AN ABSTRACT OF THE THESIS OF

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Cryoprotection in stabilized mince from Pacific whiting (*Merluccius productus*) was investigated by monitoring changes in physical and chemical properties during 32 weeks of frozen storage. The effects of 4 different cryoprotectants were evaluated by torsion test, color analysis, extractability of salt soluble proteins, and formation of dimethylamine (DMA) and 2-thiobarbituric acid (TBA). The quality of the stabilized mince was significantly higher than the control (mince without cryoprotectants) when compared by shear strain, salt soluble proteins, and DMA. The results show that the functionality of the proteins in the mince can be protected by using cryoprotectants with Polydextrose® being the most effective of the 4 tested. The effect of food-grade protease inhibitors on the gel-forming characteristics of Pacific whiting mince was also investigated. Four levels (1, 2, 3, and 4%) of different protease inhibitors (beef plasma protein, whey protein concentrate, egg white liquid, and egg white powder) were added to the stabilized mince before heating and effects on texture and color were evaluated. Shear strain was significantly increased by increasing the level of

inhibitors. Beef plasma protein was most effective and presented significantly higher strain than the other inhibitors tested. Due to higher concentration of proteolytic enzymes in the mince, an increased amount of protease inhibitors is needed compared to surimi to prevent proteolysis during heating.

Physical and Chemical Changes in Stabilized Mince from Pacific Whiting During Frozen Storage

by

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

Edda Magnusdottir, Author

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PHYSICAL AND CHEMICAL CHANGES IN STABILIZED MINCE FROM PACIFIC WHITING DURING FROZEN STORAGE

I. INTRODUCTION

Pacific whiting (*Merluccius productus*) is the most abundant commercially harvested groundfish found off the Pacific coast of the contiguous United States. The annual catch has been ~ 200,000 metric tons (mt) in the US over the last decade with approximately 250,00 mt 1994 (OCZMA, 1994). Shoreside processing of Pacific whiting in Oregon was ~ 65,000 mt in 1994 (Talley, 1995). This represents more than a ten-fold increase since 1991 when joint venture operations with foreign processing vessels were excluded from the quota (Radtke, 1995). These numbers illustrate the abundance of this resource at present and the potential for utilizing it in the most economical way. Most of the harvest of Pacific whiting in Oregon is processed into surimi or frozen fillets. Surimi, which is a washed stabilized mince, is an intermediate product that is used as a main ingredient in crab analogs and other related products. The seasonal availability of Pacific whiting (April-October) limits the shore-based plant's operation. A solution to this problem was proposed by Simpson et al., 1994. They produced quality surimi from stabilized mince (6% w/w sucrose and 0.2% w/w polyphosphates) that had been stored at -20°C for 6 mo (Simpson et al., 1994b). No significant differences were found between the surimi made from stabilized mince stored at -50°C and -20°C and surimi made from fresh mince while unstabilized mince and headed and gutted fish produced surimi of low-grade quality (Simpson et al., 1994a). When fish mince is washed in surimi production, the water soluble proteins

are removed and lost in the waste water. The sarcoplasmic proteins, composed of enzymes and myoglobin, constitute ~ 20% of the total muscle proteins (Lanier, 1993).

The percentage of connective tissue proteins, largely composed of collagen, is very low, 3%, in fish muscle compared to 16-28% in beef muscle (Lanier, 1993). The high percentage of myofibrillar protein in fish contributes to the excellent gel-forming ability of fish proteins compared to meat proteins. The connective tissue in hake muscle *(Merluccius merluccius L.)* measured by Montero et al. (1990) was 1.7%.

The yield in surimi production from the whole fish is ~ 15-20%, whereas, in mince the yield is close to 32%, or almost twice that of surimi (Toyoda et al., 1992). The difference is due mostly to removal of sarcoplasmic proteins by the washing steps in surimi production, but some myofibrillar proteins are also lost as well as fat and minerals (Lin et al., 1995). Frozen storage can result in protein denaturation and loss of protein functionality in fish mince. Research has shown that the addition of cryoprotectants, such as sorbitol and sucrose can limit the degree of denaturation in washed fish mince such as surimi (MacDonald et al., 1990; Matsumoto and Noguchi, 1992) and that surimi can be made from stabilized mince (Simpson et al., 1994)

The overall objective of this research was to study the texture of the mince itself (without washing) and determine whether cryoprotectants could prevent the loss of functional properties in Pacific whiting mince during frozen storage . The specific objectives of this research include: (1) to estimate the cryoprotective effects of several ingredients, and (2) to estimate the effects of different inhibitors on proteolysis in Pacific whiting mince.

The ultimate goal is to aid the seafood industry in using unwashed, stabilized fish mince in seafood products. Stabilization of protein functionality during frozen storage would render mince readily available for product development. Advantages would be to increase the yield of fish proteins, lower processing costs (no washing steps), and decrease fish waste compared to present surimi production.

Literature Review

Fish Mince

Fish mince is skinned and deboned flesh of fish that are processed from headed and gutted fish, fillets, V-cuts or frames. In automated fish deboners/mincers, fish are fed into a hopper and transferred by a rubber conveyor belt to a drum perforated with 4 to 7 mm openings. The flesh is squeezed by the belt through holes of the drum and separated from skin, scales and bones which remain on the outside of the drum (Toyoda et al., 1992; Hsieh et al., 1992). The higher the belt pressure on the deboner the higher the yield of minced fish forced through the holes. However, elevated belt pressure can lead to lower frozen storage stability due to increased cellular rupture in the mince, affecting the gelling properties, water holding capacity and lipid oxidation (Hsieh et al., 1992). Higher fat content in mince than surimi is more likely to cause oxidation with rancid taste and off-flavor.

Nutritional and Economic Considerations

Unwashed fish mince has the nutritional advantage over surimi as it contains water-soluble vitamins, minerals and fats which remain in the mince. High levels of calcium have been reported in fish mince compared with fish fillets due to small bone fragments in the mince as a result of the mechanical deboning (Lin, 1992; Hsieh et al., 1992). A number of other minerals have also been measured in Pacific whiting (Gordon and Roberts, 1977). Fish oils, rich in unsaturated fatty acids, have shown potential in the prevention of heart disease, cancer, and other diseases. Although low in fat (~ 1-2%), 70% of the lipid in Pacific whiting are unsaturated fatty acids (Bonnet et al., 1974). Yoon et al. (1991) suggested that sarcoplasmic proteins retard freeze-induced contraction of the myofibrillar proteins during frozen storage. However, sarcoplasmic proteins affect texture by interfering with the three-dimensional network of the myofibrillar proteins during heating (Okada, 1964). The resulting lower gel strength could limit the variety of products made from mince. Research on how to protect the functional properties of the fish proteins during frozen storage could lead to economic benefits because of lower processing costs of the mince and higher yield of the fish proteins. This is especially important now as many of the conventional fish stocks resources in the world continue to decline and there is a shift toward higher utilization of what is harvested.

Textural Properties

The myofibrillar proteins, myosin and actin, are responsible for the textural properties of fish mince. Protein gels are formed by intermolecular interactions resulting in a three-dimensional network of protein fibers that promotes structural rigidity (Fennema 1976). 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). Parameters as temperature, pH, salt, and protein concentration alter the degree of cross-binding by changing the quarternary structure of the protein or polymer molecules and the number of connections between them that determine the elastic nature of the gel structure (Paul and Palmer, 1972). The myofibrillar protein form an irreversible gel that is initiated by heat. The gel exhibits a high water-binding capacity and very strong elastic qualities depending on the content of the myofibrillar protein (Ishioroshi et al., 1979). Salt added during comminution of fish mince increases water-binding capacity of myofibrillar proteins by increasing the negative charges to attract water molecules and, thus, enhancing water binding (Chung and Lee, 1991). During grinding the mince with salt, the cell walls are disrupted and the content of the cell is no longer separated from the extracellular constituents. During heat-setting of surimi (washed mince), there are three temperatures where major textural transitions take place: setting phenomena at 40°C; a weakening in gel structure at 60°C, often attributed to proteases; and intermolecular and intramolecular reactions which take place to form a heat-set gel beginning at 80°C. Above 105°C, there is a general decline in gel strength (Pigott, 1986). 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). Salt added during comminution of fish mince increases the water-binding capacity of myofibrillar proteins by increasing the negative charges to attract water molecules and, thus, enhancing water binding (Chung and Lee, 1991). Phosphates with their anionic groups 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 majority of water in muscle is in the form of free water (Hamm, 1960). Measurements of free water released under application of force have been used as indicators of the water-binding properties of proteins and are commonly referred to as water-holding capacity (Regenstein et al., 1979).

Denaturation during Frozen Storage

Most researchers agree that denaturation of fish myofibrillar proteins during frozen storage is due to formation of protein aggregates, side to side, from myosin monomers as proposed by Connell (1959) and Buttkus (1970). The exact mechanism is still not understood, but evidence favors the crosslinking of myosin chains via covalent bonding (Buttkus, 1970), as well as via secondary forces such as hydrogen bonding or hydrophobic interactions (Shenouda, 1980). Moisture plays a major role in the denaturation of myofibrillar proteins during frozen storage. The formation of ice crystals, dehydration of proteins and increase in salt concentration promote protein aggregation and loss of solubility (Shenouda, 1980). Sarcoplasmic proteins undergo a decrease in activity as do the myofibrillar proteins during frozen storage, but solubility is unchanged (Matsumoto and Noguchi, 1992).

Salt Extractable Proteins

Rapid texture deterioration occurs during frozen storage. Fish mince has been found to deteriorate in frozen storage at about twice the rate of that of frozen fillets (Bligh and Regier, 1977). Changes in texture are connected to protein changes, especially of the myofibrillar proteins, myosin and actin, that are responsible for the two-step process of gel formation. The proteins are partially unfolded in the first step by addition of salt and/or heating and then become more reactive. The main function of the salt is to help 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 et al., 1979). A decrease in the amount of salt soluble actomyosin is regarded as a primary criterion of freeze denaturation and/or aggregation (Matsumoto, 1980).

Dimethylamine - (DMA)

Trimethylamine oxide (TMAO) is found in large amounts in gadoid species (i.e., cod, haddock, hake, pollock, cusk, whiting) and causes several storage problems (Hebard et al., 1982). TMAO in frozen fish is degraded into dimethylamine (DMA) and formaldehyde (FA) in equal molar quantities by TMAO demethylase. FA is believed to cause the toughness of fish flesh through the methylene crosslinking of muscle proteins (Regenstein et al., 1982). Such interactions lead to a dry and spongy

texture of the fish muscle. Textural changes are more apparent than the change in taste and odor during frozen storage. DMA has been used as a spoilage indicator of gadoid fish during frozen storage. Ragnarsson and Regenstein (1989) investigated crosslinking in both gadoid and non-gadoid species. They observed cross-linked polymers formed at -7°C in gadoid species; whiting (*Merlangius merlangus*) after 3 days, and in cod (*Gadus morhua*) after 20 days, but not in the non-gadoid species tested. TMAO is reduced to trimethylamine (TMA) by bacteria in fresh fish at low temperature. The fishy odor associated with TMA is produced when TMA reacts with fat in the muscle of fish (Hebard et al., 1982). TMA has been used as an index of microbial spoilage in unfrozen fish or of pre-freezing quality. TMAO demethylase is found in kidneys, blood, and dark muscle. The enzyme is resistant to freezing but demethylation does not occur at temperatures below -30°C (Hsieh et al., 1992).

Lipid Oxidation

Lipid oxidation in fish is an important factor related to quality loss in refrigerated and frozen storage (Flick et al., 1992), particularly in fatty fish. According to The International Institute of Refrigeration (1991), the frozen storage life of lean fish (i.e. cod, haddock) is 8 mo at -18°, 18 mo at -25°C, and 24 mo at -30°C while fatty fish, such as, sardines and salmon have a storage life of 4, 8, and 12 mo, respectively. Air enhances oxidation and the mincing process involves incorporation of air into the mince and distribution of hematin compounds (myoglobin, hemoglobin, and cytochromes) both of which catalyze lipid oxidation (Hsieh et al., 1992). Oxidation of the lipids results in rancidity of the mince and an off-flavor. Oxidative

rancidity has been reported in silver hake (*Merluccius bilinearis*) fillets and mince stored at -10°C for 8 weeks with the rate in mince twice the fillet rate (Hiltz et al., 1974). Oxidation can be controlled by low temperature, dark storage, use of antioxidants and elimination of oxygen by vacuum packaging. Flick et al. (1992) reported vacuum packaging as a more effective method of reducing oxidation than the addition of antioxidants. Another important factor in increasing the shelf life of mince is to avoid contact of flesh with iron parts of the deboner to prevent accelerated oxidation of lipids from metal ion catalysts.

Color

The mincing process often introduces pieces from viscera, blood, and skin fragments into the muscle tissue resulting in discoloration upon oxidation (Hsieh et al., 1992). The freshness of fish and thorough separation of blood and viscera before mince processing are the main factors necessary to obtain a lighter and more uniform color (Hsieh et al., 1992).

Cryoprotection/Stabilization

In the 1960's, Nishiya and his co-workers in Japan discovered the role of cryoprotectants in preserving the functionality of fish proteins during frozen storage (Nishiya et al., 1961). The key in Nishiya's patent was the prevention of denaturation of the muscle actomyosins during frozen storage by using 10% sucrose and 0.3%

polyphosphates. Later, half of the sucrose was replaced by sorbitol to reduce the sweet taste of surimi (Okada, 1992).

Cryoprotectants

A cryoprotectant is a compound that prevents loss of the functionality of a protein during frozen storage. Cryoprotection occurs only in comminuted food systems by intimate association of the cryoprotectant and the protein molecule. Many compounds other than sucrose and sorbitol have been tested for cryoprotective effects. Noguchi et al. (1976) discovered that disaccharides (sucrose, lactose) and hexoses (glucose, fructose) gave better cryoprotection than other carbohydrates studied. Based on the results of Noguchi's and other workers research on the cryoprotective effects of several compounds, the following similarities of chemical structure were assessed for cryoprotectants:

1. The molecule must have one of the essential groups;
   -COOH, -OH or $-OPO_3H_2$ and more than one of the supplementary groups:
   -COOH, -OH, -SH, $-NH_2$, $-SO_3H$, and/or $-OPO_3H_2$.
2. The location of the essential and supplementary groups on the molecule must be oriented in a particular way.
3. The molecule must be relatively small.

A mixture of 8% w/w sucrose and sorbitol and 3% w/w polyphosphates is still the industrial standard for cryoprotection in surimi. However, many other cryoprotective compounds have been discovered which can be used with equal

efficiency, and have benefits such as, reduced sweetness, lower price, and reduced caloric values.

Noguchi et al. (1976) found a marked synergistic effect in reduction of freezing damage in proteins with the addition of amino acids and carboxylic acids to sorbitol or glucose. Most effective were Na-aspartate and Na-glutamate of the amino acids and Na-salts of citrate, malate, glycolate and gluconate of the carboxylic acids. Rodger et al., (1979) compared lactose, monosodium glutamate and sodium citrate, at a 3% w/w level, for a cryoprotective effect on fish mince from cod stored on ice. Technical qualities, such as, the protein solubility of unwashed and washed mince were similar. Lactose gave better results than monosodium glutamate and sodium citrate, even though it did not show more than a marginal effect over the control at the 3% level. Dry whey, containing 50-70% lactose, has been investigated for cryoprotective effect by several workers. Madsen (1984) discovered an improvement in texture and color of minced blue whiting using a 10% skim milk powder (36.5% protein, 50.5% lactose, 1.0% fat, 8.0% ash). The mince was drum-frozen, granulated and extruded into fish sticks. The fish sticks had a slight sweet off-flavor. Zarzycki and Świniarska (1993) reported the cryoprotective effect of dry whey on minced cod and improvement of taste and color. Dondero et al. (1994) analyzed the gel-forming ability of surimi from jack mackerel during frozen storage at -18°C for 5 mo. They found that dry whey, 8% w/w, plus 0.2% polyphosphates protected the functionality of fish protein equally as well as the commercial mixture of 8% sucrose-sorbitol (1:1). Dry whey improved the sensory characteristics of the gel after 155 days at -18°C. DaPonte et al. (1985)

worked on stabilization of minced whiting (*Merlangius merlangus*) by hydrocolloids (locus bean gum, xanthan, iota carrageenan) without success.

Carpenter et al. (1986) found that disaccharides, trehalose, and maltose (made of glucose units) were very effective as cryoprotectants for the sarcoplasmic enzyme, phosphofructokinase, which is extremely labile. The nucleotides, ATP, ADP, and IMP, exhibited a protective effect on fish actomyosin stored at -20°C while the nucleotide catabolites, inosine, and hypoxanthine, destabilized these proteins (Jiang et al., 1987). Carpenter and Crowe (1988) investigated the cryoprotective effects of 28 different compounds on the enzyme lactate dehydrogenase, (a sarcoplasmic protein) and found them all (e.g., sugars, polyols, amino acids, methylamine, and inorganic salts), except for NaCl, able to protect the enzyme from damage during freeze-thawing. Polydextrose®, a branched polymer of glucose and sorbitol units, with 0.5% polyphosphates, provides good cryoprotection of pollock surimi equivalent to sucrose/sorbitol mixture and phosphates (Park and Lanier, 1987). Polydextrose® adds no color, flavor (including sweetness), or odor which makes it advantageous as a cryoprotective agent in food products (Park et al, 1993). Polydextrose® has been approved by the FDA for use in reduced or low calorie foods (Sych et al., 1990), but it has not yet been approved for muscle food (Park, 1994). Park et al. (1988) assayed sucrose/sorbitol, Polydextrose®, and a 10 D.E. maltodextrin, Maltrin® and/or phosphates on pollock surimi. The gel-forming abilities were better protected by sucrose/sorbitol or Polydextrose® than by maltodextrin. Sodium tripolyphosphate (STP) at pH 9.7 was superior to neutralized pyrophosphate (NPP; sodium acid

pyrophosphate and tetrasodium pyrophosphate, pH 7.0) and a mixture of STP/NPP (1:1).

MacDonald et al. (1990) found 12% sucrose added to minced New Zealand hoki sufficient to reduce DMA levels 60% compared to unstabilized mince stored at -20°C for 6 mo. Simpson et al. (1994b) made good quality surimi from stabilized Pacific whiting mince (6% sucrose, 0.2% polyphosphates) after 6 mo frozen storage. Lin et al. (1993) found that the gel of minced squawfish stabilized with sucrose/sorbitol had a high textural quality after 3 mo of frozen storage. As additives, the polyols lactitol (derived from the catalytic hydrogenation of lactose) and Palatinit® (produced from sucrose) present several attractive properties including reduced caloric value of approximately 50%, and a relative sweetness of 0.40 and 0.50, respectively, compared to the sweetness of sucrose as 1.0. Sych et al (1990) compared the cryoprotective effect of lactitol dihydrate, Polydextrose®, and Palatinit® on cod surimi to sucrose/sorbitol during frozen storage. Palatinit® (isomalt), lactitol, and Polydextrose® stabilize surimi proteins equally well as the sucrose/sorbitol mixture. Sych et al. (1991a and b) reported good cryoprotective effect of lactitol dihydrate (optimal 5.7-6.4%) and 0.5% polyphosphates on cod surimi. Maltrin® M-100 and M-250 (dry glucose syrups) have been suggested as good cryoprotectants with additional benefits of lower sweetness and lower price. Maltrin® M-100 with average D.E. (Dextrose Equivalent) of 10 and M-250 with D.E. average of 25 have low to medium effect in reducing sugar level, a low to moderate browning tendency, and a low to moderate sweetness (Anderson, 1993). MacDonald and Lanier (1994) discovered the cryoprotective effect of sodium lactate in an extract of tilapia actomyosin during

freeze-thawing, and found an optimum concentration of 6.0% on a percentage basis more effective than sucrose. Sodium lactate is GRAS (generally recognized as safe) for use according to good manufacturing practices. Lanier et al. (1995) reported the use of CRYODEX®, a dextran, as a cryoprotectant in surimi with properties equal to or greater than conventional sucrose/sorbitol mixtures.

The Mechanism of Cryoprotection

The mechanism of cryoprotection is not yet fully understood. Sugars and polyols stabilize proteins through their interaction with the surrounding water (MacDonald, 1992; Park, 1994). Their spatial structure, i.e. the configuration of hydroxyl groups in various sterioisomers is thought to account for their cryoprotective effect (Noguchi et al., 1976). Sucrose and sorbitol stabilize proteins both against freezing and heat denaturation (Park and Lanier, 1987, 1990). Conversely, NaCl is used to lower the denaturation temperature of proteins, and it promotes freezing denaturation of myofibrillar proteins (Park et al., 1987). Carpenter and Crowe (1988) proposed that the cryoprotection of proteins in solution was accounted for by the exclusion of the solutes from the protein surface. The only mechanism common to a wide variety of compounds (sugars, polyols, amino acids, methylamine, and inorganic salts), all with cryoprotective effect on proteins, is the exclusion of the solute (cryoprotectant) from the surface of the protein. Protein denaturants, such as, urea and guanidine HCl act by binding to the protein surface (Crowe et al., 1990). Carpenter and Crowe (1988) concluded that it does not seem likely that stabilization of proteins during freezing involves direct interaction with the solute.

It is generally accepted that cryoprotectants cause increased hydration of proteins, thus, preventing the molecules on the surface of the proteins from interacting together and aggregating. The reason for this phenomenon is described by thermodynamics (Arakawa and Timasheff, 1982). The native state of the proteins in sugar solutions is thermodynamically more favorable than in the denaturated state. The enthalpy is lower in the native state (recognized by differential scanning calorimetry technique).

According to Back et al. (1979), hydrophobic interactions are more stable in sucrose and glycerol solutions than in pure water. They recommended this as the mechanism by which sugar and polyols, in general, stabilize proteins against denaturation. Another mechanism for high molecular weight cryoprotectants has been postulated. High molecular weight polymers form glasses (amorphous structure) at higher temperatures than low molecular weight compounds (MacDonald, 1992). By increasing the concentration of the cryoprotective polymers in solution, the Tg (glass transition temperature) occurs at temperatures higher than the normal freezing point. The water is immobilized, formation of ice crystals is shut down and the glassy state of the solution immobilizes the proteins and keeps them in their native state (Levine and Slade, 1988).

MacDonald (1992) differentiates between "cryoprotection" with low molecular weight sugars and polyols which thermodynamically favor the maintenance of the native proteins state and "cryostabilization" in which primarily high molecular weight polymers effectively raise the glass transition temperature and assure a less reactive glass state in the system.

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II. PHYSICAL AND CHEMICAL CHANGES IN STABILIZED MINCE FROM PACIFIC WHITING DURING FROZEN STORAGE

Abstract

Cryoprotection in stabilized Pacific whiting mince was investigated during 32 weeks of frozen storage. Four different formulas of cryoprotectants were used: sucrose/sorbitol; sucrose/M-250 (corn syrup solid); Polydextrose®; and dry whey, added as 8% w/w. Quality loss in both samples and control (mince without cryoprotectants) was estimated after 1 day of frozen storage (t_0), and after 1, 4, 8, 16, and 32 weeks of frozen storage. Protein denaturation was evaluated by torsion, extractability of salt soluble proteins and actomyosin, and the formation of DMA. Changes in lipid oxidation and color were also evaluated throughout storage. The cryoprotectants improved the functionality of the proteins as recognized by significantly higher strain in the stabilized mince at each time interval tested, except at t_0, and after 1 week. A higher percentage of salt soluble proteins, especially actomyosin, was extracted from stabilized mince and significantly less formation of DMA were observed after 16 weeks of frozen storage. Lipid oxidation remained low, with no difference between samples and the control after 32 weeks of frozen storage. Whiteness, after 32 weeks, was higher in the sample with Sucrose/M-250 than the others. Polydextrose® gave ~ 20% higher strain than the other cryoprotectants and 55% higher than the control after 32 weeks of frozen storage, indicating considerable cryoprotection of the myofibrillar proteins.

Introduction

A number of investigations have focused on the stabilization of proteins in surimi, a washed refined mince, during frozen storage (MacDonald and Lanier, 1991; Park, 1994b). Fewer research efforts have been made in the area of protein stabilization of raw mince itself. Simpson et al. (1994b) investigated cryoprotection of Pacific whiting mince and found that 6% w/w sucrose was sufficient to stabilize the myofibrillar proteins in mince allowing researchers to produce high-grade surimi after 6 mo frozen storage. The question remained, however, about cryostabilization and functional properties of the mince itself, a complex mixture of myofibrillar and sarcoplasmic proteins, lipids, vitamins and other constituents many of which are removed in surimi processing. Sarcoplasmic proteins do not form gels with the same gel strength as myofibrillar proteins. The sarcoplasmic proteins, removed by washing in surimi production, are known to interfere with the three-dimensional network formed by heating myofibrillar proteins (Yoon et al., 1991). The textural properties reflect the characteristics of a network structure formed through gelation by interactions of protein-protein and protein-water (Schmidt, 1981). The most widely used fish species for mince production are the gadoid species (cod, haddock, pollock, hake, whiting and cusk) (Hsieh et al., 1992). The gadoid and the elasmobranch (cartilage fish, such as, sharks, etc.) families contain higher concentrations of trimethylamine oxide (TMAO) than other fish species. TMAO degrades in frozen fish to dimethylamine (DMA) and formaldehyde (FA). FA is believed to cause a cottony or spongy texture of the fish tissue through cross-linking of muscle proteins

(Shenouda, 1980; Regenstein et al., 1982). Hsieh et al. (1992) described the sensory effects of FA crosslinking on proteins as follows: "The water is loosely retained in the tissue which causes the product to behave like a sponge. At first bite the moisture is all released and the remaining dry sponge has a cottony texture." The degradation of texture, flavor, and odor of stored seafood is also attributed to oxidation of unsaturated lipids. Processing operations as mincing promotes lipid oxidation, loss of water and formation of DMA (Shenouda, 1980; Flick et al., 1992). The objective of this research was to stabilize Pacific whiting mince using different cryoprotectants and to determine quality loss during frozen storage. Freshness of the mince was evaluated by K-value analysis (K-value = ratio of degradation products to total nucleotide content), as the proteins are very suseptible for degradation and loose their funtional properties within 2 days of harvest. Mince was mixed with 4 different cryoprotectants or mixtures of cryoprotectants and stored frozen at -20°C for 32 weeks. Torsion test (shear stress and shear strain) was choosen to evaluate the textural quality changes that occurred in the mince with different cryoprotectants compared to a control (mince without cryoprotectants) during frozen storage. At different time intervals samples were investigated by torsion, extractability of salt soluble proteins, formation of DMA and TBA analysis for oxidation, followed by color analysis.

Materials & Methods

Fish Source and Sample Preparation

Pacific whiting were caught off the Oregon coast, stored onboard the vessel in refrigerated sea water and off-loaded within 24 hr of capture. The fish were filleted at a local seafood processing plant, stored in ice, and transported to the OSU Seafood Laboratory the same day. The fillets were processed into mince by passing through Autio grinder, (Model 601 Hp, with 1/8" plate, Autio Co. Astoria, OR). A protease inhibitor, beef plasma protein, (AMP 600N, AMPC, Inc., Ames IA), 1%, was added to the fish mince before freezing and cryoprotectants to protect the functionality of the proteins. Each formula contained 0.3% w/w Brifisol S-1 (tetrasodium pyrophosphate and sodium tripolyphosphate - B. K. Ladenburg Corp., Cresskill, NJ) and 8% w/w of one of the cryoprotectants; 4% sucrose (C & H Pure Cane Sugar, Concord, CA) and 4% sorbitol (ICI Specialties, Wilmington, DE); 4% sucrose and 4% M-250 (Maltodextrin 250, Grain Processing Corp., IO); 8% Polydextrose® (Pfizer Inc., NY), and 8% dry whey (Tillamook County Creamery Association, OR), containing 73% lactose, 13-14% protein, 4.5-4.8% moisture, 1.25-1.5% butterfat and 8.5% ash as reported by the producer. The disaccharides, sucrose and lactose, the sugar alcohol, sorbitol, and the dextrin molecules in M-250 and Polydextrose® were the stabilizing agents. The polyphosphates act as a water-binding improver and a cryoprotective agent along with other cryoprotectants. Mixing was done in a Hobart mixer, (Model A-200, The Hobart Mfg. Co., Troy, OH) for 4 min at $T < 8°C$. The stabilized mince was vacuum packed in semi-rigid, 500 g, plastic containers. The samples were frozen

in a blast freezer overnight at -30°C and stored at -20°C. Samples were tested after one day in freezer (t_0), and 1, 4, 8, 16, and 32 weeks.

K-value Measurements

Nucleotide analyses were used to calculate K-value, an indicator of freshness in fish. Adenosine 5′-triphosphate (ATP) and its degradation products; adenosine 5′-diphosphate (ADP), adenosine 5′- monophosphate (AMP), inosine 5′-monophosphate (IMP), inosine (HxR) and hypoxanthine (Hx) were determined by modification of the methods of Burns and Ke (1985) and Ryder (1985). Five g of minced fish were homogenized in 40 ml of 7.5% chilled perchloric acid (PCA) solution for 90 sec with homogenizer (model PT 10/35, Brinkmann Instruments, Westbury, NY), centrifuged at 250 xg at 4°C after standing in a cold lab for 15 min. The extract was neutralized with 10 ml of 1.3 M KOH in 0.2 M KH_2PO_4 buffer and stirred for 10 min in cold lab. After settling for 5 min, the supernatant was filtered through 0.45 μm disposable filter disc and aliquots of 5 ml stored frozen at -50°C until immediately before use. The extract was analyzed by a Bio-Rad Series 800 high performance liquid chromatograph equipped with a Model 2700 solvent delivery system and Model 1706 UV/Vis monitor (Bio-Rad Lab., Richmond, CA) and a 6800 SX integrator (Leading Tech., Inc., Beaverton, OR). Separations were completed on a reverse phase column (ODS-5S 250 mm x 4 mm) at room temperature with gradient profile of the mobile phases; 0.1 M KH_2PO_4 and HPLC grade methanol. Wavelength was adjusted to 254 nm, range 0.04, flow rate 0.9 ml/min and sample size 20 μl. Nucleotide standards were obtained from

Sigma Chemical Co., St. Louis, MO. The K-value is the ratio of the sum of HxR and Hx to the sum of ATP, ADP, AMP, IMP, HxR, and Hx.

Gel Preparation

The mince samples were thawed overnight at 0-4°C. Moisture content in the mince was determined, in triplicate, by a microwave procedure with a sample of 2.5-4.5 g mince spread evenly over one glass microfibre filter, covered with another filter and dried in a microwave oven for 10 min at 80% power (Morrissey et al., 1993). Moisture and NaCl were adjusted to 78% and 2%, respectively in the stabilized mince samples. The mince was mixed in a vacuum chopper (Model 5289, Stephan Machinery Corp., Columbus, OH) for 4 min and salt and ice/water added while mixing. The fish paste was vacuum-packed to eliminate air pockets before it was transferred to a sausage stuffer (Model 14208, The Sausage Maker, Buffalo, NY) and extruded into stainless steel tubes (i.d.=19 mm) which had been sprayed with a lechitin-based release agent, and sealed with screwed caps. The tubes were cooked in a water bath at 90°C for 15 min, immediately chilled in ice-water and the gel samples were taken from the tubes and kept in sealed plastic bags at 4°C overnight.

Torsion Test

Textural changes in the heat-set gels were evaluated by torsion as shear stress and shear strain at mechanical failure. The stress is indicative of gel strength and correlates with sensory hardness. The strain is related to protein functionality of the

gel. It is indicative of gel deformability and correlates to sensory cohesiveness or elasticity (Hamann and Lanier, 1987). The gel samples were allowed to reach room temperature by standing for 2 hr before testing torsion. Meanwhile, the samples were cut to 28 mm lengths in a template with a gel cutter (NFI, 1991). The sample ends were glued to styrene discs with cyanoacrylate glue. The samples were hourglass-shaped in a torsion cutter (Gel Consultants, Model 91, Raleigh, NC) and the minimum diameter adjusted to 1 cm by a caliper. Shear stress and shear strain, at mechanical failure, were determined in a modified Brookfield viscometer (Gel Consultants, Inc., Raleigh, NC) connected to a computer that collected and calculated the data using equations developed by Hamann (1983). Eight subsamples were used for each determination.

Color Measurements

Color of 3 samples from each treatment was measured by a Minolta CR300 colorimeter (Osaka, Japan). CIE (Commission Internationale de l'Eclairage) L* (lightness), a* (red to green), and b* (yellow to blue) were measured to evaluate the effect of long term frozen storage with different additives on the color of the mince. Whiteness was calculated according to the traditional Japanese method, L*-3b* (Park, 1994a).

Salt Soluble Protein - (SSP)

Extraction of 0.6 M KCl-soluble proteins and actomyosin was done according to the method of Noguchi and Matsumoto (1970) as modified by Hsu (1993). Twenty grams of fish mince were homogenized with ~ 235 ml of chilled 0.6 M KCl solution (pH 7.0) in an Osterizer blender (Model 860-61K, Oster Corporation, Milwaukee, WI). After centrifugation, the supernatant was filtered through a two-fold cheese cloth and brought to 250 ml before dilution for protein determination. Protein concentration of the extracted salt soluble proteins and actomyosin was determined by Lowry assay (Lowry et al., 1951) using BSA as a standard. The concentration of the SSP is expressed as milligrams of protein per g mince.

2-Thiobarbituric acid - (TBA)

The TBA reaction between malonaldehyde and 2-thiobarbituric acid was used to determine oxidative rancidity in the mince. TBA values were determined according to the method of Sinnhuber and Yu (1977). Samples of 200-400 mg were prepared, in triplicate, for each determination. The mince for these measurements was not vacuum packed like previous samples but was kept loosely packed during frozen storage, as vacuum packaging would have inhibited oxidation.

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, 6%

PCA instead of 6.25% TCA 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.

Statistical Analysis

Statistical analysis of data was carried out using ANOVA test (one way analysis of variance). Difference between analysis was determined by least significant difference multiple range test (Statgraphics, version 5.0, Manugistics, Inc., Rockville, MD). Values were considered significant when $p < 0.05$.

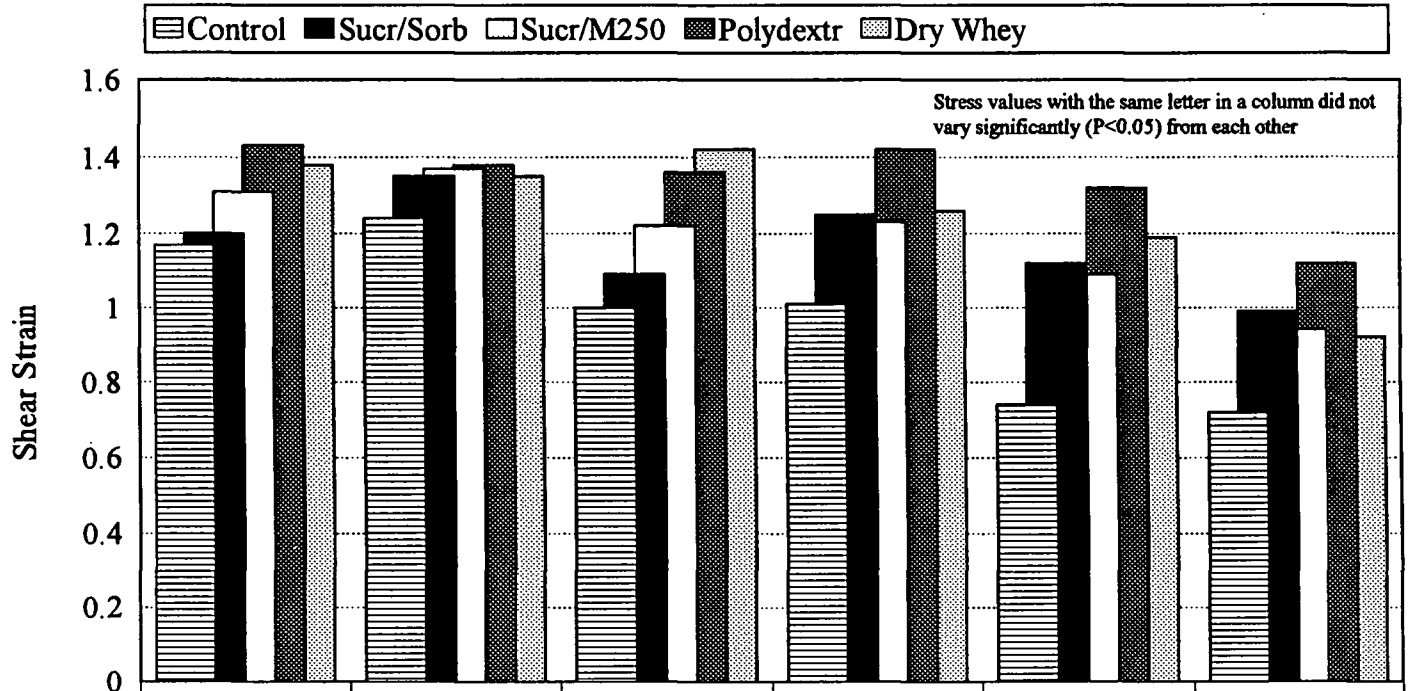
Results & Discussion

K-value of Pacific Whiting Mince

The K-value calculated for fresh mince was 27.6%, indicating fresh samples. K-values for fresh Pacific whiting fillets were reported as 15% (Hsu et al., 1993a) and 10% (Simpson, 1994b). K-values around 20% are considered an indicator of high-quality fish (Ehira and Uchyama, 1974). It is reasonable to expect higher K-value from mince rather than fillets, as the mince has gone through a longer processing period before being frozen.

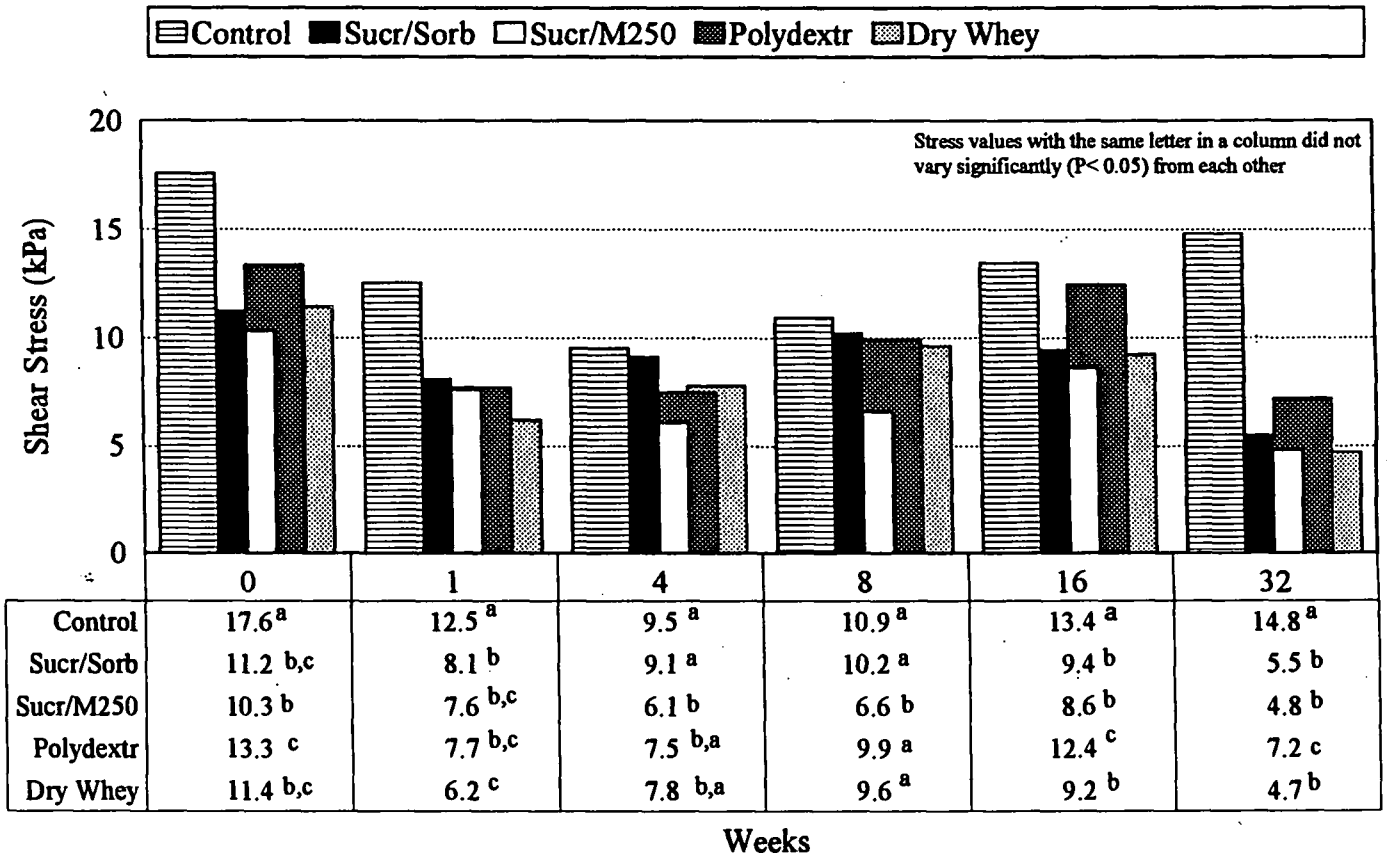
Texture Analysis

Water content in stabilized mince samples, originally from 74.4 to 75.5%, was adjusted to 78% during gel preparation. As a consequence of added water the ratio of protein/water became lower in the stabilized mince than in control. The effects of cryoprotectants on protein functionality are shown in Fig. II.1 and II.2. Strain (Fig. II.1) in stabilized mince was significantly higher than in the control (mince without cryoprotectants) at each time interval tested after the first week. Torsion test at t_0 (after 1 day of frozen storage) showed a strain from ~ 1.2-1.4. The mince samples, stabilized with Polydextrose®, were ~ 20% higher in strain than other stabilized samples and 55% higher than the control after 32 weeks. The three samples; with sucrose/sorbitol, sucrose/M-250 and dry whey were all similar in strain after 32 weeks (0.92-0.99). Significantly higher stress values were measured in control at t_0 and after 1 week than in stabilized mince and also after 16 and 32 weeks (Fig. II.2). The low stress values in the stabilized mince at t_0 declined to ~ 50% during 32 weeks of frozen storage while the control decreased 16% . The higher ratio of protein/water in the control could be causing higher stress in control than in the stabilized mince. Stress can also be influenced by other factors; such as, starch content, while strain is influenced mostly by functional properties of the proteins. The stress in control was lowest at week 4 and increased after that. A similar trend was seen in the stabilized mince with increased stress after 16 weeks, but it had already decreased considerably after 32 weeks. Increased stress over time could be caused by toughening as a result of protein linkages associated with DMA+FA and reduced water holding capacity (Hsu



	0	1	4	8	16	32
Control	1.17 [a]	1.24 [a]	1 [a]	1.01 [a]	0.74 [a]	0.72 [a]
Sucr/Sorb	1.2 [a]	1.35 [a]	1.09 [a]	1.25 [b]	1.12 [b]	0.99 [b]
Sucr/M250	1.31 [b]	1.37 [a]	1.22 [b]	1.23 [b]	1.09 [b]	0.94 [b]
Polydextr	1.43 [c]	1.38 [a]	1.36 [c]	1.42 [c]	1.32 [c]	1.12 [c]
Dry Whey	1.38 [b,c]	1.35 [a]	1.42 [c]	1.26 [b]	1.19 [b]	0.92 [b]

Fig. II.1 - Effect of Cryoprotectants on Gel Deformability (shear strain) in Pacific Whiting Mince



	0	1	4	8	16	32
Control	17.6 [a]	12.5 [a]	9.5 [a]	10.9 [a]	13.4 [a]	14.8 [a]
Sucr/Sorb	11.2 [b,c]	8.1 [b]	9.1 [a]	10.2 [a]	9.4 [b]	5.5 [b]
Sucr/M250	10.3 [b]	7.6 [b,c]	6.1 [b]	6.6 [b]	8.6 [b]	4.8 [b]
Polydextr	13.3 [c]	7.7 [b,c]	7.5 [b,a]	9.9 [a]	12.4 [c]	7.2 [c]
Dry Whey	11.4 [b,c]	6.2 [c]	7.8 [b,a]	9.6 [a]	9.2 [b]	4.7 [b]

Fig. II.2 - Effect of Cryoprotectants on Gel Strength (shear stress) in Pacific Whiting Mince

et al., 1993b). Simpson et al. (1994a) also reported low and highly variable values of shear stress from 7 to 15 kPa, in stabilized Pacific whiting mince.

Color Analysis

The overall trend in CIE L* values of lightness was from 76 to 80. L* (lightnesss) was significantly higher in the stabilized mince samples than the control and significantly highest in mince with dry whey. Several studies report improved color from dry whey on mince according to sensory tests (Madsen, 1984; Zarzycki and Świniarska, 1993). The green hue, -a*, was low with a range of ≈ 0 to -2.73, but significantly more intense in all the stabilized mince samples than in the control, indicating that the cryoprotectants imparted a greenish hue on the mince. Yellowness (b*) was significantly higher in mince with dry whey and lower in mince with sucrose/M-250 than in other samples including control, with Polydextrose® as the second highest. Whiteness (Table II.1) was calculated according to the traditional Japanese method, L*-3b*, to compare the samples, as the greenish hue was low and not suitable for differentiating between the samples and the control. Whiteness after 32 weeks was highest in sucrose/M-250, 44.5, while much lower in Polydextrose®, 38.7, and in dry whey, 35.4.

Effect on Salt Soluble Protein

The extractability of SSP started to decrease rapidly after 8 weeks of frozen storage. Cryoprotectants significantly increased extractability of salt soluble proteins

Table II.1 - Whiteness (L* - 3b*) in Pacific Whiting Mince during 32 weeks of Frozen Storage

Cryoprotectants:	t = 0	1 week	4 weeks	8 weeks	16 weeks	32 weeks
Control	44.78	45.9		43.21	41.1	41.91
Sucr/Sorb	46.06	48.88	45.72	47.8	42.82	42.94
Sucr/M-250	42.57	49.73	43.54	47.2	46.58	44.49
Polydextr	40.99	43.81	42	38.79	36.03	38.67
Dry Whey	31.19	35.74	33.02	36.33	32.63	35.4

in the mince, after 16 weeks of frozen storage, compared to the control (see Fig. II.3 and II.4). Actomyosin was not measurable in the control after 16 weeks of storage but only decreased 20% with Polydextrose®. While 79-87% salt soluble proteins were extracted in the stabilized mince, less than 20% were extracted from the control after 32 weeks of frozen storage. The results are in accordance with Hsu et al. (1993a) with SSP extractability in Pacific whiting fillets during 10 mo of frozen storage and indicate considerable protective effect from the cryoprotectants on myofibrillar proteins in the mince.

Chemical Changes

Formation of DMA was significantly lower in 3 of the stabilized mince samples compared to the control (Fig. II.5). The cryoprotectants Polydextrose®, sucrose/M-250, and sucrose/sorbitol reduced significantly the rate of DMA formation compared to the control (36-52% of control) after 32 weeks. DMA in dry whey was 85% of the control after the same time. The high formation of DMA could have been catalyzed by Fe^{+2} originated in the dry whey and from the sarcoplasmic proteins (Spinelli and Koury, 1981). DMA in samples with Polydextrose® and sucrose/M-250 increased about 3-fold, while samples with dry whey increased 4-fold and the control reached 6-fold the initial value of DMA. The results are similar to those of Simpson et al. (1994a) who used 12% sucrose as a cryoprotectant in Pacific whiting mince at -20°C. They also showed that low temperature was very effective in reducing the rate of DMA formation when compared at -50°C vs -20°C. Dingle et al. (1977) found fish with 0.5 mM formaldehyde per 100 g fish unacceptable because of toughness and

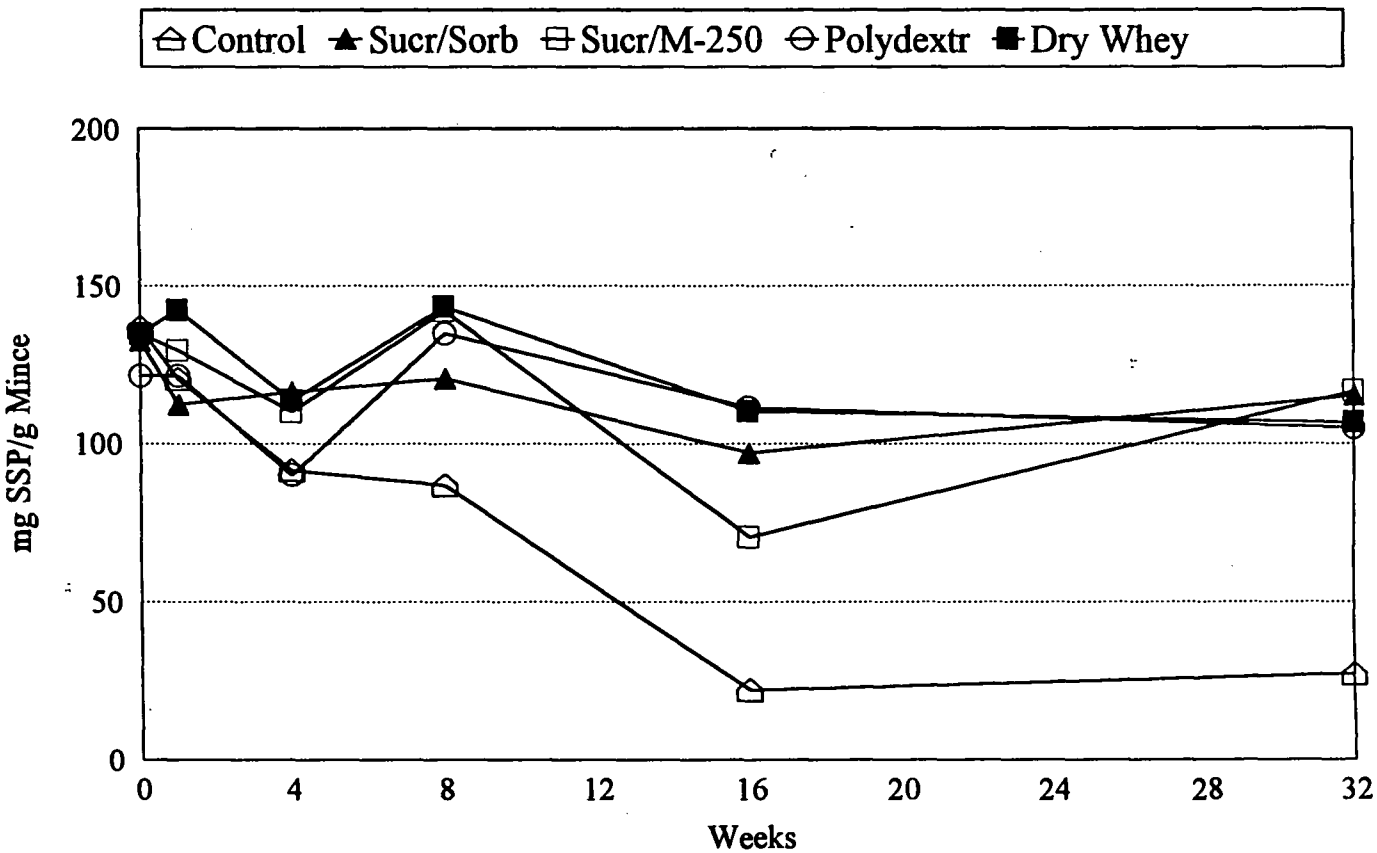


Fig. II.3 - Effect of Cryoprotectants on Extractability of Salt Soluble Proteins in Pacific Whiting Mince

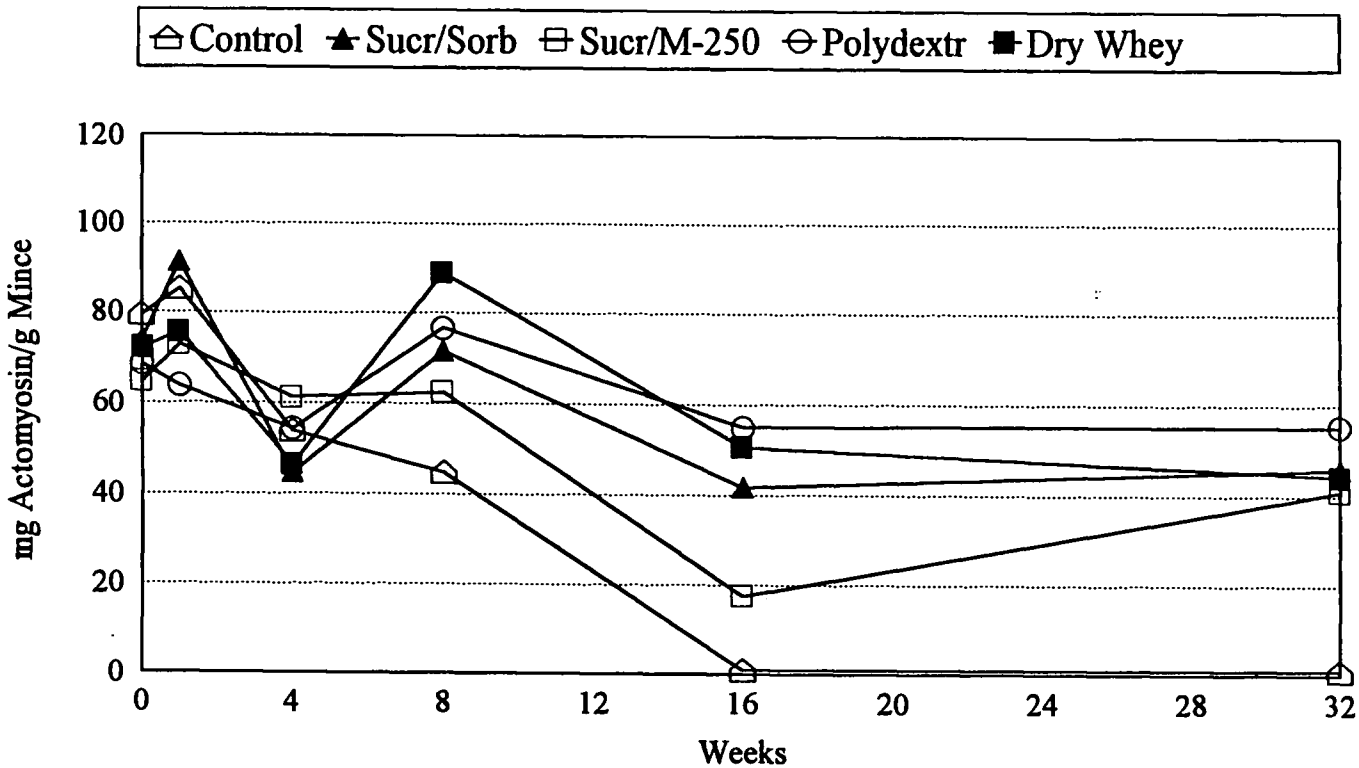


Fig. II.4 - Effect of Cryoprotectants on Actomyosin Extractability in Pacific Whiting Mince

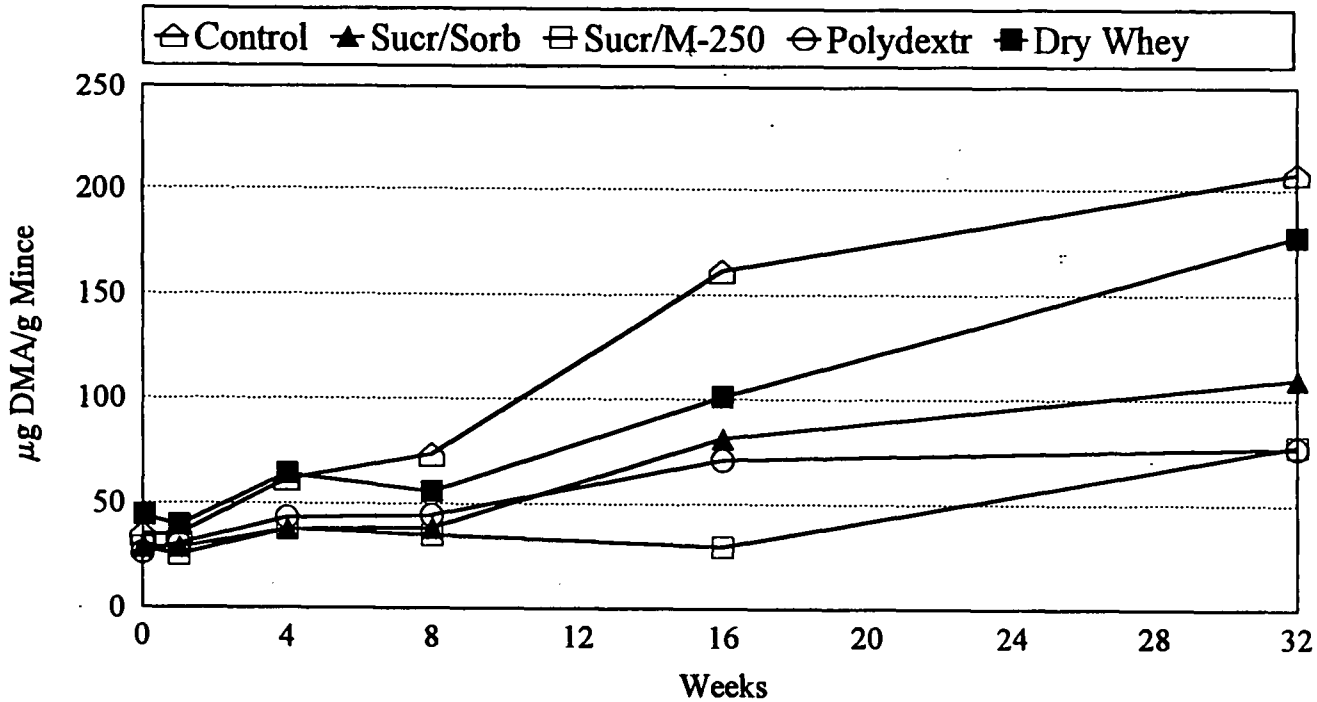


Fig. II.5 - Effect of Cryoprotectants on DMA Formation in Pacific Whiting Mince

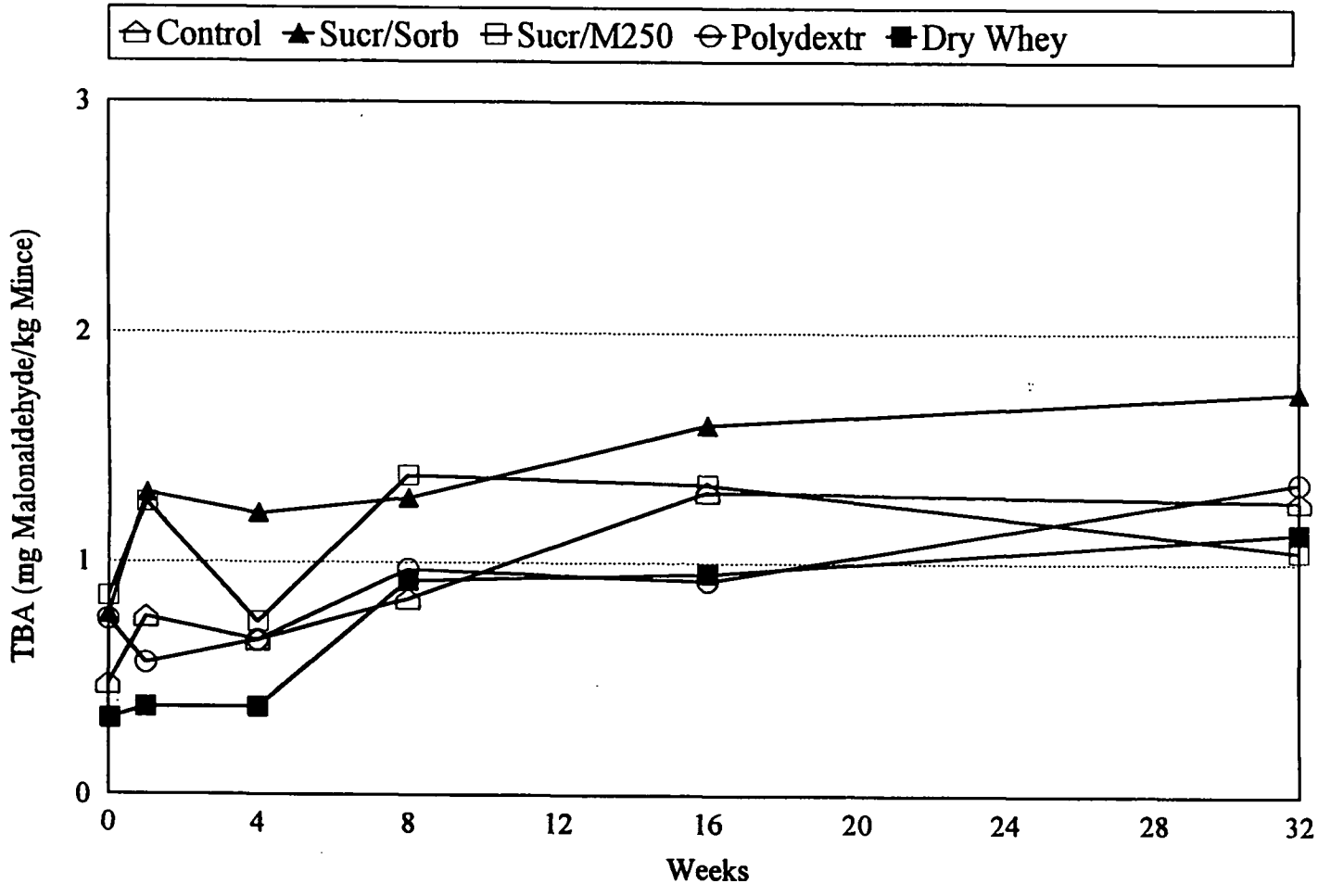


Fig. II.6 - Effect of Cryoprotectants on TBA (2-thiobarbituric-acid) Formation in Pacific Whiting Mince

sponge-like texture. This is equivalent to DMA of ~ 225 μg/g fish. DMA ranged 77-110 μg/g fish after 32 weeks of frozen storage; whereas, the control reached 207 μg/g fish, respectively. TBA was low throughout storage or < 1.75 mg/kg mince, both in samples and control (Fig. II.6). Hiltz et al. (1976) measured oxidation in minced silver hake (*Merluccius bilinearis*) after 4 - 7 weeks at -10°C, with maximum values of 3.6 mg malonaldehyde/kg mince. Silver hake have more fat (2-4%) than other gadoid species such as Pacific whiting (1-2%), which could contribute to the higher levels of oxidation along with higher storage temperature.

Conclusions

Mince stabilized with Polydextrose® was significantly higher in strain than other samples from 8 weeks of frozen storage and throughout the 32 weeks. DMA content in samples with Polydextrose® and sucrose/M-250 was only 37% of that in control after 32 weeks. All cryoprotectants significantly improved the extractability of SSP compared to control. Polydextrose® was most effective in conserving the extractability of actomyosin. Lipid oxidation was low, with no difference between control and samples. These results show that quality loss in minced Pacific whiting can be significantly reduced by adding cryoprotectants to the mince before frozen storage. Advantages to using stabilized mince as a raw material would include: increased yield and utilization of the whole fish for primary products, lower production costs per lb of product and a smaller capital investment when compared to surimi.

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III. EFFECT OF ADDED INHIBITORS ON PROTEOLYSIS IN PACIFIC WHITING MINCE DURING HEAT-SET GELATION

Abstract

Different levels of several food grade proteolytic inhibitors were added to stabilized mince from Pacific whiting to determine their effect on textural quality and color. Increased amounts of inhibitors increased gel strength as measured by torsion. All inhibitors were effective in inhibiting proteolysis during heating. BPP (beef plasma protein) was the most effective in increasing shear strain while WPC (whey protein concentrate) containing 96% protein, increased most the shear stress. Color of samples with BPP was darker than in other samples, but WPC lightened the color.

Introduction

Post-mortem proteolysis in Pacific whiting is a critical problem in preserving the quality of fish during processing. Proteolytic enzymes in the muscle tissue associated with spores from Myxosporean parasites (*Kudoa paniformis*) are the primary cause of texture softening (Patashnik et al., 1982; Erickson et al., 1983). The relationship between the number of parasites and intensity of proteolysis in the fish tissue has been established while it is not known whether the parasites produce the proteolytic enzymes themselves or that the enzymes are immune response to the presence of the parasites (An et al., 1994a; Morrissey et al., 1995). To avoid proteolysis in Pacific whiting muscle tissue, it is important to process the fish within 24 hr of capture. The percentage of fish with poor texture increases from ~ 27% after 1 day in ice to over 70% after 5 days (Morrissey et al., 1992). The sarcoplasmic proteins (water soluble proteins) are removed by the washing steps in surimi production including digestive enzymes, such as, proteases, lipases and phospholipases (Ohshima et al., 1993). About 85% of protease activity in Pacific whiting is removed by the washing and dewatering processes in surimi production (Morrissey et al., 1995). It has been established (An et al., 1994b) that the cysteine proteases cathepsin B, L, and H, are highly active enzymes involved in post-mortem changes of Pacific whiting. Cathepsin B is most active in minced Pacific whiting, and cathepsin L is the predominant one in Pacific whiting surimi. Cathepsins B and H show optimal activity at 20°C in mince. They are removed by washing during surimi production. Cathepsin L is heat activated, with optimal activity at 55°C, both in fish mince and surimi which

indicates its role in protein degradation during conventional heating (An et al., 1994a). Cathepsin L has been shown to cause gel-weakening in the fish paste where the muscle protein myosin is the main component. Morrissey et al. (1993) showed the disappearance of high molecular weight proteins (above 94.0 kDa), including myosin, in Pacific whiting surimi when heated at 60°C for 30 min due to proteolysis.

Haga (1980) reported inhibition of whiting proteases by egg white (EW). Miller and Spinelli (1982) assayed the inhibitory effect of several protein inhibitors (i.e., egg white, potato powder (PP), soybean and lima bean) on proteolysis in Pacific whiting mince incubated at 45°C for 90 min. The low concentration (0.5% w/w) of inhibitors used did not cause any significant inhibition. Chang-Lee et al. (1989, 1990) tested the effect of EW, whey protein concentrate (WPC) and soy protein isolate (SPI) at 0 to 5% levels on heat-set Pacific whiting surimi gels. They found 3% EW superior for gel hardness and elasticity. Morrissey et al. (1993) found that BPP and PE at 1% level inhibited proteolysis about 70% in Pacific whiting mince, and BPP, PP, and EW were all equally effective at 3% level with more than 80% inhibition. Arrowtooth flounder has similar softening problems as Pacific whiting. Wasson et al. (1992) reported that arrowtooth flounder could be used in surimi by using BPP or EW as inhibitors. Reppond and Babbitt (1993) tested the inhibitory effect of BPP, EW and PP, 2% of each, in arrowtooth flounder and walleye pollock surimi. Porter et al. (1993) studied inhibition of proteolysis by a potato extract (PE) in both Pacific whiting and arrowtooth flounder. They found PE (0.1%) a more effective inhibitor than either egg white (3.0%) or BPP (1%). The activity of proteases in Pacific whiting surimi is highest at 55°C and at 20°C in Pacific whiting mince due to different types of

cathepsins (An et al., 1994a). Patashnik et al. (1982) showed that the enzymes in Pacific whiting fillets are totally inactivated by rapid cooking for 10 min (core temperature of the food 70°C within 1.5 min). This can be achieved by microwave cooking or deep fat frying. Recently, ohmic heating which heats surimi paste to 90°C in less than 2 min has proven successful in decreasing proteolysis in Pacific whiting (Yongsawatdigul et al., 1995).

Enzyme concentration in mince is » than in surimi where more than 80% of the protease activity has been removed during processing (Morrissey et al., 1995). The objective of this study was to test the effect of different levels of common, food grade inhibitors on textural quality in Pacific whiting mince during heat-set gelation and on color.

Materials & Methods

Fish Source and Sample Preparation

Machine-cut butterfly fillets from Pacific whiting were processed at a local seafood processing plant, stored on ice and transported to the Oregon State University Seafood Laboratory within 24 hr of capture. The fillets were immediately skinned and passed through an Autio grinder with a 1/8" plate (Model 601 Hp, Autio Co. Astoria, OR). Cryoprotectants were added to the fish mince, 4% w/w sorbitol (ICI Specialties, Wilmington, DE), 4% w/w sucrose (C & H Pure Cane Sugar, Concord, CA), and 0.3% w/w Brifisol S-1 (tetrasodium pyrophosphate and sodium tripolyphosphate - B. K. Ladenburg Corp., Cresskill, NJ). The mince, with additives, were mixed in a Hobart

mixer (Model A-200, Hobart Mfg. Co., Troy, OH) at low speed for 6 min at T ≤ 8°C. The stabilized mince was vacuum packed in semi-rigid, 500 g, plastic containers. The samples were frozen in a blast freezer overnight at -30°C and subsequently stored at -20°C and stored for one week before testing. Sample preparations and testing were repeated once on a separate lot of fish.

Gel Preparation

The mince samples were partially thawed at room temperature. The moisture content in the mince was determined, in triplicate, by a microwave procedure (Morrissey et al., 1993) and gel samples prepared according to NFI (1991) . The mince with moisture adjusted to 78% and salt 2% was mixed for 6 min in a vacuum chopper (Model 5289, Stephan Machinery Corp., Columbus, OH) at a temperature < 8°C. The mince was mixed with additives at a low speed for 3 min: 1.5 min for mince, 1 min with added salt, and 0.5 min with the appropriate amount and type of inhibitor. The fish paste was then mixed at high speed under a vacuum (~ 50 kPa) for 3 min and ice/water was added to adjust the moisture to 78%. The inhibitors used were: beef plasma proteins (BPP) (American Meat Protein Corp., Ames IA), whey protein concentrate (WPC) with 96% protein (Davisco Int. Inc., 620 N. Main, MN), which presumably gives better protease inhibiton than WPC with lower protein content, egg white solid (EWP) and egg white powder (EWP_{new}) from two companies (Egg City, Moorpark, CA, and Monark Egg Corp., Kansas City, MO) and egg white liquid (EWL, eggs bought from a local grocer and the separated egg whites stored frozen). Each formula contained 1, 2, 3, or 4% of one of the inhibitors. The moisture

of the inhibitors was determined in triplicate (AOAC, 1990) and used in the calculations to adjust moisture. The percentage of EWL in the samples is based on solid content. Measured moisture in egg white, 89.3%, was subtracted from 100% and the difference, 10.7% used in calculations for protein content. Because of higher moisture and lower protein concentration in liquid egg white than in the powdered inhibitors, the final moisture in samples with 3 and 4% EWL was 1-2% higher than in the other samples. The fish paste was transferred to a sausage stuffer (Model 14208, The Sausage Maker, Buffalo, NY) and extruded into stainless steel tubes (i.d.=19 mm), sprayed with a lecithin-based release agent, and sealed with screwed caps. The tubes were cooked at 90°C for 15 min, chilled in ice-water for 1 hr and the gels kept in sealed plastic bags at -4°C overnight. Heating rate is potential in mince production as the proteolytic enzymes in the mince, which cause gel weakening during heating, show optimal activity at elevated heat (20-40°C). The protease inhibitors used in the mince were thought to compensate for the relatively slow rate in conventional heating, by inhibiting degradation of the proteins during heating. Eight subsamples were measured from each lot and used for each determination.

Torsion Test

The cooked gels were tempered at room temperature and prepared for torsion testing as described in NFI, 1991. Shear stress and shear strain at mechanical failure were determined in a modified Brookfield viscometer (Gel Consultants, Inc., Raleigh, NC) connected to a PC computer that collected and calculated data using equations

developed by Hamann (1983). An average of 8 subsamples were used for each determination.

Color Measurements

The color of 5 subsamples from each treatment was evaluated by a Minolta CR300 colorimeter (Osaka, Japan) and averaged for each determination. CIE (Commission Internationale de l'Eclairage) (L* black [0] to light [100], a* red [60] to green [-60], b* yellow [60] to blue [-60]) values of the different treatments were evaluated to see the effects of different types and amounts of inhibitors on the color and compared to control (mince without inhibitor). Whiteness was calculated according to the traditional Japanese method, L*-3b*, (Park, 1994).

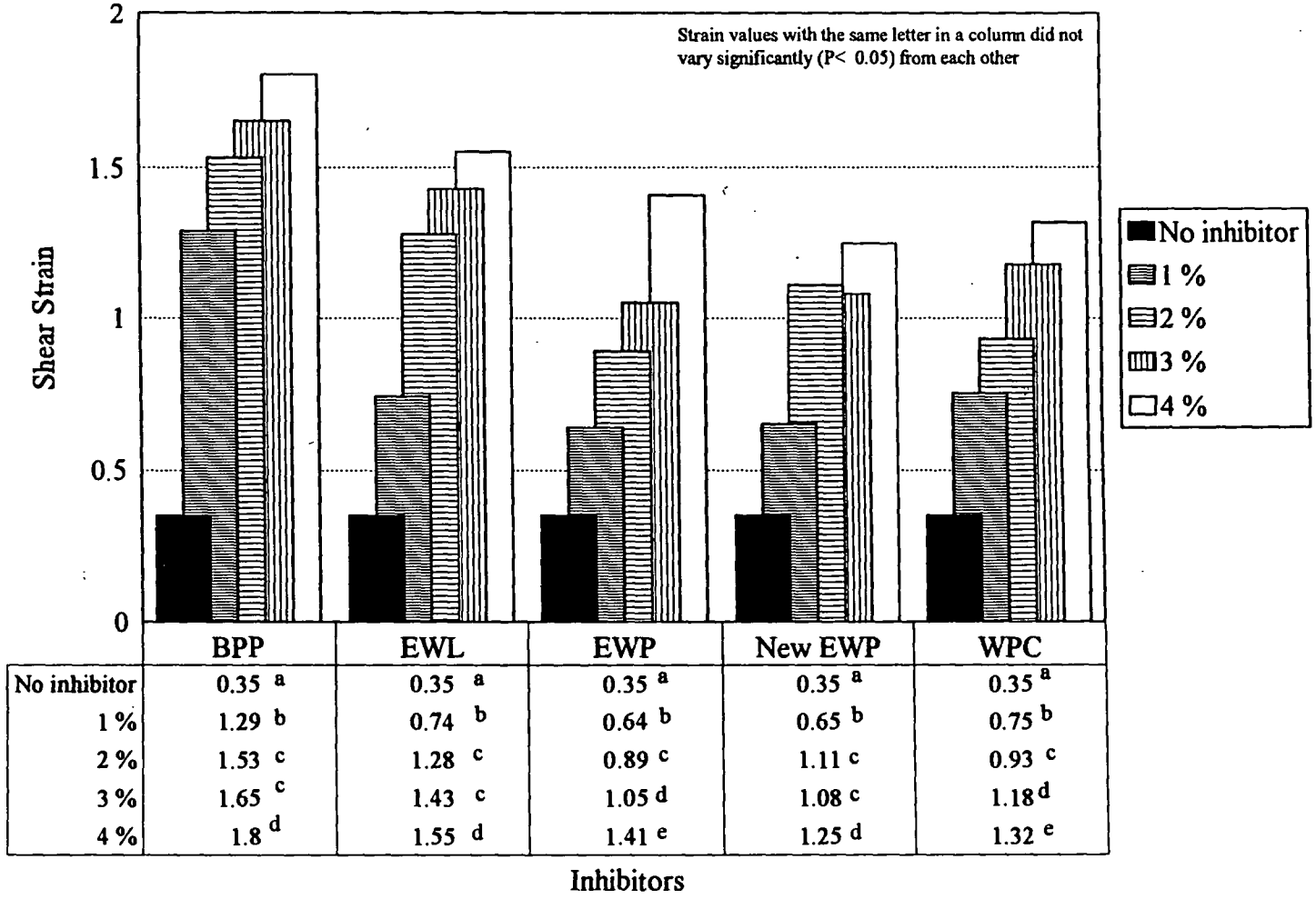
Statistical Analysis

Statistical analysis of data was carried out using one way analysis of variance. Differences among mean values were established by using the Least Significance Difference (LSD) multiple range test (Statgraphics, version 5.0, Manugistics, Inc., Rockville, MD). Values were considered significant when $p<0.05$.

Results & Discussion

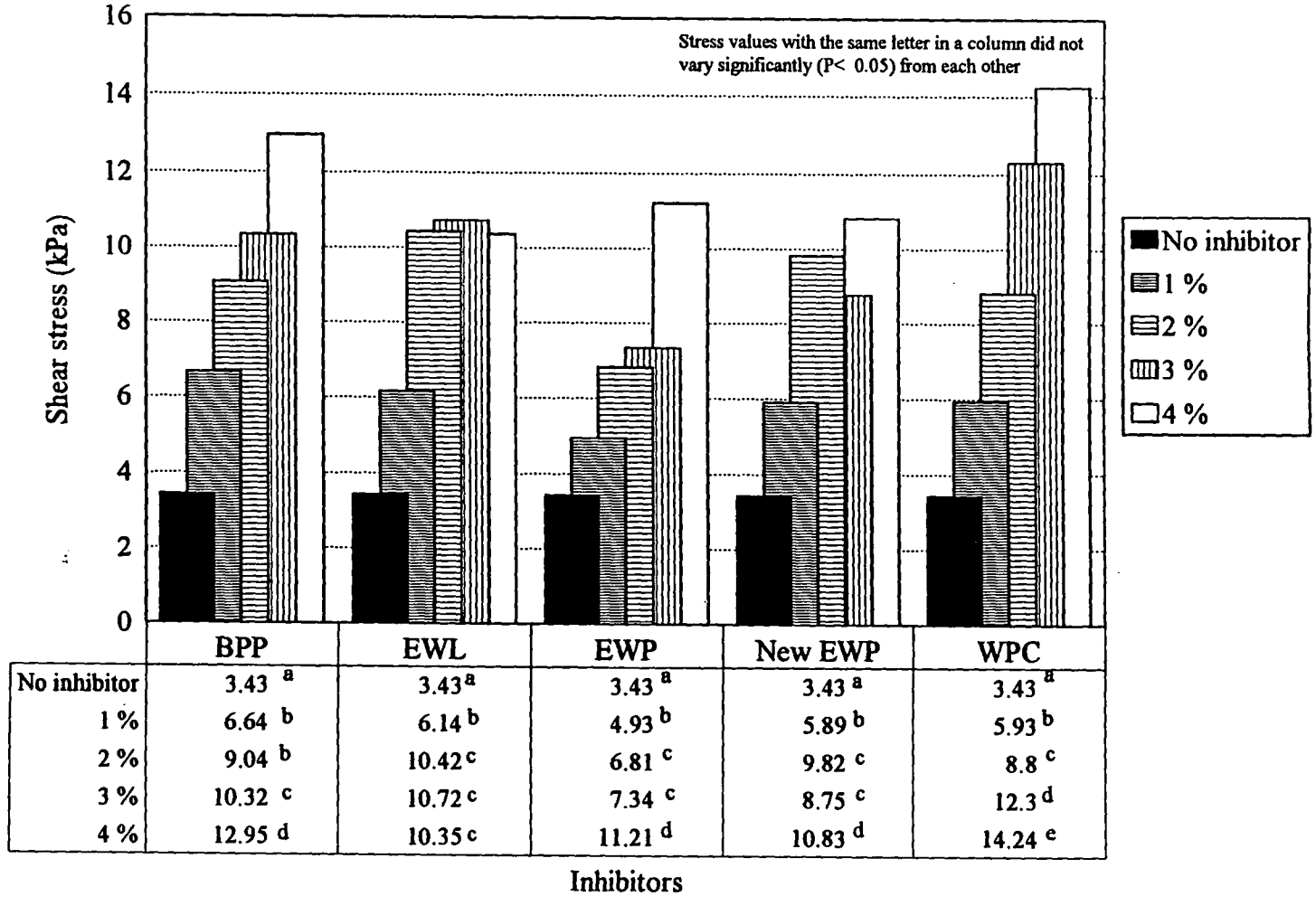
Torsion Test

Shear strain (Fig. III.1) increased with the increased level of inhibitors. The strain was highest in samples with BPP > EWL > WPC > EWP_{new} > EWP at all levels. Strain was basically the same in the 2% BPP sample as in the 4% EWL sample. Strain in samples with EWP and WPC were relatively low. Samples with the two types of EWP and WPC showed similar strain at the level of 4% as the sample with 1% BPP. WPC was most effective in increasing stress (Fig. III.2). The 4% WPC sample showed 140% higher stress than the 1% sample. BPP was the next most effective inhibitor used. Stress values in 2, 3, and 4% EWL were all similar and also samples with 2 and 3% EWP. Hamann (1990) described surimi gels below 10 kPa in shear stress as weak gels. In an attempt to increase strain and stress, samples with 3% BPP and 1% EWP were prepared and gel strength measured. Strain did not increase from 3% BPP (1.70 instead of 1.65), but stress reached 16.04 kPa, which was the highest measured value. The effects on texture were higher from EWL than EWP. Another method to minimize gel-weakening is to increase the heating rate to 70-80°C where proteolytic enzymes are inactivated. New research on Pacific whiting surimi, heated by conventional method in a water bath and by ohmic heating with a rapid heating rate, show greatly improved textural properties, or more than a twofold increase in stress and strain (Yongsawatdigul et al., 1995).



	BPP	EWL	EWP	New EWP	WPC
No inhibitor	0.35 a	0.35 a	0.35 a	0.35 a	0.35 a
1 %	1.29 b	0.74 b	0.64 b	0.65 b	0.75 b
2 %	1.53 c	1.28 c	0.89 c	1.11 c	0.93 c
3 %	1.65 c	1.43 c	1.05 d	1.08 c	1.18 d
4 %	1.8 d	1.55 d	1.41 e	1.25 d	1.32 e

Fig. III.1 - Effect of Increased Levels of Inhibitors on Shear Strain in Pacific Whiting Mince



	BPP	EWL	EWP	New EWP	WPC
No inhibitor	3.43 a	3.43 a	3.43 a	3.43 a	3.43 a
1 %	6.64 b	6.14 b	4.93 b	5.89 b	5.93 b
2 %	9.04 b	10.42 c	6.81 c	9.82 c	8.8 c
3 %	10.32 c	10.72 c	7.34 c	8.75 c	12.3 d
4 %	12.95 d	10.35 c	11.21 d	10.83 d	14.24 e

Fig. III.2 - Effect of Increased Levels of Inhibitors on Shear Stress in Pacific Whiting Mince

Color Analysis

The inhibitors had diverse effects on color (Table III.1). BPP lowered the L* value of the mince from 79.5 (control) to 76.4, but increased concentrations did not further reduce L*. The other inhibitors increased lightness gradually, WPC showed significantly higher lightness than the other inhibitors and increased from 80.1 to 81.5 with increased concentration. There was no significant difference in green hue or yellowness between the treated samples. The range in green hue in samples with 4% inhibitor was -2.4 to -2.7. BPP showed an increased yellowness (b*) from 8.63 to 11.37 with increased concentrations. EWP gave almost constant color, from 10.63 to 10.99, and the same trend was observed in samples with EWL, from 9.27 to 9.20. In samples with WPC, the color decreased after 2% (9.64, 9.74, 8.74, 8.68) probably because of added white color from the inhibitor. Table III.2 shows whiteness, (L* - 3b*), where lightness and yellow color are used to estimate the effect from the inhibitors according to this traditional Japanese method..

Conclusions

The proteolytic inhibitors used were all effective in improving gel-texture. BPP was most effective in increasing shear strain and WPC shear stress. A mixture of 3% BPP and 1% EWP_{new} showed the highest stress measured and similar to 4% WPC. BPP significantly darkened color in mince samples compared to the other inhibitors, with WPC significantly highest in lightness. These results show the potential of

Table III.1 - Effect of Inhibitors on Color in Pacific Whiting Mince

	L* = lightness				a* = green hue				b* = yellow hue			
Inhibitor:	1 %	2 %	3 %	4 %	1 %	2 %	3 %	4 %	1 %	2 %	3 %	4 %
BPP	76.7	76.6	76.4	76.7	-2.02	-1.71	-2.53	-2.7	8.63	9.89	9.61	11.1
EWL	79.1	78.8	79	79.6	-1.96	-2.24	-2.49	-2.69	9.27	9.34	9.18	9.2
EWPn	79.9	80.7	81	81.5	-1.95	-2.25	-2.28	-2.38	10.63	10.78	10.92	10.99
EWP	79.8	80.2	80.2	79.9	-1.98	-2.01	-2.15	-2.37	9.33	9.25	9.27	9.71
WPC	80.1	80.5	81.3	81.5	-2	-2.07	-2.25	-2.45	9.64	9.74	8.74	8.68

Control (Stabilized Mince without Inhibitor) L* 79,46 a* -1,77 b* 8,32

Table III.2 - Whiteness (L*-3b*) in Mince with Different Levels of Inhibitors

Inhibitors:	1%	2%	3%	4%
BPP	50.8	46.9	47.6	42.5
EWL	51.3	50.8	51.5	52
EWP	50	50.5	50.3	49.5
WPC	51.2	51.3	55.1	55.5

Whiteness in control (stabilized mince without an inhibitor) 54.5

inhibiting proteolysis due to heat-activated enzymes to minimize gel-weakening during heating in minced Pacific whiting

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IV. SUMMARY

Strain values of the stabilized mince and control were relatively low, or 1.2 - 1.4 at t_0, compared with strain values in surimi, ~ 2.2 - 2.4 at the same time. Quality loss in the mince was significantly decreased by the cryoprotectants tested during frozen storage as measured by strain, extractability of SSP and DMA formation.. Polydextrose yielded significantly higher strain, lower DMA and higher extractability of actomyosin than the other cryoprotectants. Mixture of sucrose and M-250 (corn syrup solid) matched the effect of Polydextrose® in preventing DMA formation and improved color.

The proteolytic inhibitors were all effective in improving gel-texture in the stabilized mince, with BPP most effective in increasing strain and WPC in increasing stress. WPC significantly imparted light color in mince compared to the other inhibitors whereas BPP darkened color. Strain was increased by increased amount of proteolytic inhibitors and reached highest 1.8 with 4% BPP.

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APPENDICES

APPENDIX A:

CRYOPROTECTIVE EFFECT OF DRY WHEY ON PACIFIC WHITING SURIMI

Introduction

As early as 1976, Noguchi et. al reported the cryoprotective effect of lactose, the disaccharide found in milk. Because of the low price and sweetness of lactose and the high costs of some cryoprotectants such as sorbitol, several economic advantages may exist for use of lactose over currently used commercial cryoprotectants. The majority of the Pacific whiting harvest is used for surimi, a washed fish mince, that uses cryoprotectants to stabilize the proteins during frozen storage. Many compounds with cryoprotectant activity have been discovered to stabilize the muscle protein (mostly myosin) against freeze-induced degradation. Noguchi et. al (1976) studied 28 compounds for their cryoprotective effect and discovered disaccharides (sucrose, lactose) and hexoses (glucose, fructose) gave better cryoprotection than other carbohydrates studied. In their research a 0.1 M solution of lactose (3.7%) was added to isolated carp actomyosin and stored at -30°C for 7 weeks. The decrease of protein solubility, indicative of protein degradation, was less in samples with hexoses and their disaccharides than in those with other saccharides during 7 weeks of storage. Rodger et al. (1979) compared lactose, monosodium glutamate, and sodium citrate at 3% w/w level, for cryoprotective effect on fish mince from cod stored on ice for 5 or 21 days before freezing at

different temperatures. Lactose gave better results than monosodium glutamate and sodium citrate in improving texture, although it showed only a marginal effect over the control at the 3% level. Dry whey, obtained by removing moisture from whey and leaving other constituents in their relative proportions, contains about 72% lactose (Anon., 1976) and has been investigated for cryoprotective effect by several researchers. Madsen (1984) reported improvement of texture and whitening of the color of minced blue whiting by using a 10% w/w whey powder which contained 36.5% protein, 50.5% lactose, 1.0% fat, and 8.0% ash. The mince was drum-frozen, granulated, and extruded into fish sticks. The fish sticks had a slight sweet off-flavor. Nielsen et al. (1985) compared the cryoprotective properties of whey powder, lactose, and fish protein hydrolysates on minced cod (*Gadus morhua)* from the Baltic Sea. High concentrations, or 11%, of lactose and whey powder (protein 17.5%, lactose 71%) failed to maintain the gelling properties during 1 month storage at the temperature tested (-10°C). Zarzycki and Świniarska (1993) reported cryoprotective effect of dry whey on Baltic cod. Different amounts of dry whey (1, 2.5, 5, and 7.5%) were tested and samples stored at -20°C for 22 weeks. The 7.5% concentration gave the best cryoprotection, and after thermal treatment samples with whey had better taste and color than the control. Dondero et al. (1994) found a mixture of dry whey, 8% w/w, plus 0.2% polyphosphates protected the functionality of the jack mackerel surimi very similar to the commercial mixture of 8% sorbitol/sucrose, (1:1) for 5 months at -18°C. Dry whey also improved the sensory characteristics of the gels compared to the control.

The objective of this study was to investigate the cryoprotective effect of dry whey (≥ 70% lactose) on Pacific whiting surimi. Dry whey of 5 and 8% was mixed with 4% sucrose and the cryoprotective effect compared to the industrial standard of 8% sorbitol/sucrose, (1:1). The surimi samples went through 10 freeze/thaw cycles where they were thawed at room temperature for 8 hr and frozen again for 16 hr at -20°C. This was repeated daily for 10 days.

Materials & Methods

Preparation of Surimi Samples

Pacific whiting was harvested off the Oregon coast, stored on-board in refrigerated sea-water, and off-loaded within 12 hours of capture. The fish were headed and gutted at a local seafood processing plant, and the meat separated from bones and skin. The resulting mince was washed and dewatered, and the raw surimi stored in ice and transported to the Oregon State University Seafood Laboratory within 8 hr. Three different mixtures of cryoprotectants were added to the raw surimi. Each batch was mixed in a Hobart mixer, (Model A-200, Hobart Mfg. Co., Troy, OH) for 6 min at temperature below 10°C. All 3 formulations contained 4% w/w sucrose (C & H Pure Cane Sugar, Concord, CA) and 0.3% w/w Brifisol 414 polyphosphates (BK-Ladenburg Co., Ladenburg/Neckar, Germany) with the addition of: (1) 5%, (2) 8% dry whey (Tillamook County Creamery Association, OR) or (3) 4% sorbitol (ICE Americas Inc., Wilmington, DE). The surimi batches were vacuum packed in semi-rigid 500 g plastic trays before freezing. Freeze/thaw studies were undertaken using

the following procedures; samples were thawed after 1 day in freezer for ~ 8 hr and subsequently frozen at -20°C for 16 hr. This was repeated 10 times in 10 days. The parameters measured were 0, 1, 3, 5, 7, and 9 cycles of freeze/thaw. The period one cycle means that the frozen samples had been thawed and frozen again and measured after thawing.

Gel Preparation and Torsion Test

The method is the same as described in chapter II, except that 2% beef plasma protein was used instead of 1%. The average of 8 subsamples were used for determination of each shear stress and shear strain value.

Color Measurements

Color was evaluated by a Minolta CR300 colorimeter (Osaka, Japan) by using the CIE (Commission Internationale de l'Eclairage) tristimulus values for the LAB space; L* (black [0] to light [100], a* red [60] to green [-60], b* yellow [60] to blue [-60]). The average of 5 subsamples from each treatment was used for each determination. Whiteness was calculated as L*-3b* according to a Japanese method (Park, 1994).

Dimethylamine - (DMA)

DMA content was determined according to the method of Dyer and Mounsey (1945) with modifications as described in chapter II.

Water Holding Capacity

The percentage of water holding capacity (WHC) or bound water in the uncooked samples was measured by the press technique method. After thawing, ~ 0.5 g of sample was weighed out on filter paper (Whatman No. 1) and the sample pressed between two plexiglass sheets for 1 min at 500 psi (pounds per square inch) using a Carver Laboratory Press (Fred S. Carver Inc., NY). The percent of free water was calculated according to the following formula:

$$\% \text{ Free water} = \frac{(\text{Total surface area-meat film area } (cm^2)) \times 9.47 \text{ mg water}/cm^2}{\text{total moisture (mg) of sample}}$$

The constant 9.47 mg/cm^2 was used instead of 61.10 mg/in^2 as in the method (Tsai and Ockerman, 1981). The percentage of free water in the samples was estimated by the average of three subsamples.

pH Measurements

The pH was measured in samples after each freeze/thaw cycle. Ten grams of sample was diluted in 90 ml distilled water, homogenized for 2 min, and measured with a Corning pH meter, (Model 250, Corning Ciba Diagnostics Co., Corning, NY).

Statistical Analysis

Statistical analysis of data was carried out using one way analysis of variance. Differences among mean values were established by using the Least Significance

Difference (LSD) multiple range test (Statgraphics, version 5.0, Manugistics, Inc., Rockville, MD). Values were considered significant when $p<0.05$.

Results & Discussion

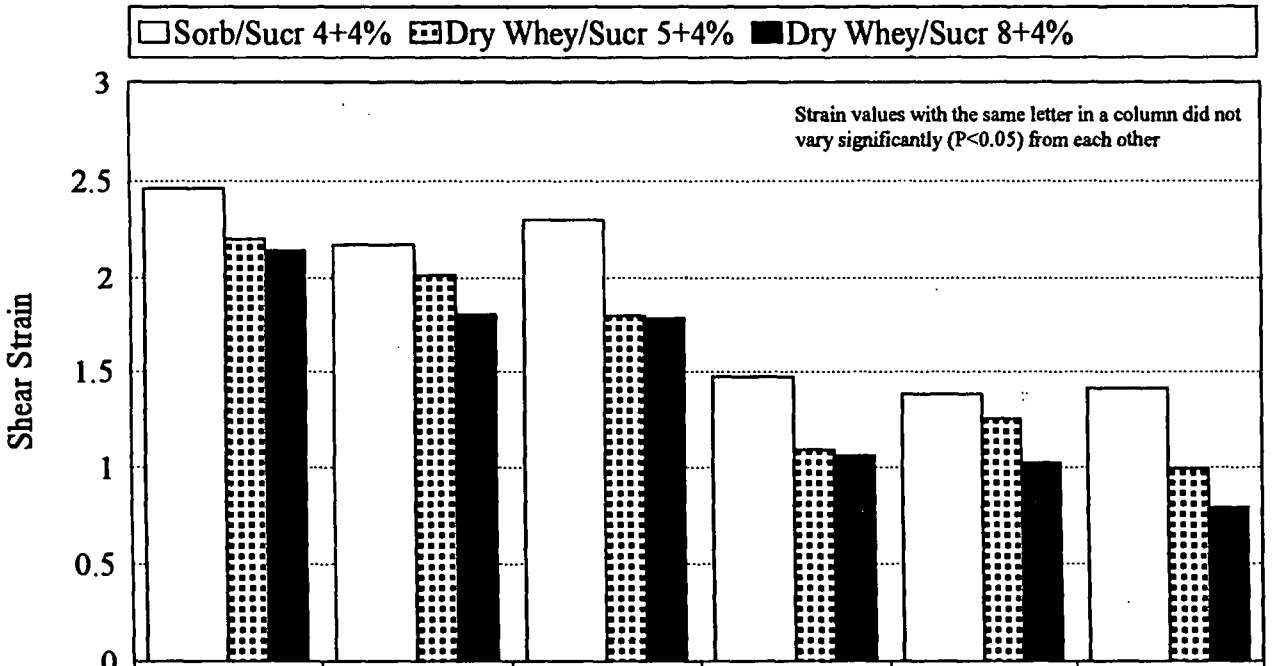
Proximate Analysis on Dry Whey

Proximate analysis of dry whey gave the following results: lactose 71.88%, protein 11.92%, ash 7.23%, moisture 6.46%, lipid 2.51%.

Torsion Test

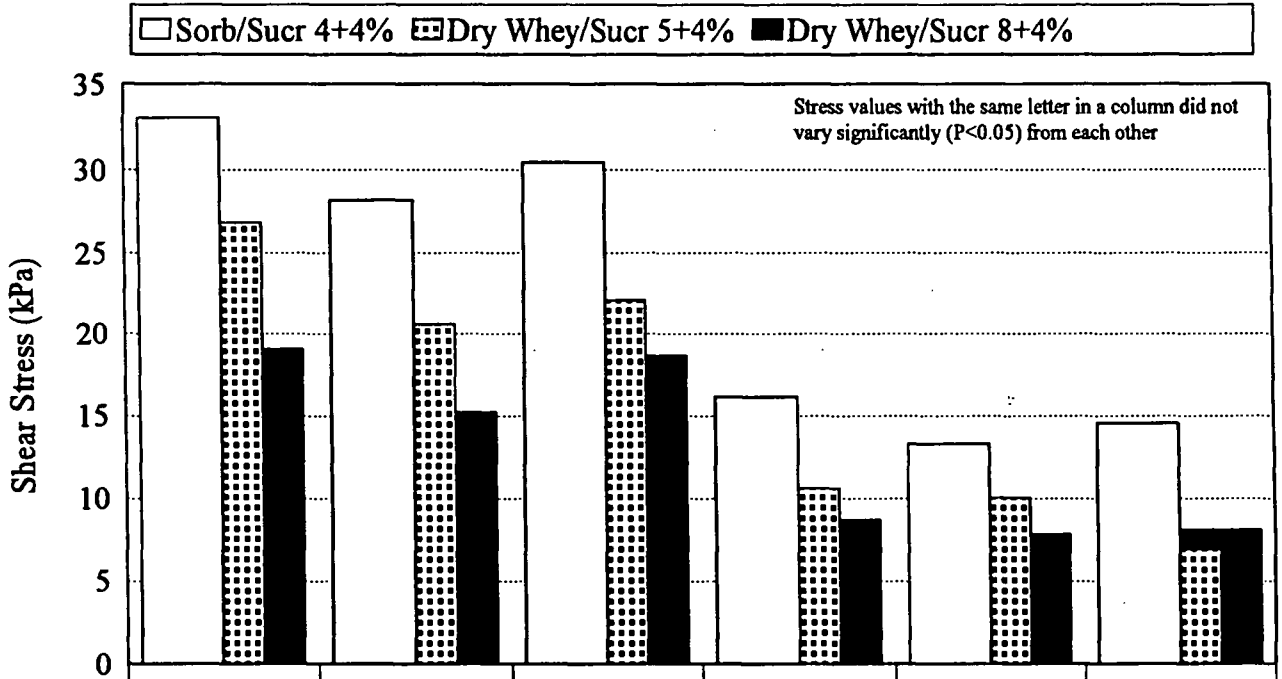
The ratio of protein:water in samples with 8% dry whey was 1:4.19 or somewhat higher than in samples with 5% dry whey, 1:4.41, and in sorbitol/sucrose samples, 1:4.42, because of proteins in the dry whey. The strain value of good quality surimi commonly ranges from 2.0 - 3.0 (NFI, 1991). A strain value depends on the functional quality of proteins. The strain values in surimi (Fig. A.1) at t_0 (after 1 day in frozen storage) ranged from 2.14 - 2.46, and the sample treated with sorbitol/sucrose showed higher strain than the 2 samples containing dry whey and sucrose. After 9 cycles of freeze/thawing, the surimi with sorbitol/sucrose contained 57% of the original strain and the surimi with dry whey, 5 and 8%, retained 45 and 37%, respectively.

Shear stress (Fig. A.2), which is indicative of gel hardness or strength, was 33 kPa in surimi with sucrose/sorbitol at t_0, and 26.9 and 19.0 in the whey samples, 5 and



	0 #	1 #	3 #	5 #	7 #	9 #
Sorb/Sucr 4+4%	2.46 a	2.17 a	2.3 a	1.47 a	1.38 a	1.41 a
Dry Whey/Sucr 5+4%	2.2 b	2.01 b	1.79 b	1.09 b	1.25 b	0.99 b
Dry Whey/Sucr 8+4%	2.14 b	1.8 c	1.78 b	1.06 b	1.02 c	0.79 c

Fig. A.1 - Effect of Dry Whey/Sucrose and Sorbitol/Sucrose on Shear Strain in Pacific Whiting Surimi



	0 #	1 #	3 #	5 #	7 #	9 #
Sorb/Sucr 4+4%	33.05 a	28.19 a	30.44 a	16.12 a	13.27 a	14.54 a
Dry Whey/Sucr 5+4%	26.86 b	20.58 b	22.06 b	10.62 b	10.03 b	6.87 b
Dry Whey/Sucr 8+4%	19.02 b	15.23 c	18.64 b	8.7 b	7.85 c	8.11 c

Fig. A.2 - Effect of Dry Whey/Sucrose and Sorbitol/Sucrose on Shear Stress in Pacific Whiting Surimi

8% respectively. Stress remained high in all samples after 3 freeze/thaw cycles (30.44-22.06-18.64). There was a significant difference in stress between the 2 samples with dry whey after each of the first 5 cycles, which levelled out after 7 and 9 cycles. The biggest changes occurred after 5 cycles with relatively slow changes in following cycles. The sample with sorbitol/sucrose showed high stress value after 3 cycles, 30,000 kPa, compared to 5% dry whey sample with 22,000 kPa, and 18,600 kPa in 8% dry whey sample. According to the industry a stress value of 20,000 kPa is considered high. This shows that dry whey can be used as an alternative for other cryoprotectants although it is not as effective as the traditional mixture of sorbitol and sucrose.

Color Analysis

L* values (lightness) in samples with sorbitol/sucrose were lower (81-82) than in the whey samples (82-83) and (83-84), 5 and 8% respectively. The trend of increased lightness from sucrose/sorbitol < dry whey/sucrose < 8% dry whey/sucrose continued throughout the experiment, with the exception of L* 84.5 in 5% whey after 5 cycles and L* 86.95 in 8% whey after 9 cycles. Green hue (-a*) did not change much (-3.5 to -3.1), with samples containing sorbitol/sucrose somewhat closer to -3 than the others. Samples with dry whey were measured with more yellow hue (Table 1) than sorbitol/sucrose, and the 8% whey sample higher than the 5%. Whiteness, (L*-3b*) (Table 2), was significantly higher in sorbitol/sucrose samples than in whey samples (60-55-53) until the last cycle. After 9 cycles the difference had diminished, and the yellow color of the sample with 8% dry whey was not stable.

Table A. 1 - Whiteness (L*-3b*) of Surimi with Sorbitol/Sucrose and Dry Whey/Sucrose

Cycles of Freeze/Thawing	Sorb/Sucr 4+4%	Dry Whey/Sucr 5+4%	Dry Whey/Sucr 8+4%
0 #	60.39	54.9	53.14
1 #	60.74	54.99	51.78
3 #	62.09	55.15	53.73
5 #	60.1	55.3	50.41
7 #	60.27	55.77	51.24
9 #	55.94	52.95	54.54

Table A. 2 - Yellow hue (b*) in Surimi with Sorbitol/Sucrose and Dry Whey/Sucrose

Cycles of Freeze/Thawing	Sorb/Sucr 4+4%	Dry Whey/Sucr 5+4%	Dry Whey/Sucr 8+4%
0 #	6.93	9.14	10.01
1 #	6.96	9.15	10.53
3 #	6.86	9.16	10.08
5 #	7.49	9.74	10.97
7 #	7	8.82	10.45
9 #	8.59	9.76	10.75

Dimethylamine - (DMA)

There was almost no difference between the samples in DMA formation after 0 and 1 freeze/thaw cycle (Fig. 3). DMA was below 25 μg/g surimi in each sample. In following measurements, DMA increased by the number of freeze/thaw cycles. The DMA content increased rapidly after 3 cycles, especially in the 5% whey sample, and after 9 cycles, it had reached between 200 and 300 μg/g in whey samples, but was considerably less in the sucrose/sorbitol sample, 131 μg/g. The DMA produced in the 5% whey sample was higher than the DMA in 8% whey sample. Dingle et al. (1977) found fish with 225 μg/g DMA unacceptable because of toughness and sponge-like texture. DMA in stabilized mince ranged from 77-110 μg/g fish after 32 weeks of frozen storage, whereas control reached 207 μg/g fish.. This shows that 9 cycles of freeze/thawing is equivalent to much longer storage than 32 weeks.

Water Holding Capacity

The WHC of the samples with sucrose/sorbitol was somewhat higher than that for the dry whey samples (Fig. 4). There was no clear trend except that the samples lost WHC throughout the experiment and they all had a similar percentage of free water after 9 freeze/thaw cycles (38-39%).

pH Measurements

pH increased during the experiment in samples with sorbitol/sucrose (6.7 - 6.9) and samples with 5% dry whey (6.7 - 6.8) with peaks after 3 and 9 cycles, but it

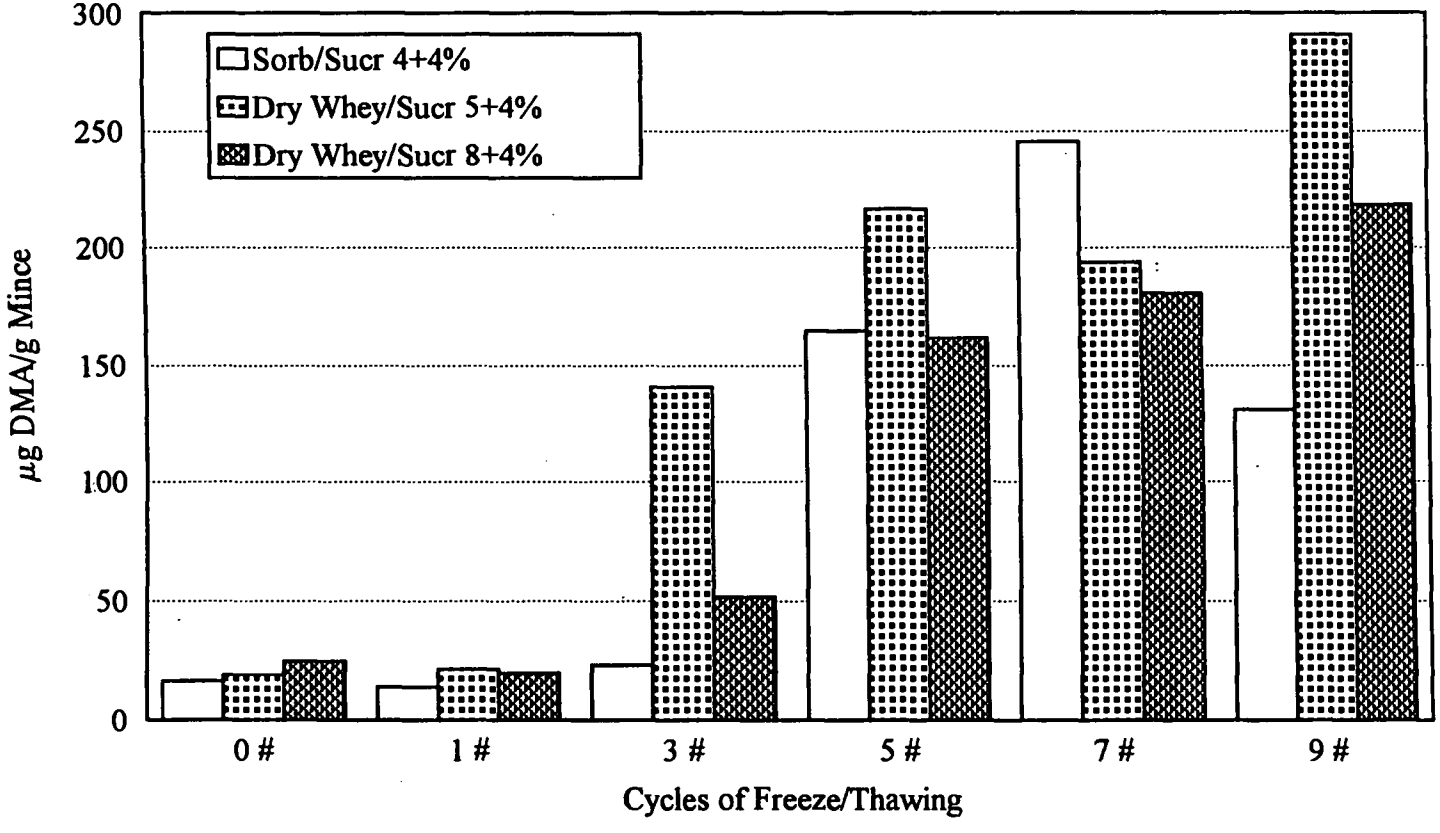


Fig. A.3 - Effect of Dry Whey/Sucrose and Sorbitol/Sucrose on DMA Formation in Pacific Whiting Surimi

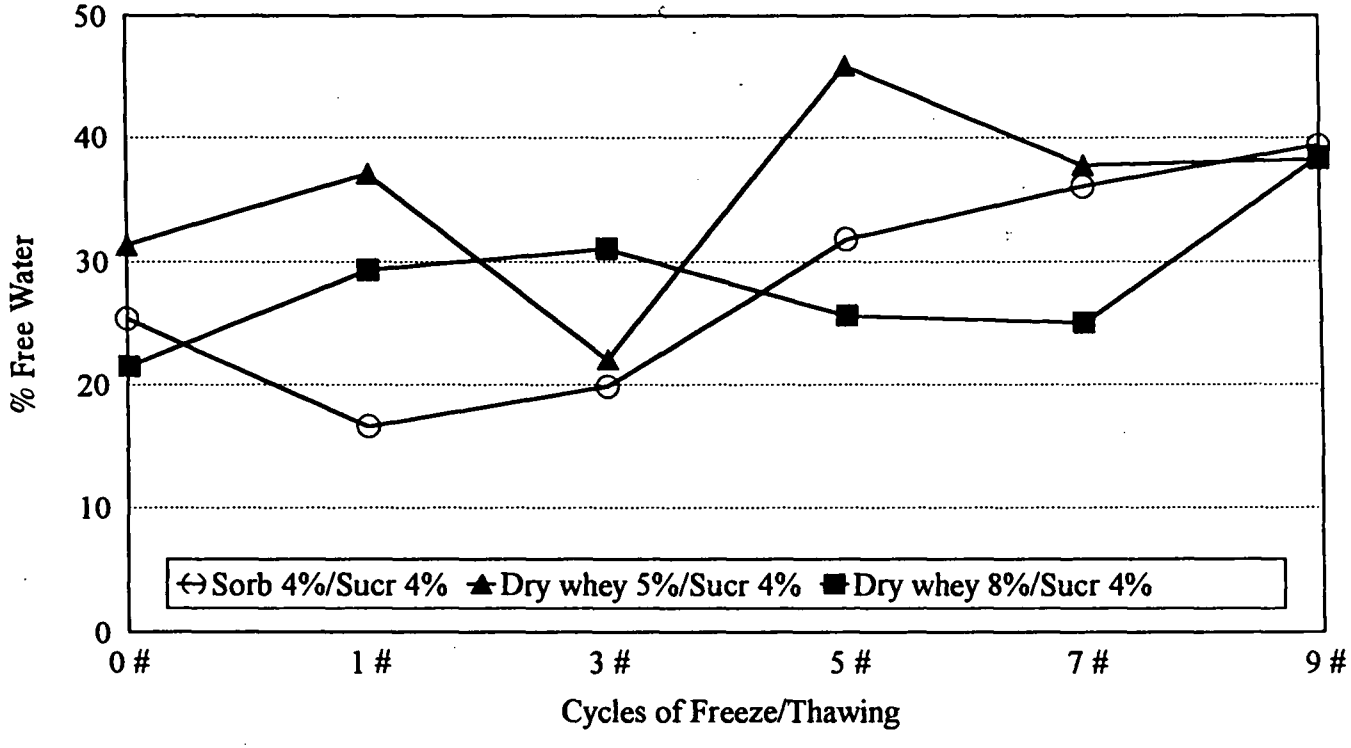


Fig. A.4 - Percentage Free Water in Surimi after Freeze/Thaw Cycles

decreased in samples with 8% dry whey, from 6.6 to 6.1 with a peak after 5 cycles. Fukuda et al. (1981) reported increased denaturation of the myofibrillar proteins of mackerel at pH below 6.5. Chung et al. (1993) found that the strain and stress values in Pacific whiting surimi were dependent on pH, and a decrease of 0.5 units between pH 6-7 would dramatically decrease torsion results. Therefore, it is assumed that the low pH in samples with 8% dry whey is the reason for lower stress and strain in these samples than the ones with 5% dry whey.

Conclusions

A mixture of dry whey/sucrose protected the functionality of the muscle proteins in surimi, although to a lesser extent than the sorbitol/sucrose mixture. After 7 and 9 cycles, gels with 5% dry whey produced significantly higher strain than samples with 8% dry whey. Shear stress was significantly higher for sucrose/sorbitol gels than for samples with whey after every freeze/thaw cycle. Samples with 5% dry whey were also significantly higher than dry whey at 8% levels after all cycles except the final freeze-thaw cycle. The highest increase in DMA, after 5 freeze/thaw cycles, coincides with the highest decrease in strain and stress.

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APPENDIX B:

THIN LAYER FREEZING OF MINCE

There are many factors that affect the storage stability of fish mince. Freshness of the fish is probably the most important factor. The time from harvest to processing is very critical for species like Pacific whiting because of tissue softening that takes place within 2 days of catch. Other variables include processing methods and storage temperature. Other important factors are stabilization of the proteins during frozen storage and the freezing rate.

Fast freezing of muscle tissue leads to formation of many small ice crystals in the tissue while slow freezing creates big ice crystals which damage the cell structure as they grow and accelerate the denaturation of proteins in the muscle. Madsen (1984) compared the frozen storage stability of minced cod frozen in a drum freezer and a plate freezer and found improved stability by using the drum freezer. The objective in this preliminary study was to measure the freezing rate of different thicknesses of stabilized mince from Pacific whiting frozen in a plate freezer to simulate the drum freezer technique. An evaluation of how the mince comes off in freezing sheets and breaks apart was also undertaken. The goal is to reduce changes that take place in the mince while freezing, especially at a slower freezing rate.

Materials & Methods

Fresh Pacific whiting fillets, processed at a local seafood processing plant, were stored in ice, and transported to the OSU Seafood Laboratory on the same day and ground by passing them through a 1/8 plate of an Autio Grinder, (Model 601 Hp, Actio Co., Astoria, OR). Two batches of mince were prepared; with 4% and 8% sucrose, w/w, respectively, and 0.3% polyphosphate. The mince and additives were mixed in a Hobart Mixer (Model A-200, The Hobart Mfg. Co., Troy, OH) for 6 min at 4°C. Samples of surimi (a washed mince) from Pacific whiting were also tested, with and without cryoprotectants (4% sucrose+ 4% sorbitol, and 0.3% polyphosphates). Mince samples were frozen in a plate freezer and the time to reach -10, -20, -30, and -40°C was recorded by a data logger, with 3 thermocouples inserted in each sample, and 1 placed between the space bar and the freezing plate and 1 on the freon pipe. Samples of ~ 450 g were placed on a sheet of steel or aluminum, 1 mm thick, previously sprayed with PAM (American Home Food Products, Inc., NY) and a rolling pin with specially prepared end plates (designed in the Department of Bioresource Engineering, OSU) used to determine the thickness of each sample, i.e., 3, 5 and 8 mm. A sheet of wax paper was used between the mince and the rolling pin to prevent sticking. Wooden frames were used as space bars to press the sheet with the sample closer to the freezing plate to avoid any air spaces. Hydraulic pressure was used to press the plates and frames together. A Data Logger (Model 3020 T, Electronic Controls Design Inc.) with a multiplexer, Series 3000, collected information from the thermocouples every minute. Three thermocouples were placed in the middle

of each mince sheet to measure the temperature, and one thermocouple was placed inside the freezer between the space bar and the freezing plate to check the temperature inside the freezer. One to three sheets with mince (3, 5, or 8 mm thick) were frozen each time in a plate freezer (Model no. "12", Amerio Plate Freezer, APV Crepaco Inc., Rosemont, Illinois) and the easiness of scraping the mince off the sheets with a spatula (6.5 cm width) evaluated. The samples were stored frozen at -20°C for further analysis.

Results

The freezing rate calculated from the data collected was slower than expected. The time needed to reach certain temperature in a 3 mm thick layer of mince with 4% sucrose was measured and found to be; 23 min for -10°C, 29 min for -20°C, 36 min for -30°C and 49 min for -40°C, with starting temperature of -27°C. The higher sucrose concentration the longer time needed to reach the appropriate temperature.

The steel sheets were easier to use than the aluminum ones, as the mince was easily scraped off with the aluminum. Raw surimi (a washed mince without additives), were difficult to spread out, and the same was found with the surimi containing cryoprotectants (sucrose/sorbitol, 8%). In general, it was easier to work with the mince with sucrose, probably because of more flexibility from the sugars. The ease of scraping off the 3 mm layer of mince did not seem to depend on the percentage of sucrose added (4% or 8%), rather the freezing temperature. At low temperature (-40°C), both 4 and 8% sucrose samples were almost glued to the sheet.

The 8 mm layers with 8% sucrose were more easily scraped off than mince with 4% sucrose. The frozen mince came off in flexible flakes, sometimes even in one piece. Only the samples that were appeared glued to the sheets came off in small brittle fragments.