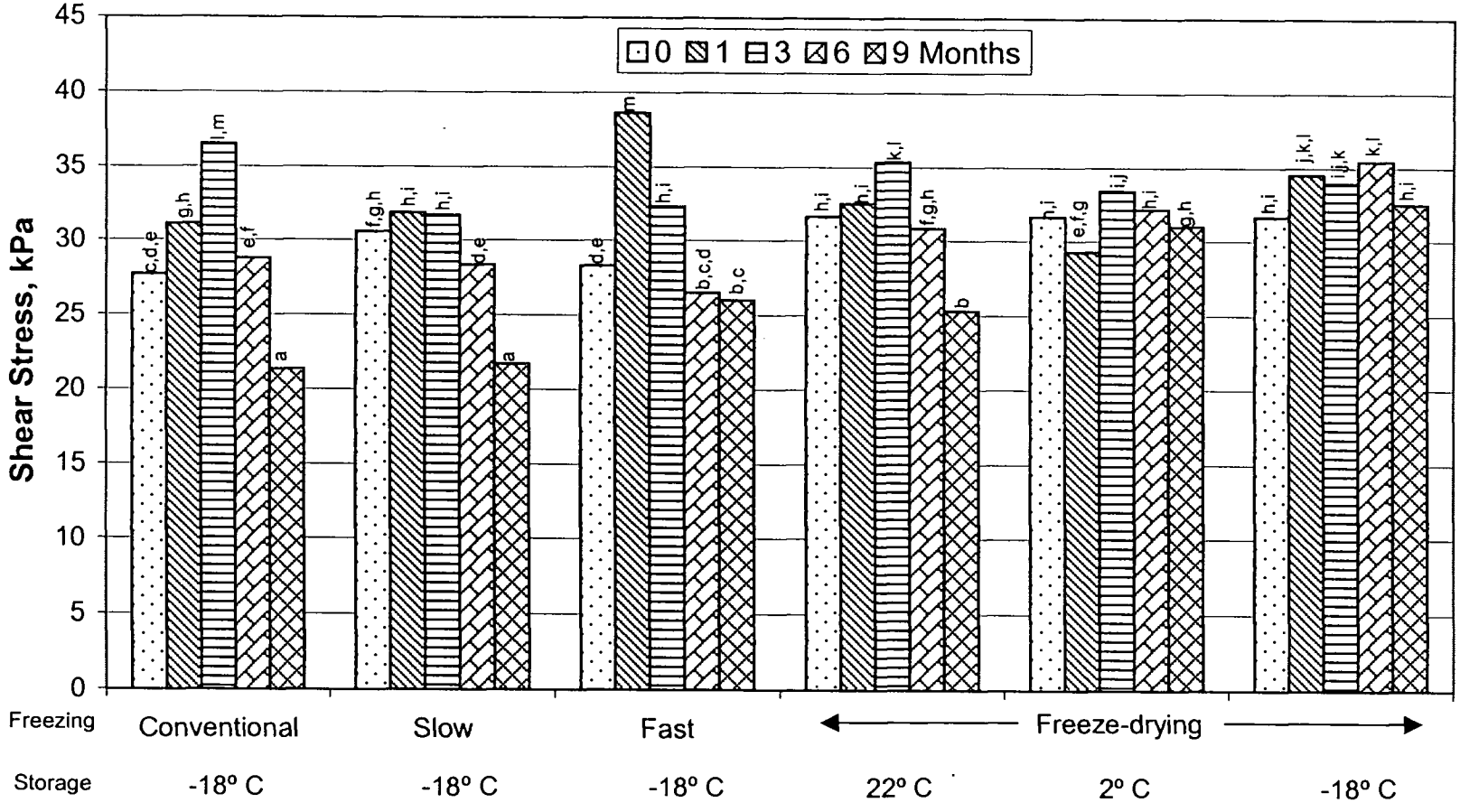

Fig. 3.9- Shear stress values for Cook A (90° C 15 min)

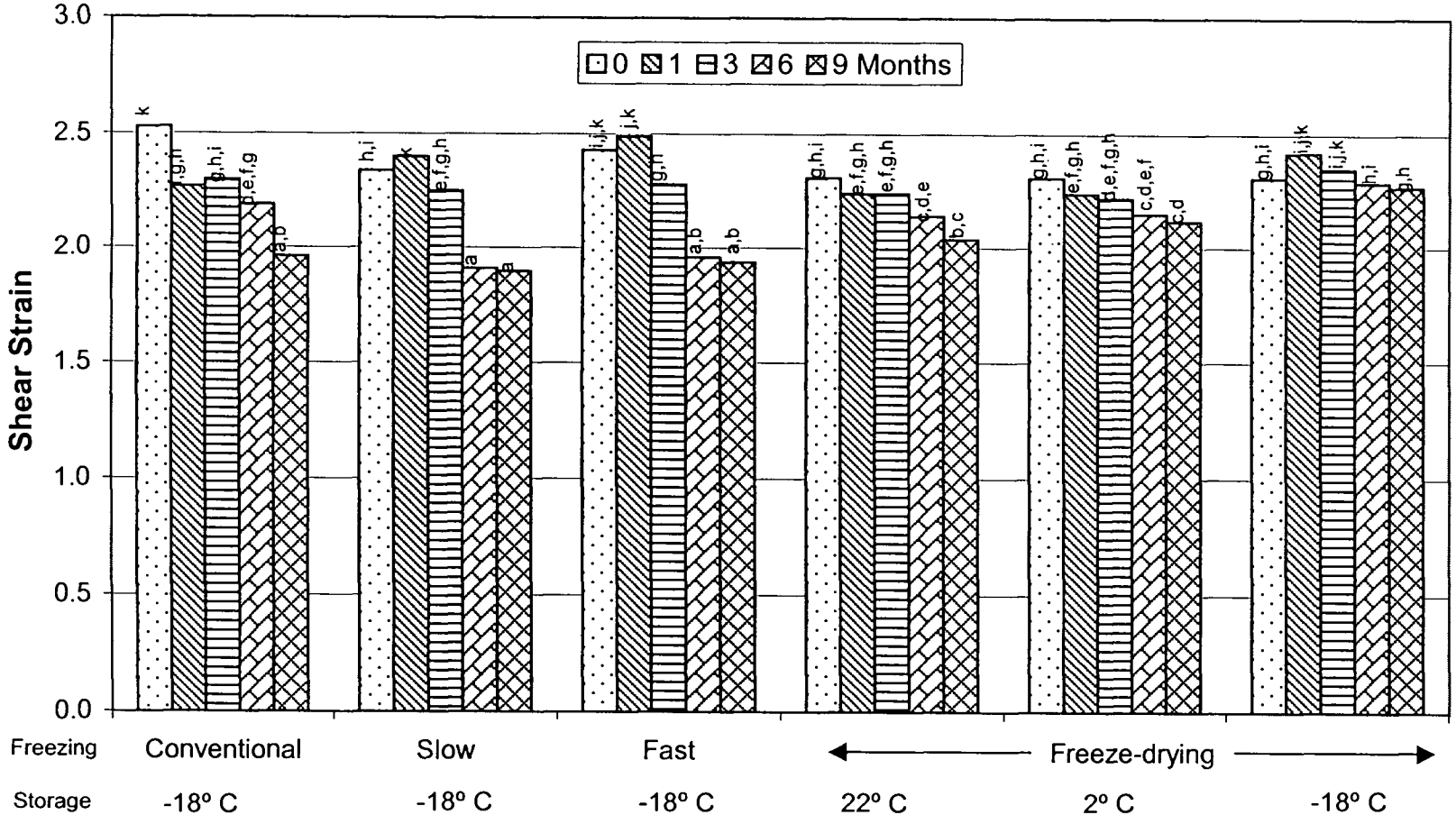

Fig. 3.10- Shear strain values for Cook A (90° C 15 min)

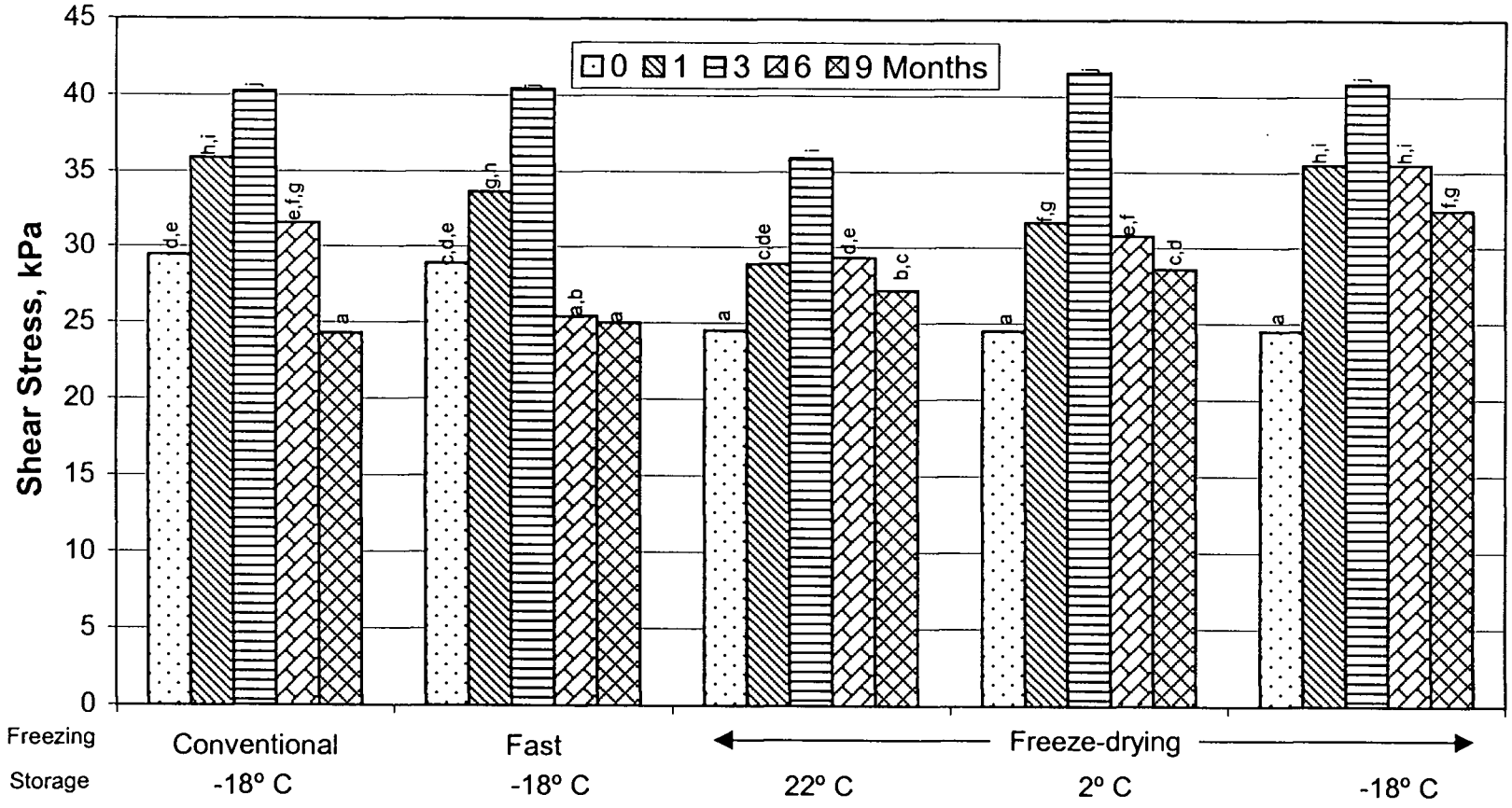

Fig. 3.11- Shear stress values for Cook B (60° C 40 min; 90° C 15 min)

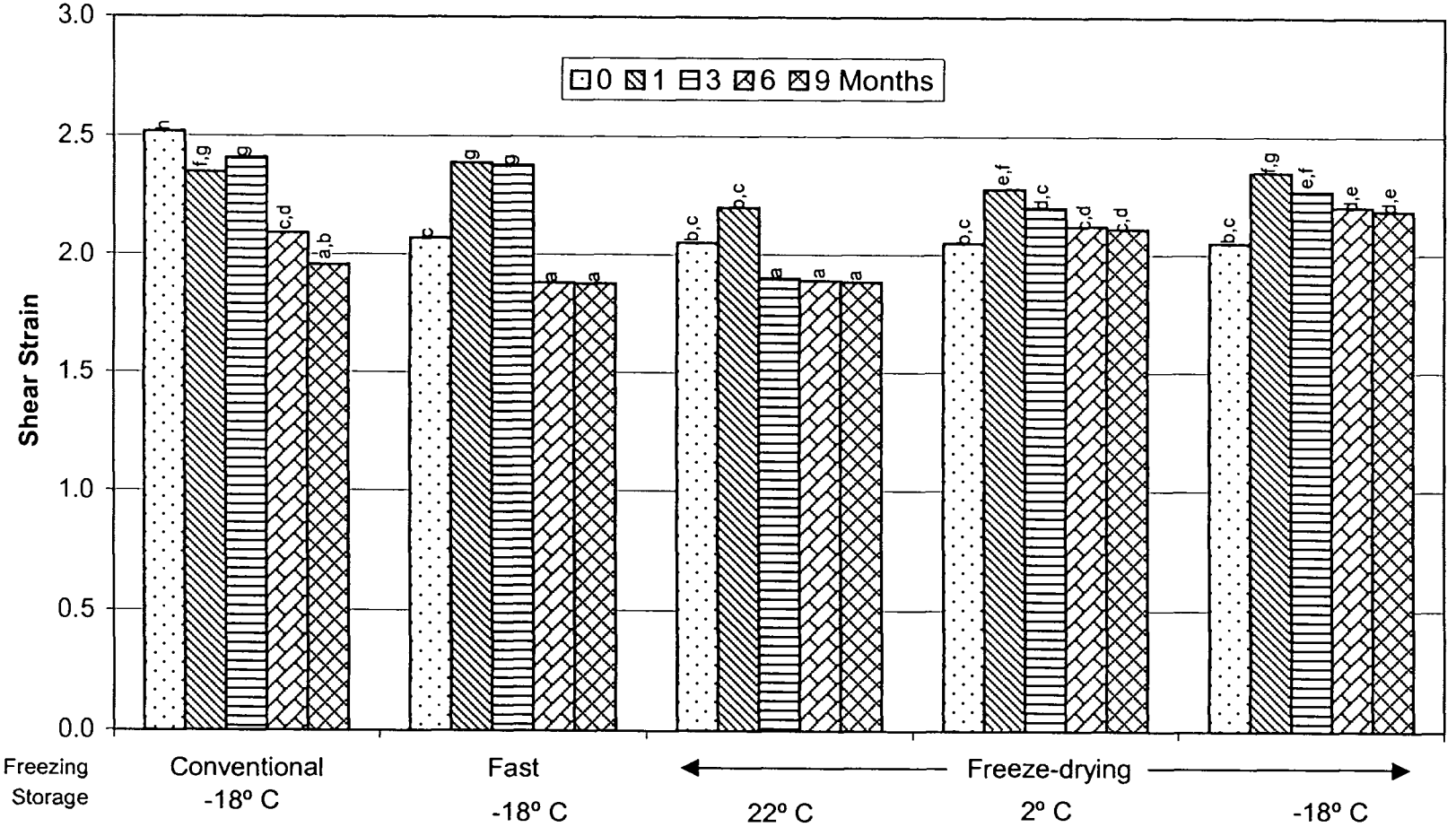

Fig. 3.12- Shear strain values for Cook B (60° C 40 min; 90° C 15 min)

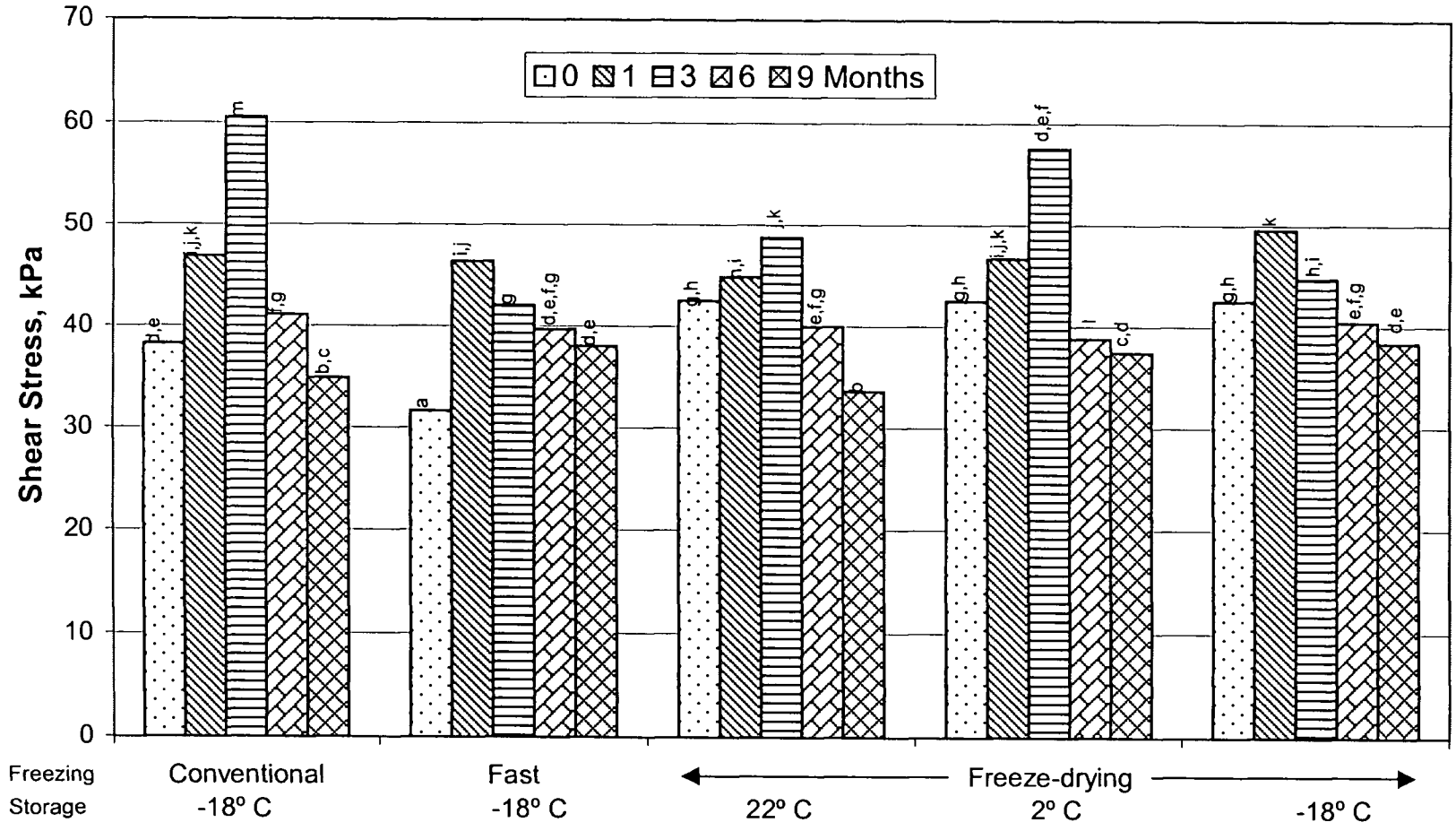

Fig. 3.13- Shear stress values for Cook C (22° C 3 hrs; 90° C 15 min)

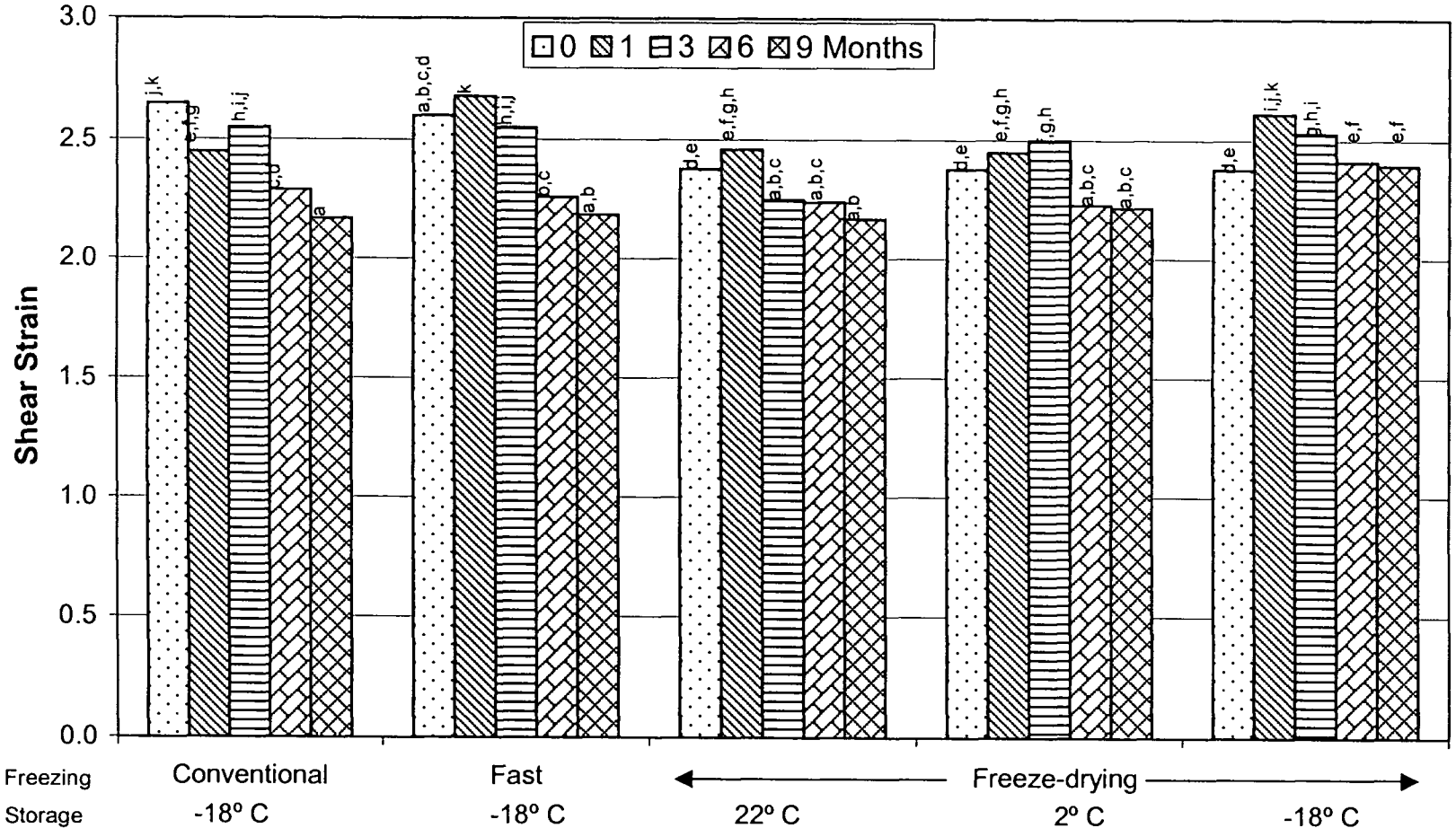

Fig. 3.14- Shear strain values for Cook C (22° C 3 hrs; 90° C 15 min)

type (sarcoplasma, myofibril, stroma), pH, and the degree of denaturation of proteins (Park 1994; Lanier 1986, Howe and others 1994.)

Pacific whiting surimi undergoes proteolytic degradation of myofibrillar components, which inhibits optimal gel formation when it is heated relatively slowly. Degradation is highest at ~55°-60° C, resulting in losses of surimi gel strength (Chang-Lee and others 1989; Morrissey and others 1993). This suggests that gels from Cook B should have resulted in much lower stress and strain values than Cook A due to proteolysis, which was not observed in this study. Both gels from Cook A and B resulted in similar values, which are not really discernible from each other.

This increasing trend in stress values may have been due to the concentration levels of cryoprotectants (sorbitol and sugar) and inhibitor (beef plasma protein) that were used. Traditionally, 8% cryoprotectants and 1% BPP is added to commercially produced whiting surimi (Morrissey and others 1993). The samples used in this study contained almost 10% cryoprotectants and 1.5% BPP. Previous studies have reported that higher levels of cryoprotectants produced more cohesive gels due to the better protected proteins in frozen surimi (Yoon and Lee 1990; Pipatsattayanuwong and others 1995; Hamann and MacDonald 1992). BPP has also been shown to greatly increase stress values at concentrations above 1% (Morrissey and others 1993; An and others 1992). This leads to the assumption that the high level of cryoprotectants

maintained muscle protein functionality fairly well in the freezing process and subsequent frozen storage. The level of enzyme activity and the age of the fish were unknown and may be another possible explanation as to why differences were not noted between Cook A and Cook B. Protease concentrations can vary in fish from school to school. Factors that could affect these levels are feeding habits, water temperature, fish size, freshness, and fishing methods (Park and Morrissey 2000). A gradual decrease in protease activity by washing has also been reported by Chang-Lee and others (1989).

The highest stress values were observed in the sample from Cook C as anticipated (Fig. 3.13). The setting process induced greater gel strength and higher water holding capacity in surimi gels (Kimura and others 1991).

At 9 months, shear stress values decreased due to ice crystal growth (Lawrence and others 1986; Sikorski and others 1976) and denaturation, which caused changes in functional properties (Sikorski and others 1976; Connell 1959; Matsumoto 1979; Shenouda 1980). The freeze-dried samples maintained the highest stress values ($p<0.05$) during the 9 month study. Without the presence of water, ice crystallization, denaturation, and changes in intermolecular conformation appears to be greatly reduced.

Strain, on the other hand, was not as greatly affected over time, though changes were observed as illustrated in Figs. 3.10, 3.12, and 3.14. A slight increase in values was observed from 0 time to 1 month. Strain

values then decreased slightly as storage time increased but was not significant ($p<0.05$) until after 6 months, except for the freeze-dried surimi kept in the freezer. The quality of surimi, in terms of texture characteristics, is regarded as commercially acceptable when a true shear strain value is >1.9 (Hamann and Lanier 1987). After 9 months of storage, all freeze-dried samples had exceeded this minimum level. Conventional, slow, and fast frozen samples were at the minimum level of 1.9, which is still commercially acceptable quality. Freeze-dried samples stored in the freezer maintained the highest quality with only a 2% decrease in strain values after 9 months. Conventionally frozen, fast frozen, slow frozen, freeze-dried room temperature, and freeze-dried refrigerator decreased by 23%, 20%, 19%, 12%, and 8%, respectively.

By comparing the results of the conventionally frozen block samples, which reached ~-25° C in 2.8 hours, and the slow frozen samples, which took ~21 hours to reach -16° C, no significant differences ($p<0.05$) resulted for rheological properties between the samples up to 9 months. However, there were striking visual differences between the two block forms. The conventionally frozen samples had a smooth white appearance with no visible ice crystals. The slow frozen blocks, on the other hand, were more translucent, exhibiting a darker appearance. The slow frozen blocks appeared to be glossy and ice crystals were visible. In addition, when the block was tempered it had a crystalline look. It also tempered faster than

the conventionally frozen block. From their appearance, it seemed that the slow frozen samples would have resulted in lower gel strengths but this was not the case. However, further study is required to confirm this observation and the samples should be tested beyond 9 months storage (i.e., 12 and 24 months).

Color

All surimi samples exhibited some changes in L*, a*, and b* values throughout the study (Table 3.3). Both L* and a* values remained relatively consistent over time with L* ranging from 77-79 and a* ranging from 0.2-0.7. The greatest degree of change was noted in the b* values in the freeze-dried samples, especially in those samples held at room temperature (2.14-7.70). The sample held in the refrigerator also increased more than those held in the freezer.

This change was probably the result of a Maillard reaction. In the presence of heat and amino acids, sucrose, even as a non-reducing sugar, is able to undergo a non-enzymatic browning reaction (BeMiller and Whistler 1996; Troller 1978). The 'heat' is a result of heat accumulation over time. It is this heat accumulation that drives the reaction. Therefore, the samples stored in the colder temperatures of the refrigerator and freezer underwent slower reaction rates, but the non-enzymatic browning

Table 3.3 - Color of Pacific whiting gels prepared after various freezing and storage conditions

Freezing Treatment (Storage Temp)	Months of Frozen Storage	L*	a*	b*	Whiteness
Conventional (-18°C)	0	78.96^{gh}	0.43^{cd}	1.57^{bcd}	74.25^{j}
	1	77.16^{bc}	0.65^{def}	0.74^{fg}	74.94^{e}
	3	77.96^{cde}	0.26^{b}	1.74^{cde}	72.74^{gh}
	6	77.57^{ab}	0.26^{ab}	1.73^{cde}	72.38^{g}
	9	77.39^{cde}	0.49^{c}	1.50^{a}	72.89^{j}
Fast (-18°C)	0	78.74^{fg}	0.59^{efg}	1.73^{cde}	73.55^{hij}
	1	77.78^{bc}	0.62^{fg}	1.27^{ab}	73.97^{ij}
	3	78.37^{ef}	0.55^{def}	1.18^{ab}	74.83^{j}
	6	78.35^{def}	0.15^{a}	1.98^{ef}	72.41^{g}
	9	78.13^{cde}	0.55^{def}	4.15^{i}	65.68^{c}
Freeze-Dried/ (22°C)	0	79.40^{h}	0.53^{efg}	2.14^{fg}	72.98^{gh}
	1	77.74^{bc}	0.55^{def}	2.25^{fg}	70.99^{e}
	3	77.93^{cd}	0.50^{cde}	4.15^{i}	65.48^{c}
	6	77.70^{bc}	0.42^{c}	5.73^{j}	60.51^{b}
	9	77.11^{a}	0.50^{cde}	7.70^{k}	54.01^{a}
Freeze-Dried/ (2°C)	0	79.40^{h}	0.53^{efg}	2.14^{fg}	72.98^{gh}
	1	77.43^{ab}	0.54^{def}	1.50^{bc}	72.93^{ghi}
	3	78.10^{cde}	0.67^{gh}	1.79^{cde}	72.73^{gh}
	6	78.06^{cde}	0.20^{ab}	3.60^{h}	67.26^{d}
	9	77.09^{a}	0.40^{c}	4.10^{i}	64.79^{c}
Freeze-Dried/ (-18°C)	0	79.40^{h}	0.53^{efg}	2.14^{fg}	72.98^{gh}
	1	77.79^{bc}	0.71^{gh}	1.67^{cde}	72.78^{gh}
	3	77.74^{bc}	0.74^{h}	1.62^{cd}	72.88^{gh}
	6	78.37^{ef}	0.49^{cde}	2.43^{g}	71.08^{ef}
	9	77.75^{bc}	0.49^{cde}	1.87^{de}	72.14^{fg}

Means in the same column followed by different superscripts are significantly ($p<0.05$) different within the same color parameter.
0 (storage time) indicates gels were made directly after freezing.

reaction still occurred. The rate was faster in the freeze-dried samples because of the relatively high concentration of amino acids and sucrose due to the removal of water. Due to the elevated concentrations of amino acids and sugar, the reaction rate increased exponentially compared to the other frozen samples that had ~75% moisture content.

Another reaction that might have caused color changes is lipid oxidation. Conventionally produced surimi is washed free of blood, myoglobin, fat, and membrane, which is detrimental to the final quality of the surimi gels (Park and Morrissey 2000). Further refining is done to remove fine bones, scales, and connective tissue. While these are very effective procedures they do not completely eliminate these undesirable materials. These materials, such as membrane lipid are highly reactive compounds and over time they will undergo oxidation resulting in dramatic increases in the b* values (Hultin and Kelleher 2000a). The reactions can be slowed in colder temperatures thus, the smaller degrees of change in the samples stored in the freezer.

Hultin and Kelleher (2000b) have been working on a new acid-aided process, which could produce functional protein isolates with minimal contamination by eliminating the membrane lipid. This could help stabilize the b* value, which would result in maintaining higher whiteness values.

Summary and conclusions

In all samples SEP decreased while DMA increased. Stress values increased up to 3 months then decreased substantially. Strain values did not fluctuate greatly and had a small increase at 1 month then decreased substantially over time. Overall, the freeze-dried samples stored in the freezer maintained the highest quality throughout the duration of the study. Plate frozen (conventional), slow frozen, and flake frozen (fast) samples acted essentially the same. There was little variance between the three, even though the slow frozen samples were visibly different with visible ice crystals and a crystalline appearance. This indicates that a freezing rate of 2 hours compared to that of 15 min or 24 hours does not effect the overall quality of shelf life of the product during 9 months of study. This may be largely due to the role of the cryoprotectants. However, it would be necessary to evaluate the samples after 1-2 years of storage.

While expecting gel values, stress and strain, to decrease over time this did not occur as rapidly as anticipated. This was most likely due to the levels of cryoprotectants and BPP used, which helped to prevent the degradation of the high molecular weight proteins important for gelation. In addition, not knowing the enzyme activity of the fish used and extent of washing that was done during production may have played a role in extending the shelf-life of the product. This was further supported by the

gel electrophoresis results, which showed no noticeable protein degradation occurring in Cook B.

Low temperature storage is important for retaining good functionality in freeze-dried surimi. This was also noted in a study by Niki and Igarashi (1982). While the same types of reactions still occurred in the freeze-dried samples they could not be stopped entirely. Removing the water from the surimi however, seemed to greatly reduce any effects of ice crystallization, denaturation, and changes in intermolecular conformation.

Therefore, it is suggested that while still having to incorporate frozen storage costs, freeze-dried surimi would be a good alternative to traditional surimi production. It would greatly reduce transportation and shipping costs and take up much less storage space when in the powder form. It also lends to greater ease in utilization and eliminates cumbersome blocks that need to be tempered and broken before use.

With the discovery of Hultin and Kelleher (2000b) acid-aided process, which removes membrane lipids through centrifugation, there is a great potential to produce a shelf-stable freeze-dried product that neither requires cryoprotectants or frozen storage to maintain quality. This is a very new area of study and further investigation is necessary before any final conclusions can be made.

On the downside, freeze-drying at this point may not be a feasible process to do aboard factory trawlers, which are the primary producers of

surimi. Freeze-drying plant locations are limited to the shore side facilities. It is also certain that it greatly increases the overall production costs of surimi manufacturing. Information on freeze-dried surimi is very limited to date, therefore it is highly recommended that further research be done in this area.

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Chapter 4

Improved Torsion Test Using Molded Surimi Gels

J. Hoffman and J.W. Park

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Abstract

The effects of cross-section diameter on shear stress and strain and effects of individual variation in measuring diameter were studied. Gelation properties of surimi using milled and molded gels were compared. The possibility of skin formation using various cook times was also evaluated. Shear stress values were significantly affected by diameter accuracy, whereas the effect was not as significant for shear strain values. Individual variation in measurement was also greatly noted. Molded gels resulted in significantly lower strain values than milled samples, whereas stress values were significantly higher in molded gels than in milled gels. Using a lechitin-based spray appeared to eliminate skin formation on all samples.

Keywords: torsion, surimi gel, rheology, gelation, skin formation

Introduction

For the evaluation of gelling ability, surimi is comminuted with salt, stuffed into casings or stainless steel tubes, and cooked at various temperatures. The cooked gels are subjected to a variety of tests including rheological testing, such as the fold test, punch test, and torsion (twisting) test (Lee 1984; Lanier and others 1985). The torsion test, using the Hamann gelometer (Gel Consultants, Raleigh, NC) has been successfully adopted as a fundamental analysis to measure fracture properties of surimi gels (Kim and Park 2000). The test is used to measure shear stress, indicating textural strength, and shear strain, denoting textural cohesiveness. Over the last 15 years, more than one hundred research papers have been published using the torsion method. This test requires surimi gels to be milled into a dumbbell (hourglass) shape. With the assumption of a linear torque vs. angle of twist, a rotational rate of 2.5 rpm, a groove width of 1.27 cm, and 1.0 cm cross-section diameter at the center of the groove, typical equations for fracture shear stress (τ) and uncorrected shear strain (γ) were developed by Hamann (1983):

τ = 1580 x the torque in the Brookfield viscometer = Pa

γ = 0.150 x time to fracture – 0.00848 x the torque in the Brookfield viscometer = (dimensionless)

However, maintaining an exact 1.0 cm cross-section diameter is not an easy task. Milling is a timely process and may give structural damage to soft gels depending on the type of gels and/or cutting device. Therefore, the use of molded gels instead of milled gels was thought to ensure a consistent gel diameter, eliminating human errors in milling and measurement, and also save time.

The objectives of this study were to evaluate the effect of cross-section diameter on shear stress and strain, to compare gelation properties between milled gels and molded gels, and to determine the effect of skin formation on molded gels. The effect of individual variation in measuring diameter was also evaluated.

Materials and methods

Frozen Pacific whiting surimi (medium grade) was obtained from Point Adams Packing (Hammond, OR). The samples were transferred to the OSU Seafood Lab, portioned into smaller blocks (~500g) with a band saw, and vacuum-sealed in oxygen/moisture impermeable bags. The blocks were stored at –18° C until testing was conducted.

Gel preparation

All samples were obtained from the same surimi block (10 kg). The moisture content of the block was determined to be 75% (AOAC 1995). All

gel formulations were adjusted to 78% moisture and 2% salt content. The surimi was comminuted in a vacuum chopper (Model 5289, Stephan Machinery Corp., Columbus, OH). The partially frozen samples (~ -5° C) were chopped to –1.5° C, salt was added, a vacuum was applied and then further mixed to a final temperature of 5° C. The paste was vacuum-packed in plastic bags to eliminate air bubbles before it was placed into a sausage stuffer (Model 14208, The Sausage Maker, Buffalo, NY) and extruded into either stainless steel tubes (I.D.=19mm) as shown in Fig. 4.1 or molded tubes (groove diameter 1.0 cm) (Fig. 4.2). All tubes were sprayed with a lechitin-based release agent (Pam®, butter flavor, International Home Food Products, Inc., Madison, NJ) before filling and sealed with screwed caps. The samples used to test diameter differences in gel strength were cooked at 90°C for 15 min. To determine skin formation the samples were cooked at 90°C for 15, 30, 60, and 90 min. After cooking, the samples were immediately chilled in ice water for 30 minutes. The gelled samples were removed from the tubes and stored in sealed plastic bags overnight at 5° C before conducting gels tests. All tests were performed in triplicate.

Rheological properties

To eliminate the effects of varying gel temperature at measurement, the gels were held at room temperature for 2 hours before analysis. The samples in the stainless steel tubes were cut into 2.9 cm lengths using a gel

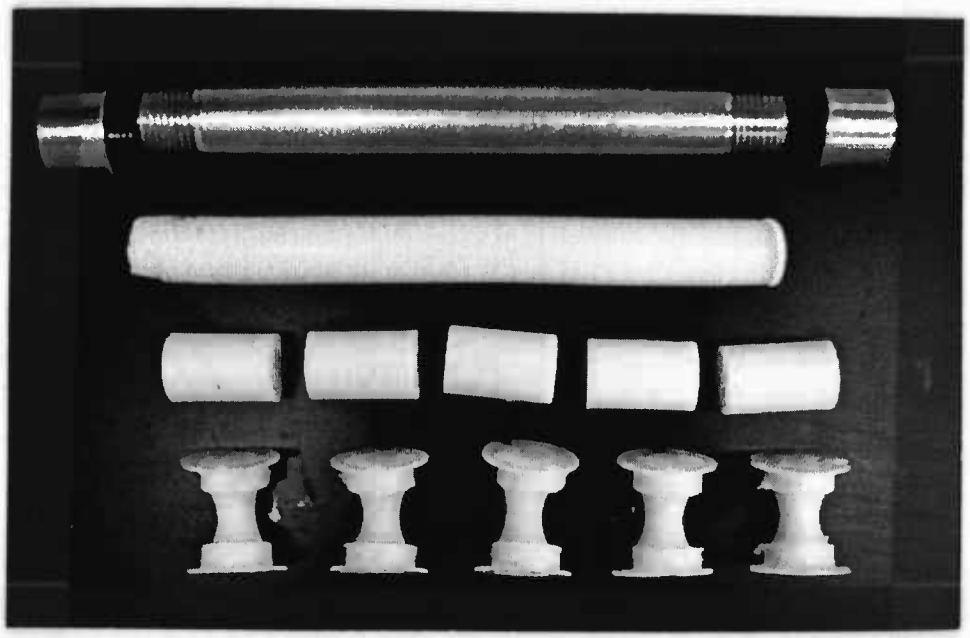

Fig. 4.1- Milled surimi gel samples with disks glued to ends. Ready for torsion testing.

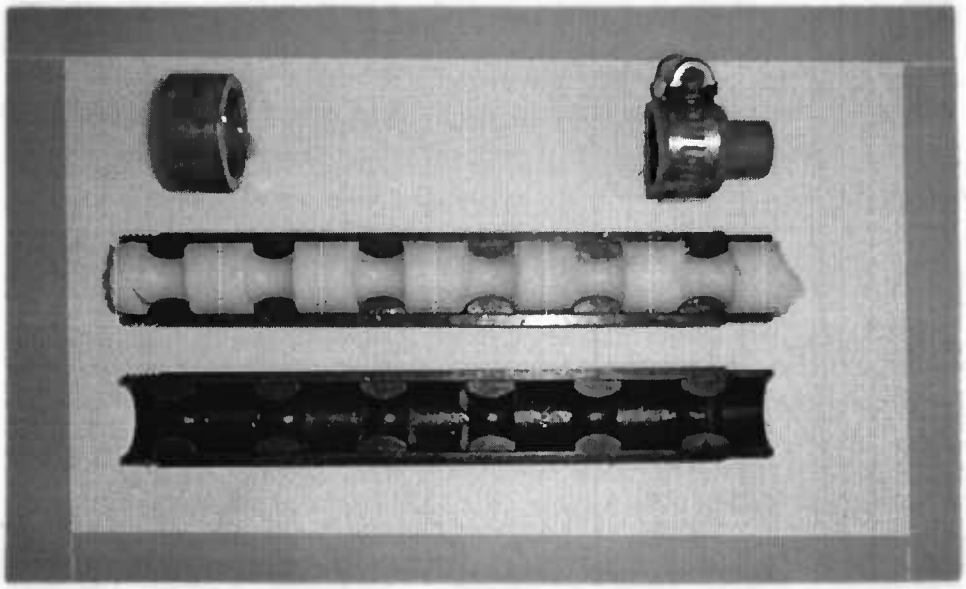

Fig. 4.2- Molded surimi gel samples before being cut into segments for torsion testing.

cutter (Lanier and others 1991) and both ends were glued to plastic disks with Super Glue (Dupro, LocTite Corp., Rocky hill, CT). Each segment was milled using a milling machine (Gel Consultants Inc., Model 91, Raleigh, NC) into an hourglass shape with a minimum 1.0 cm diameter in the center of the groove as measured using calipers.

The samples in the molded tube were cut into segments and glued to plastic disks in the same manner, except no milling was required. Each gel segment was then placed in a Hamann Torsion Gelometer (Gel Consultants Inc., Raleigh, NC). By twisting the samples, shear stress and shear strain were evaluated by a computer, which collected and calculated the data using equations developed by Hamann (1983). At least six samples per treatment were subjected to torsion measurement.

For the effect of diameter on shear stress and shear strain, gels were milled with a wide range (7.0-13.5 mm) of diameters and subjected to torsion testing.

For evaluation of individual variation in diameter measurement, ten samples were randomly selected and milled to arbitrarily chosen diameters. These samples were then subjected to ten panelists who then measured the diameter of the gels using calipers.

Statistical analysis

Analysis of variance (Statgraphics, version 3.1, Manugistics, Inc, Rockville, MD) was used for the torsion data to determine the significance between milled samples and molded samples, and using various cook times. Least significant difference (LSD) at $p<0.05$ was used to determine significant differences between mean values.

Results and discussion

Variance in diameter

Shear stress values were extremely affected by the accuracy of the 1.0 cm cross-sectional diameter and increased as diameter increased (Fig. 4.3). Stress values fluctuated between ~25 and ~40 kPa depending on the cross-section diameter, 0.90-1.10 cm. The difference in diameter by 0.1 cm resulted in 30-40% larger or smaller stress values. Shear stress (Y) can be calculated as a function of diameter (X) where $Y= 7.92X - 46.50$, with a regression coefficient of 0.91 (Fig. 4.3). Kim and Park (2000) also demonstrated similar trends as affected by various diameters. The effect of cross-section diameter on shear strain was not as significant as shear stress, but there was a trend of a reduction in strain values as diameter increased (Fig. 4.4). Shear strain (Y) can also be calculated as a function of diameter (X) where $Y= -0.0723X + 3.0426$, with a regression coefficient

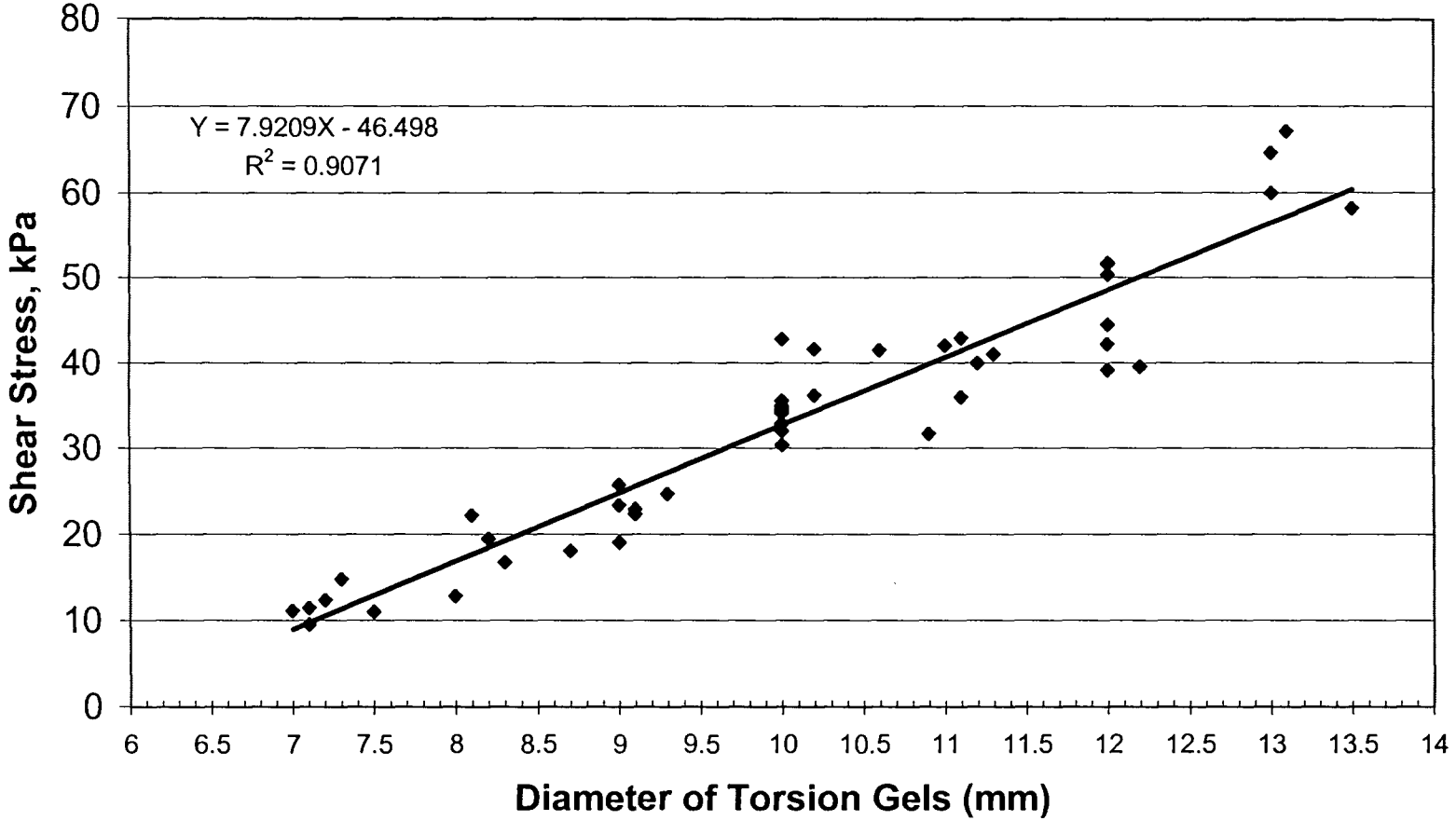

Fig. 4.3- Effects of diameter on shear stress using milled samples

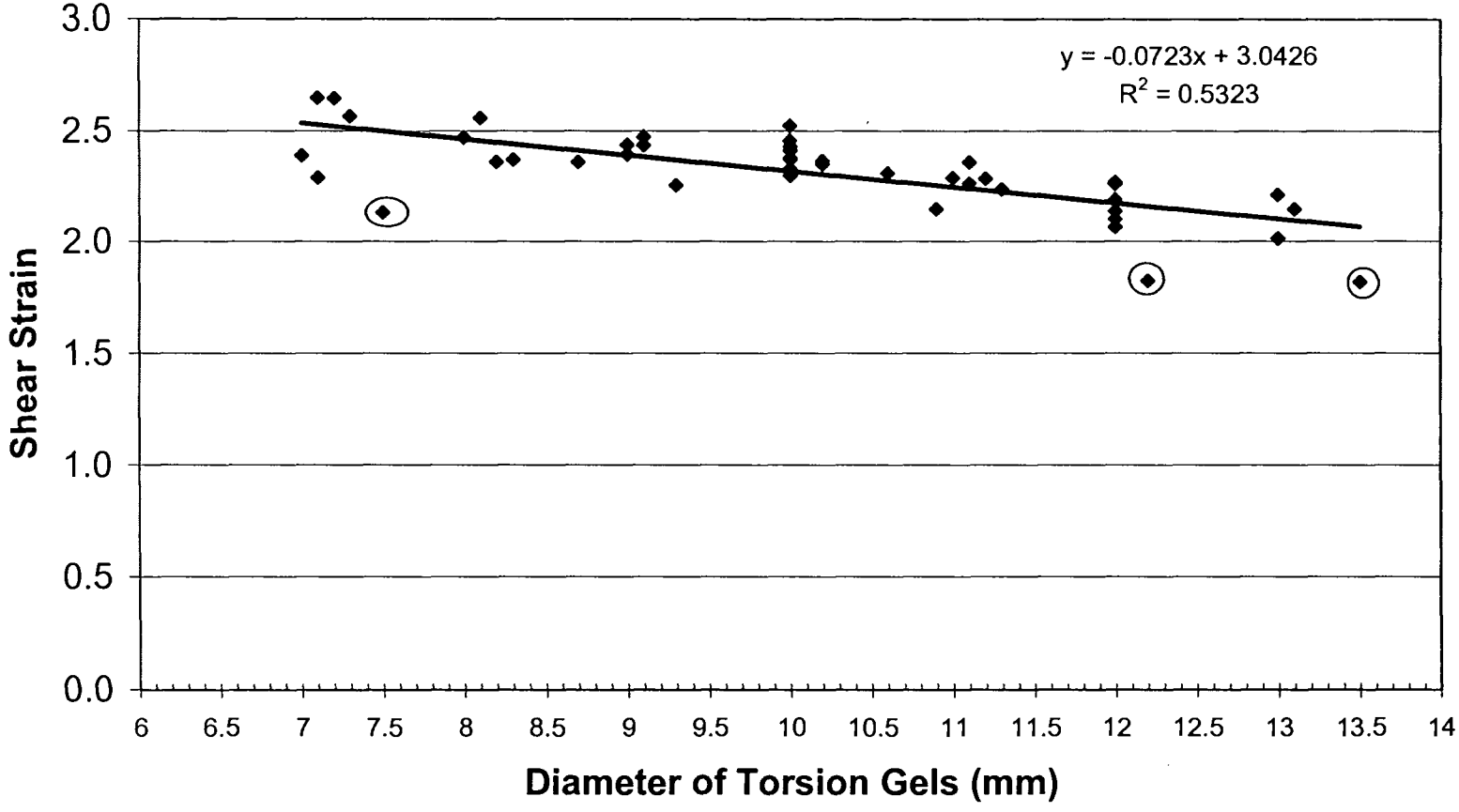

Fig. 4.4- Effects of diameter on shear strain using milled samples

of 0.53 (Fig. 4.4). This relatively low coefficient was thought to be due to three data points (circled in Fig. 4.4). When these points were removed the regression coefficient became 0.62. Our results indicate a cross-section diameter of the milled gel must be maintained at exactly 1.0 cm for accurate gel analysis when a torsion test is used.

Fig. 4.5 illustrates the individual variation between panelists in measuring the cross-sectional diameter of the milled gels. Using the same milled sample (i.e.: #1 in Fig. 4.5), ten panelists measured its diameter and answered with ten different measurements (1.43 the largest and 1.28 the smallest). These results demonstrate the difficulty of maintaining a precise diameter for torsion testing. In addition to the results of varying diameters on gel strength (Fig. 4.3 and 4.4), individual variation (human error) in measurement causes significant gel strength differences to be calculated from the same sample.

Milled vs. molded gel samples

Molded gels provide consistency in diameter by eliminating the introduction of human error in milling and measuring. Stress values between the milled and molded samples were relatively consistent within each sample group, but exhibited significant differences ($p < 0.05$) between the two sample types with the molded values being larger, as seen in Fig. 4.6. Strain values of the molded samples proved to be significantly lower

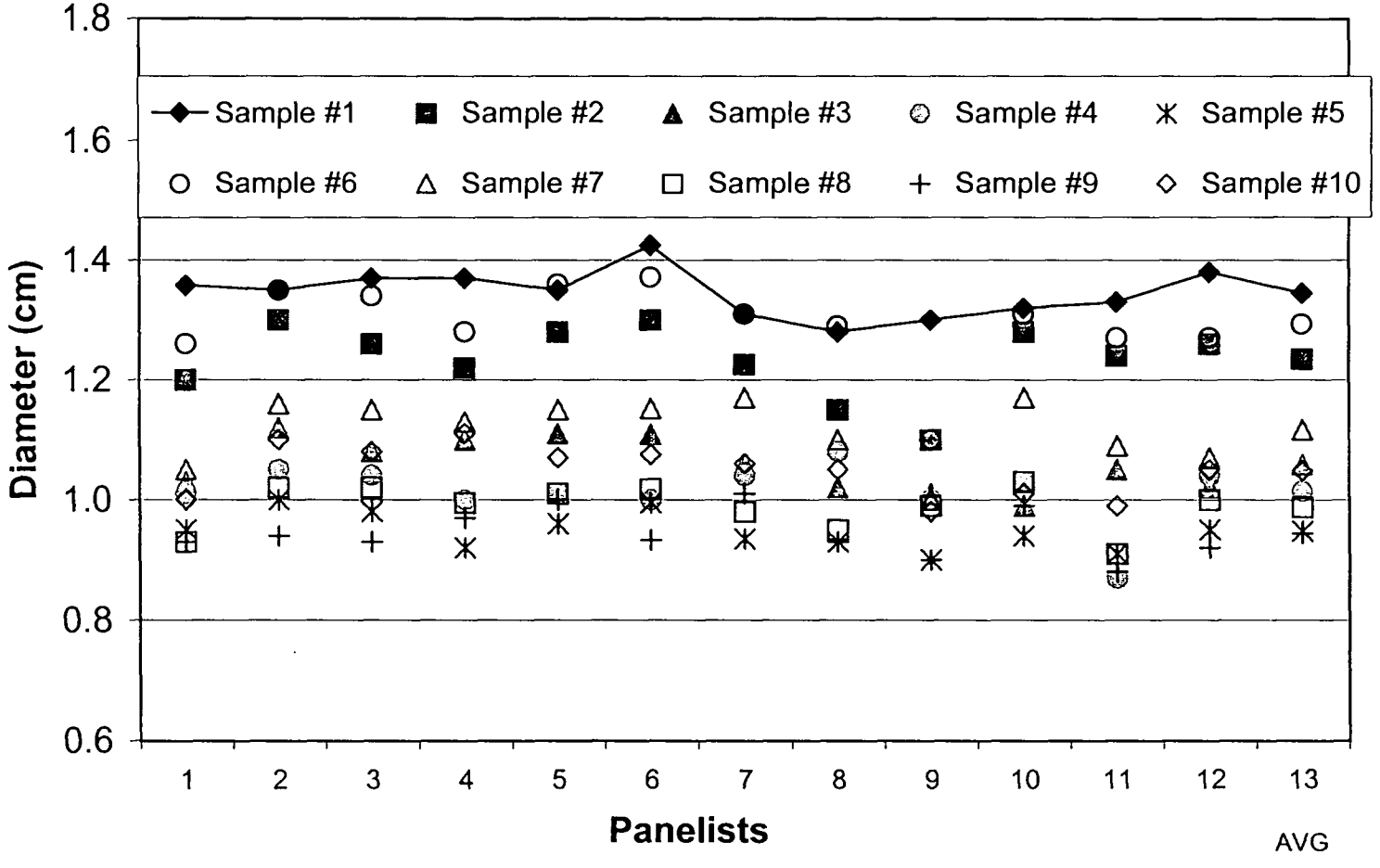

Fig. 4.5- Individual variation in measuring the cross-sectional diameter of milled surimi gels

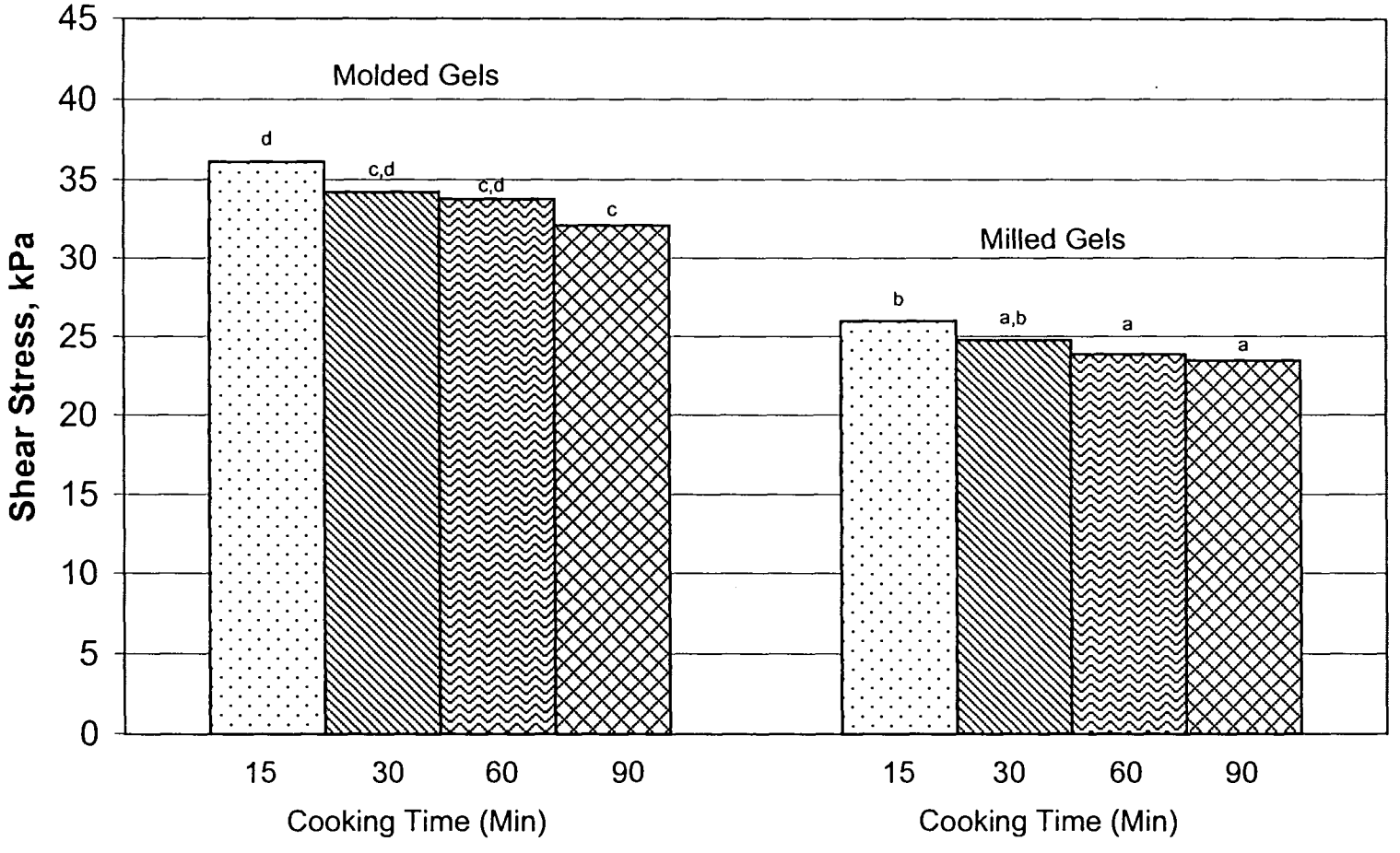

Fig. 4.6- Stress values comparing molded and milled samples with cook time variance. Different letters denote significant differences ($p<0.05$) between samples.

($p < 0.05$) than the milled samples (Fig. 4.7). Using the molded tubes, a new database of torsion values would need to be established to determine the quality trends of the different grades of surimi. With this information, product specifications could be recalculated based on rheological values measured using the molded tubes. Molded tubes could be used as a successful replacement for the stainless steel tubes because they can eliminate common errors in milling gels and measuring the diameter.

Skin formation

As cooking time increased, stress and strain decreased in both the molded and milled samples indicating possible textural disintegration. Stress values of the molded samples were significantly higher ($p<0.05$) than those of the milled samples (Fig. 4.6). Stress was significantly lower ($p<0.05$) between 15 min and 90 min cooking for both milled and molded samples (Fig 4.6). Strain values of the molded samples remained significantly lower ($p < 0.05$) than the milled samples for all of the cooking temperatures (Fig. 4.7), but no difference was found between cooking times. No increase in stress and strain, as cooking time was prolonged, indicated that there was no skin formation. No skin formation is probably due to the use of a lechitin-based spray that was used to coat the tubes prior to extruding the surimi paste. It has been reported in a previous study investigating the melting of a low fat mozzarella cheese where skin

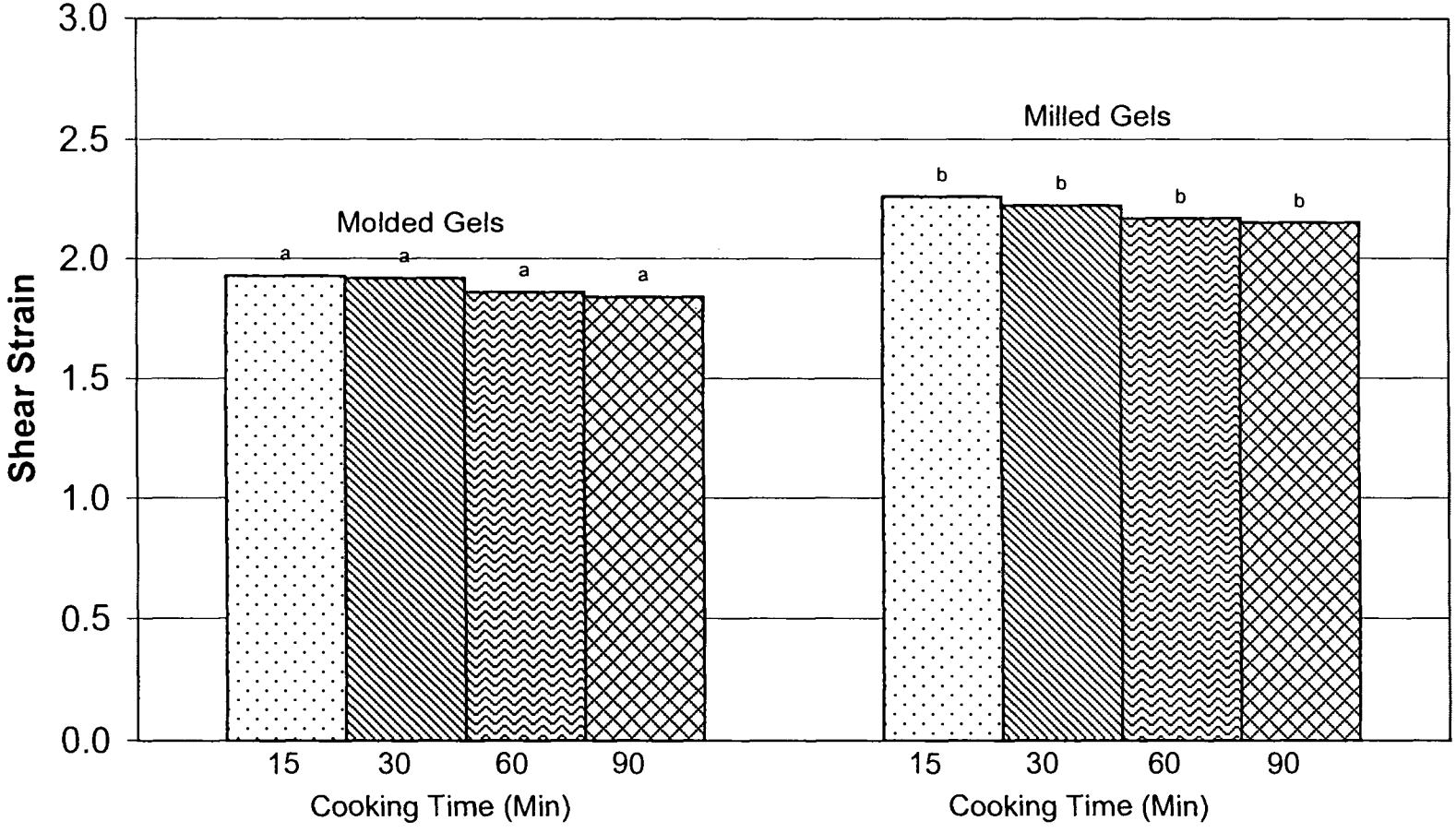

Fig. 4.7- Strain values comparing molded and milled samples with cook time variance. Different letters denote significant differences ($p<0.05$) between samples.

formation can be prevented by lightly coating a low fat mozzarella cheese surface with a hydrophobic material (i.e., Pam®) prior to baking it (Rudan and Barbano 1998). This hydrophobic surface coating prevents moisture loss from the surface and subsequent skin formation allowing a low fat mozzarella cheese to melt similarly to full fat mozzarella cheese (Rudan and Barbano 1998).

Conclusion

Using the dumb-bell shaped gels that are molded instead of milled can save time and maintain accurate diameter control, which is critical to maintaining the accuracy of the torsion test. Without milling, individual variation in measuring the same gel and any possible damage to microstructure can also be eliminated. Therefore, molded gels can result in more precise gel evaluation when torsion testing is used.

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Chapter 5

Conclusion

J. Hoffman

Freeze-dried Pacific whiting surimi stored in the freezer maintained the highest quality for the duration of the 9 month study. While freeze-drying greatly reduced the rate of denaturation the reactions could not be stopped all together and cold storage was still required to maintain higher quality. The surimi gels made from all storage parameters and freezing rates still produced commercially acceptable gels up to the 9th month of the study but further testing is required to determine the point that this quality is lost.

Stress values increased up to the 3rd month then decreased substantially as storage time extended. In general, strain values decreased over time. This is an indication that the higher concentrations of cryoprotectants (9%) used in the surimi samples maintained muscle protein functionality fairly well in the freezing process and subsequent frozen storage.

Proteolysis effects were adequately inhibited by the 1.5% Beef Plasma Protein (BPP) as no differences were exhibited in the Cook A and Cook B samples. This was further supported by the SDS-PAGE electrophoretic patterns, which didn't show any apparent damage to the Myosin Heavy Chain (MHC) between the two cooking parameters. However, degradation was noted from the SDS- PAGE gels, as a reduction of MHC was seen at 6 months and an increase in intensity of the lower molecular weight bands. This intensity was even greater at 9 months.

Plate frozen, slow frozen, and fast frozen samples acted essentially the same throughout the study. There was little variance between the three, even though the slow frozen samples were visibly different with visible ice crystals and a crystalline

appearance. This indicates that a freezing rate of 2 hours compared to that of 10 minutes and 24 hours does not affect the overall quality of shelf life of the product during the 9 months of the study. However, this requires further investigation and the samples would need to be evaluated after 1-2 years of storage.

Freeze-dried surimi seems to be an acceptable alternative to traditional surimi production. It would greatly reduce transportation costs and requires much less storage space in the powder form. It also lends greater ease in utilization.

For the evaluation of gelling ability, cooked gels are subjected to a torsion test. This can be a timely process when using samples that require milling prior to testing. Shear stress values were significantly affected by diameter accuracy, whereas the effect was not as significant for shear strain values. Individual variation in measurement was also greatly noted. Molded gels resulted in significantly lower strain values than milled samples, whereas stress values were significantly higher in molded gels. Using a lecithin-based spray before filling the molded and milled tubes appeared to eliminate skin formation.

In conclusion, using the dumb-bell shaped gels that are molded instead of milled can save time and maintain accuracy of the test. Without having to mill the samples, individual variation in measuring the gels can be eliminated. Therefore, molded gels can result in more precise gel evaluation when torsion testing is used.

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Appendix

Appendix

An Approach to Investigate the Effects of Gel Rolling

J. Hoffman and J.W. Park

Introduction

The elasticity of surimi gels is influenced by both the quality and quantity of salt soluble proteins (Kawashima and others 1970; Nishioka and others 1983, Park and others 1988). Hydrogen polar bonds in fish gels, in contrast to hydrophobic bonds, have been reported to destabilize at high temperatures but stabilize at low temperatures, (Nemethy 1968, Niwa and others 1983). Hydrogen bonds and hydrophobic bonds in the gelation of actomyosin or myosin have been studied extensively (Hamada 1992; Niwa 1975; Niwa and others 1982, 1986; Wicker and others 1989). Niwa (1975) and Niwa and others (1983,1986) have emphasized the importance of the hydrophobic bond in setting and gelation of fish actomyosin. Hydrophobic interaction occurs when non-polar molecules are introduced into the polar environment of water (Busk 1984).

During gelation, disruption of the protein conformational structure occurs, enabling exposure of the uncovered hydrophobic regions to the solvent (Lanier and others 1982). This increase in surface exposure of hydrophobic regions is likely involved in the intramolecular interactions of gel formation. Studies of covalent bonding in heat-induced protein gels have focused on disulfide (-SS-) linkages. Heat treatment can result in cleavage of existing –SS- bond structures or exposure of covered sulfhydryl

(–SH-) groups through unfolding of the protein. Such newly formed or exposed SH groups can form new intermolecular bonds, and affect the final gel characteristics (Kim 1987).

Study of the potential cross-linking of gels is very important to understand the nature of gelation. It has been observed in industry settings that if a gel segment is quickly rolled back and forth by hand (manipulating the gel network) prior to rheological testing, it will cause an increase in deformation values without significantly affecting the force values. If the rolled gels are allowed to relax for a period of time, the values decrease again and are close to the values of the unrolled gel values. The mechanism behind this phenomenon is unknown at this time.

The objectives of this study were 1) to evaluate the fracture force and deformation as affected by rolling to determine the extent to which the deformation will increase and then relax; 2) to determine if the surimi age influences the extent of deformation/relaxation.

Materials and methods

Frozen whiting surimi was obtained from Point Adams packing plant (Hammond, OR). Frozen Alaska pollock surimi was obtained from Trident Seafoods. Moisture content of the surimi was determined, in triplicate, with

a gravity oven (VWR Scientific, 1330GM) set at 105° C for 24 hours. Samples (~5.0 g) were spread into a pre-weighed aluminum dish which were then reweighed after drying to calculate the percentage of moisture. The pollock surimi gel formulations were adjusted to equal moisture contents of 78% and a 2% salt content. The Pacific whiting surimi moisture (75%) was not adjusted. The surimi was comminuted in a vacuum chopper (Model 5289, Stephan Machinery Corp., Columbus, OH). The samples were tempered (~-5° C), chopped into small cubes then mixed to –1.5° C. Salt was added, a vacuum was applied, then further mixed to a final temperature of 5° C. The paste was vacuum-packed in plastic bags to eliminate air bubbles before placing into a sausage stuffer (Model 14208, The Sausage Maker, Buffalo, NY) and extruded into plastic sausage casings (3 in diameter). The sausages were cooked for 40 min in a 90° C circulating water bath. After cooking, the samples were chilled in ice water for 30 minutes then the gels were stored overnight at 5° C.

Textural properties were evaluated as force and deformation. To minimize the effects of various gel temperatures at measurement, the gels were kept at room temperature for >2 hours before analysis. The gels were cut into 2.9 cm long segments. Samples were subjected to a penetration test using a Rheotex machine (Sun Rheo Tex, Type SD-305, Sun Scientific

Co., Ltd., Tokyo, Japan). The segments were divided into 3 sample groups. The first sample set, control, was punched immediately with no rolling. The second sample set was rolled then tested immediately. 'Rolled' is defined as a gel segment that was rolled vigorously back and forth, on the bench-top, by hand, 8-10 times. The third sample set was rolled then allowed to relax for 1, 2, 3, 4, and 5 hours before being tested. The segments from the 'relaxed' set were stored in a sealed plastic bag to avoid moisture loss during relaxing time, which would alter the results.

Results and discussion

Deformation values were greatly affected by the rolling (Table AP.1). The deformation values of the 2 month old Pacific whiting increased 12% upon rolling and after 2 hours of rest had decreased back down to 3% above the values of the control. The 3 month old pollock surimi increased 19% upon rolling and the relaxed back to 10% above that of the control sample. The 7 month old pollock surimi increased 22% when rolled and then after relaxation decreased to 11% above that of the control. Force values after rolling and after relaxation did not change significantly in both the Pacific whiting and Alaska pollock surimi gels.

Gel Rolling Data

Sample Species	Sample Age	Control (Un-Rolled)		Rolled (Tested Immediately)		Rolled Relaxed then Tested		Relaxation Time
		Force	Deformation	Force	Deformation	Force	Deformation	
PW	2 Mos	650	1.37	641	1.54	649	1.41	2 Hrs
PW	2 Mos	639	1.36	626	1.56	623	1.39	2 Hrs
POLL	3 Mos	276	1.26	239	1.55	271	1.39	2 Hrs
						254	1.38	3 Hrs
						246	1.43	4 Hrs
						277	1.38	5 Hrs
POLL	7 Mos	286	1.16	244	1.44	261	1.33	1 Hr
						254	1.29	1.5 Hrs
						256	1.29	2 Hrs
						257	1.22	2.5 Hrs
						285	1.23	3 Hrs
						259	1.23	3.5 Hrs
POLL	7 Mos	288	1.13	289	1.51	288	1.33	2 Hrs
						282	1.25	3 Hrs
						271	1.3	4 Hrs

PW= Pacific whiting 75% moisture

POLL= Atlantic pollock 78% moisture

Table AP.1- Gel rolling data. Each values indicates an average of 5-10 measurements.

It appears that the freshness of the surimi plays an important role in the overall elasticity of the gel. The fresher surimi relaxed to a greater extent and as the surimi age increased elasticity decreased. Further research is necessary to confirm this theory.

Future research

To further examine the mechanism behind this phenomenon it is suggested that the parameters of the study be expanded. It is advised that in addition to evaluating a control sample (only moisture is adjusted) other gel samples should be made using different compounds as additives. The added compounds may elucidate the types of cross-linking in the gels. The compounds of choice would be .25M guanidine hydrochloride (G-HCl) to break hydrogen bonds, 5mM sodium-dodecyl sulfate (SDS) to break hydrophobic interactions, and 10mM ß-mercaptoethanol (ß-ME) to break disulfide bonds (Park and others, 1994).

It is also suggested that in addition to the 'punch test', a stress relaxation test and/or creep test should be performed. These other important tests used to study viscoelastic response. A stress relaxation test consists of suddenly applying a constant deformation (strain) and measuring the decay of the stress as a function of time while the strain is

held constant (Rosen 1993). The recorded relaxation curve is in the form of a force versus time, force versus stress, or modulus decay versus time relationship (Kim and Park 2000). An ideal elastic body does not relax at all and an ideal viscous body cannot maintain any stress. In viscoelastic materials, such as surimi gels, the effect is intermediate and the stress relaxes, but at a definite, not necessarily constant, rate (Kim and Park 2000).

The creep test is typically performed under dead weights, which are resting in compression. When the viscoelastic gel is subjected to an instantaneous constant load, the resulting strain is followed as a function of time (Rosen 1993) The increase in strain as a function of time is called creep or creep recovery. The recorded creep curve is in the form of a deformation vs. time, or strain or compliance vs. time relationship (Kim and Park 2000). In almost every creep test, there is an immediate deformation followed by deformation at a progressively decreasing rate, which is called retard deformation. In some instances, the deformation reaches an equilibrium state and the deformation becomes constant indicating that the material is solid in nature (Kim and Park 2000).

Anticipated results

It is thought that by adding the above compounds it may be possible to determine what type of interaction (bond) is the major influence in changes of deformation upon rolling and relaxing. Based on the findings of Park and others (1994) it seems that the hydrogen and hydrophobic bonds would cause greater increase in deformation values as the gel network is manipulated (rolled). However, this requires further investigation.

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