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The major portion of Pacific whiting (PW) is commercialized in the form of frozen surimi. Alternative products for PW were investigated focusing on fresh surimi and texturized meat from PW mince. Fresh surimi is made without additives and kept refrigerated instead of frozen. Texturized meat is a meat-like product made from PW mince through freeze-texturization.

Fresh surimi was stored at 5°C and analyzed for its total aerobic plate count (APC), shear stress, shear strain, and color during 7 days storage. Frozen surimi from PW was prepared with 0, 3, 6, and 9% cryoprotectants and was compared with fresh surimi for its gel forming ability. Fresh surimi had a shelf life of 5 days and the gel forming ability remained unchanged throughout storage time. Shear strain of fresh surimi was not

different from frozen surimi with 9% cryoprotectants but shear stress was almost 3 times higher than the frozen one.

Texturized meat from PW mince was prepared from unwashed or 1-washed mince kept frozen for 6-8 mo with or without the addition of 6% cryoprotectants. The minces were comminuted into a protein slurry, formed into patties, and frozen at -7, -18, and -50°C. The evaluations of ice formation (by microscopic study), hardness, cook loss, color, and water holding capacity were carried out during 20 days storage. The results showed that texturized meat with parallel layers was made from 1-washed PW mince. Unwashed PW mince created a sponge-like texture and had rapid quality deterioration, thus it is not recommended for this product. Cryoprotectants did not significantly affect the texture formation of the product and are not required to store mince as raw material for the texturized meat. The optimum freeze-texturized temperature for this product was -18°C or lower because it minimized quality changes during storage depending on the desirable texture. The lower the temperature (higher freezing rate), the finer the layers created.

Alternative Products from Pacific Whiting: Fresh Surimi and Texturized Mince

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.

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ALTERNATIVE PRODUCTS FROM PACIFIC WHITING: FRESH SURIMI AND TEXTURIZED MINCE

I. INTRODUCTION

The annual catch of Pacific whiting in the US has been approximately 200,000 metric tons (MT) over the last ten years (OCZMA, 1994). In 1994, the harvest off the Northwest coast (Washington, Oregon, and California) was 179,073 MT of which ~65,000 MT was utilized by the Oregon shoreside processors (Talley, 1995).

The major portion of Pacific whiting is used for surimi processing. Although a high content of proteolytic enzymes which alter gel forming ability of myofibrillar protein in surimi is present in this species (Nelson et al., 1985; Chang-Lee et al., 1989), a high grade surimi can be produced by adding food grade protease inhibitors (Groninger et al., 1985; Porter et al., 1990; Morrissey, 1992; Morrissey et al., 1993; An et al., 1992). The use of this species is preferred in the form of minced flesh which allows the inhibitors to be mixed thoroughly. Although Pacific whiting has a limited market, at present, its abundance and low price present opportunities to improve the utilization of this species. Surimi production consists of a continuous process which includes heading, gutting, deboning, mincing, extensive washing, dewatering, mixing with cryoprotectants, and freezing. Through these processes, only a 15-20% yield is obtained for surimi while about 32% yield is obtained for the initial mince before washing (Toyoda et al., 1992). Chang-Lee et al. (1990) reported a yield of 36.2% for mince and 19.5% for surimi from

Pacific whiting on a pilot scale process. This means mince has almost twice the yield than surimi.

Efforts to utilize Pacific whiting mince have been made by many researchers. Simpson et al. (1994a, 1994b) reported that a high quality surimi could be made from Pacific whiting mince, with 6-12% cryoprotectants, stored at -20°C for 6 mo. This effort was extended to a study of physical and chemical changes in stabilized Pacific whiting mince during storage (Magnusdottir, 1995). Seafood patties made from Pacific whiting mince blended with shrimp were developed at the OSU Seafood Lab (Babbitt et al., 1976). This product was reintroduced and successfully accepted by both consumers and wholesalers (Morrissey and Sylvia, 1995).

In this study, two new concepts for utilizing Pacific whiting were introduced. These are fresh surimi, a surimi made without an addition of cryoprotectants and kept refrigerated instead of frozen, and a texturized meat from mince. The production of fresh surimi can shorten the processing operation by eliminating the mixing and freezing processes. This could lead to an increased production capacity and reduced processing cost by eliminating frozen storage and addition of cryoprotectants. Furthermore, fresh surimi can be used as a raw material for low- or non-sugar surimi-based products. It is also possible to make never-frozen surimi-based seafood using fresh surimi as opposed to frozen surimi products.

Another study focused on Pacific whiting mince. Fish mince has a functional advantage over surimi or mechanically deboned mammalian or poultry meat for certain products as it exhibits a more meat-like texture (Lanier, 1994). A freeze texturization technique was applied to produce a texturized meat from fish mince. By using this

technique, fish mince can be produced into various forms with a flaky texture. Moreover, texturized meat is free of pin bones, is easy to prepare, and can be mixed thoroughly with protease inhibitors to overcome the softening problem, particularly in Pacific whiting. In addition, this product could be further processed as flavored- or seasoned-fish meat or a ready-to-eat food.

The overall objectives of this study were to investigate new ways to utilize Pacific whiting as an alternative to conventional frozen surimi focusing on 'fresh surimi' and 'texturized meat'. This includes the feasibility of using fresh surimi, development of processing conditions for texturized meat from Pacific whiting mince, and storage effects of mince on quality attributes of texturized meat products.

Literature Review

Surimi Processing

Harvesting and Handling

Post-harvest handling and freshness of fish strongly affect the quality of surimi. High quality surimi can only be made from fresh and well handled fish. Improper handling creates softening of the tissue and disruption of final quality due to bruising of muscle. Even though surimi has its maximum gel-strength when fish is instantly processed after death, it is impractical to process a large quantity of fish before they enter the rigor stage, particularly for land-based plants. Once fish enter rigor, they are difficult to clean and surimi made from these fish tend to have a fishy odor.

Toyoda et al. (1992) suggested that fish need to be stored at temperature below 5°C. During transportation, refrigerated sea water (RSW), slush ice, or champagne ice (aerated slush ice in which water and ice are mixed thoroughly) can be used as a refrigerant. Peters and Morrissey (1994) recommended use of a champagne ice system for storage of fish on vessel due to its fast cooling ability which takes only ~1 hr to cool fish to 1-2°C while longer time is required by RSW.

Before processing, fish need to be separated from other species, sorted according to size, and washed. The wash water should be soft water containing no or very little dissolved salts and metals (Sonu, 1986).

Filleting and Mincing

The filleting process removes heads, guts, backbones, and fins, yielding only boneless fillets. Internal organs of fish need to be completely removed due to their high level of proteolytic enzymes which decrease the gel-forming property of surimi (Suzuki, 1981). In Pacific whiting surimi production, the impurity score can be lowered by the removal of black fleshed fillets infected with senile myxosporidean parasites (Park, 1995a). It is estimated that 4-5% of Pacific whiting are contaminated with these black hair-like strands (Morrissey et al., 1993). These contaminants show no health hazard but give defects of "ash in snow" in surimi which are unacceptable.

After being headed and gutted, fish meat is separated from bones, scales, fins, and other large impurities using a roll-type meat separator. This machine consists of a moving rubber belt and a steel drum with numerous holes of 3-5 mm in diameter. The fish meat

on the moving belt is pressed against the drum and the fish is minced and squeezed out through the holes. This step separates the fish mince from other impurities which are not able to pass through the holes. Yield can be increased by raising the pressure between the belt and the drum, however, this may increase the amount of impurities. Fish mince obtained from deboned fillets is cleaner but the yield is less than that from deboned carcass (Pigott, 1986). As soon as the fish mince comes out of the meat separator, before the washing process, it is immediately submerged in chilled water to avoid oxidation of blood in the fish mince.

Washing and Dewatering

The main purpose of washing the fish mince is to remove fats, sarcoplasmic proteins, and compounds that contribute to fishy odor. In return, the washing provides the product with a white appearance (Shimizu, 1979), and concentrates myofibrillar proteins resulting in improved textural properties (Nishioka, 1984;Park, 1995a). The washing process begins with stirring the mixture of minced meat and cold water in the wash tank to release and float out the fat and oil from the muscle tissues (Miyauchi et al., 1975; Suzuki, 1981). Then, the water is removed by screening and dehydrators or centrifugation to 5-10% solid content. The number of washing cycles and water/mince ratio are dependent on fish species, fish freshness, type of washing unit, and expected quality of surimi (Lee, 1984). In shore-based operations, about 29 kg waste water is generated for the production of 1 kg surimi (Lin et al., 1995). A screw press is used for the final dewatering which reduces moisture content to 80-82%. This dewatered fish mince is called 'fresh surimi'.

After the first washing cycle, approximately 50% of all water-soluble materials are removed. Continued washing concentrates myofibrillar proteins by the complete removal of water-soluble components, such as blood, dark pigments (melanin), and other impurities (Miyauchi et al., 1975; Suzuki, 1981; Adu et al., 1983). Gel strength of kamaboko increased as the washing cycles are extended (Nishioka, 1984). However, Lin and Park (1995) reported a significant loss of myofibrillar proteins which became slightly soluble and were lost during the extensive washing. Moreover, by washing with 0.25%, 0.5%, or 1% NaCl solutions, the loss of myofibrillar proteins could be reduced.

Extensive washing removes most of the water-soluble salt from the fish mince and causes the meat to hydrate and swell due to the increased hydrophobicity of myofibrillar proteins. As a result, it is difficult to dewater the fish meat (Sonu, 1986). It is not uncommon to add 0.1-0.3% salt, a mixture of NaCl and $CaCl_2$, to the final washing water for the easy removal of water from the mince flesh (Park, 1995a).

Mixing with Cryoprotectants

To prevent protein denaturation during freezing and frozen storage, cryoprotectants are added to the fresh surimi. Cryoprotectants are generally comprised of 4% sugar, 4-5% sorbitol and 0.2-0.3% polyphosphate. In Pacific whiting surimi, enzyme inhibitors, gel enhancers, and color enhancers are added along with cryoprotectants. These additives are sucrose, sorbitol, sodium tripolyphosphate, tetrasodium pyrophosphate, calcium compounds (calcium lactate, calcium sulfate, calcium citrate, calcium caseinate), sodium bicarbonate, mono-or diglyceride, and partially hydrogenated canola oil (Park and Morrissey, 1994). A silent cutter is normally used in a batch-process

operation and the additives are mixed uniformly to the dewatered meat. The use of a ribbon blender in the batch-process operation is not recommended due to heat generation during blending.

Freezing and Frozen Storage

After the surimi is mixed with cryoprotectants, it is formed into 10-kg (22 lbs) blocks in polyethylene bags. They are then placed in stainless steel pans and frozen in a plate freezer. Drum freezing, which may enhance surimi quality and offer a more accessible product form, has been introduced by Lanier et al. (1992). The contact (plate) freezer, however, is still preferred by the processors due to their limited storage space (Park, 1995a). Surimi blocks are held in the plate freezer for about 2 1/2 hrs or until the center reaches -25°C (Park, 1995a). The frozen surimi blocks should be kept under -20°C with minimum temperature fluctuation during storage (Sonu, 1986).

Physico-chemical Changes during Frozen Storage

Changes in Product Quality

During freezing, the formation of low molecular weight compounds from lipid oxidation or protein degradation results in flavor deterioration, such as off-flavor, bitterness, rancidity, or an unsatisfactory fishy taste. These quality changes are dependent on many factors during storage, such as storage time, freezing rate of products, package type, and product composition (Bhattachaya et al., 1988). Irreversible protein changes

occur in muscle during freezing. Changes in protein-bound pigments may cause undesirable color and appearance. These changes include lower intensity of tissue color, reduced surface glossiness, development of freezer burn and surface dehydration, increased drip loss, and opacity or chalky appearance of muscle (Mutsumoto and Matsuda, 1967). The protein deterioration caused by freezing also significantly decreases gel forming ability, water holding ability, and fat emulsifying capacity (Holmquist et al., 1984; LeBlanc et al., 1988; Park et al., 1988; Hsu, 1990; Sych et al., 1991; Yoon and Lee, 1990). Loss of gel forming ability (shear stress and shear strain) of fish mince or surimi due to frozen storage has been reported by Park et al. (1988, 1993), Hsu (1990), Magnusdottir (1995), and Pipatsattayanuwong et al. (1995).

Textural Changes

Textural deterioration caused by freezing has been extensively studied in cod and pollock (Love et al., 1982; Babbitt and Reppond, 1987; Leblanc et al., 1988; Sych et al., 1990a). During frozen storage, minced gadoid white fish (cod, haddock, pollock, whiting) exhibits a hard and rubbery texture (Sikorski et al., 1976). This is due to the formation of dimethylamine (DMA) and formaldehyde (FA) which are the result of the decomposition of trimethylamine oxide (TMAO) by TMAO demethylase (Yamada et al., 1969; Crawford et al., 1979; Hultin, 1992). Toughness increased due to the number and strength of existing bonds between the myofibrillar proteins (Connell, 1962) and the formation of a three-dimensional network caused by FA (Amano and Yamada, 1965; Castell et al., 1973; Dingle et al., 1977; Gill et al., 1979; Poulter and Lawrie, 1979; Perkin and Hultin, 1982b).

Freeze denaturation was suggested to be due to damage caused by the growth of ice crystals, dehydration, elevation of salt concentration, change in pH, ionic strength, surface tension, or the removal of water by ice formation (Sikorski et al., 1976; Partmann, 1977; Mutsumoto, 1980; Shenouda, 1980; Suzuki, 1981; Park, 1994a). Freeze denaturation was the result of a direct side-to-side aggregation of F-actomyosin macromolecules to form insoluble polymers (King, 1966) with no conformation change (Rodger and Hastings, 1984). The aggregation of actomyosin or myosin is due to the development of cross-linking caused by formation of hydrogen bonds, ionic bonds, hydrophobic bonds, and disulfide bonds (Akahane, 1982; Mutsumoto, 1980). However, Connell (1964) and Mutsumoto (1980) did not observe a significant change of total free -SH groups during frozen storage.

Sarcoplasmic and stroma proteins are not significantly affected by freezing (Park, 1994a). Within myofibrillar protein groups, myosin is the most sensitive to freeze denaturation while actin shows only a slight change (Connell, 1960, Akahane, 1982) and troponin is the most stable myofibrillar protein during freezing (Mutsumoto, 1980).

Changes in Protein Solubility

Another property strongly affected by freeze denaturation is protein solubility (Sikorski et al., 1976). Decreased protein solubility during frozen storage is mainly a result of changes of the myofibrillar proteins (Owusu-Ansah and Hultin, 1992), but not sarcoplasmic proteins (Sikorski et al., 1976). Solubility of sarcoplasmic proteins remain unchanged except after long term storage. Yoon et al. (1991) suggested that formation of hydrogen, hydrophobic, disulfide bonds, and protein cross-linking produced by FA, during

frozen storage, reduces the solubility of proteins (Childs, 1973; Castell et al., 1973). Precipitation of actomyosin was also observed to be correlated with frozen storage time (Carche and Tejada, 1991; Ito et al., 1991; Kumazawa et al., 1990).

Effects of Washing on Fish Mince

The washing process plays an important role in surimi processing in order to manufacture high quality surimi with bland flavor, light color, and a unique textural property (Shimizu, 1979; Lanier et al., 1982). Water soluble proteins (sarcoplasmic proteins), fats, oils, pigments, enzymes, trimethylamine oxide (TMAO), minerals, impurities, and other nitrogenous compounds were markedly reduced in washed fish mince (Okada and Noguchi, 1974; Adu et al., 1983; Lin, 1992; Chou, 1993). These substances may have adverse effects on the quality of minced meat during storage (Adu et al., 1983; Tseo et al., 1983; Sonu, 1986; Regenstein, 1986; Chang-Lee et al., 1990; MacDonald et al., 1990).

Washing fish mince resulted in a 37% loss of total solids. Ash and lipids were the most reduced (80% and 65%, respectively) (Adu et al., 1983). Lin (1992) reported a reduction of 43.4% fat, 42.1% protein, and 30.9% ash in butterfish mince.

It has been reported that washing is a method of concentrating myofibrillar proteins. The washed meat had higher functional properties than unwashed (Hsu, 1990; Morioka and Shimizu, 1990; Chou, 1993). Washing resulted in hard texture development for the mince after long term storage and freeze-thaw cycles, but did not affect the texture in short term storage (Yoon et al., 1991). The gel forming ability of fish meat increased as the number of washing cycles increased (Nishioka, 1984; Mutsumoto and Noguchi, 1992;

Okada, 1964; Lee, 1984; MacDonald et al., 1990). This is due to the higher concentration of myofibrillar proteins which are responsible for gel formation during heating (Suzuki, 1981; Lanier et al., 1982). Moreover, water-soluble proteins interfered with the formation of intermolecular cross-links of myofibrillar proteins. Myofibrillar proteins were thought to favorably form cross-links with sarcoplasmic proteins causing less myofibrillar proteins to be available for gelation (Okada, 1964).

Extensive washing removed water soluble compounds and fat from fish mince which greatly improved the stability of fish mince during frozen storage (Miyauchi et al., 1975; Adu et al., 1983; Hennigar et al., 1988). The removal of water soluble components could also remove a naturally-occuring cryoprotectant from fish meat. Functional properties of unwashed fish mince were less affected by the freezing process than those of washed mince, but they were less stable over long term frozen storage (Mutsumoto and Noguchi, 1992). However, by combining the washing process (which improved storage stability) and the addition of cryoprotectants (such as sugar and sorbitol), washed fish mince (surimi) can be kept frozen with a minimum loss of functionality.

The mineral composition of fish meat was greatly affected by washing. Phosphorus, potassium, and sodium levels were reduced but the iron, copper, zinc, and chromium levels were increased in the washed mince compared to the filleted and unwashed mince (Adu et al., 1983; Lin, 1992). Lin (1992) observed a reduction in calcium level in the mince after washing, but it was higher than the calcium level in fillets. Levels of minerals in washed fish meat, however, are strongly affected by the quality of washing water (Adu et al., 1983). According to Lee (1986), washing solutions containing

high levels of calcium and magnesium may improve textural properties, while high levels of iron and manganese may create an undesirable color of washed mince.

Furthermore, temperature and salt content of the washing solution greatly affect the quality of washed fish mince. Watanabe et al. (1990) reported that washing with 49°C water accelerated rigor-mortis, ATP degradation, and resulting in an 8-9 times rise of ATPase activity in carp muscle compared to washing with 10°C water. Lin and Park (1995) determined the loss of myofibrillar proteins during washing by measuring myosin heavy chain (MHC) in the solutions. The loss of MHC was the least at 0.5-1.0% salt concentration. At salt concentration higher than 1.0% or lower than 0.5%, the loss of MHC increased. The greatest loss of MHC, 42.9%, was found when fish mince was washed in a water contained no salt (0.0% NaCl) for 4 washing cycles.

Texture Properties of Fish Mince/Surimi

The most important textural property of fish mince is gelling property. Gelation of fish mince is thermo-irreversible (Lanier, 1995). Fish myofibrillar proteins, responsible for the gelation property, can form a strong and elastic gel upon heating. Unlike myofibrillar proteins of mammalian or avian, fish myofibrillar proteins can be heated to higher temperatures without sacrificing gel strength or water holding capacity (Hamann and MacDonald, 1992). Their gel forming ability is greatly improved through a cold temperature preincubation (setting) (Montejano et al., 1984). Surimi has better cohesiveness and binding ability than other food proteins or hydrocolloids (Lanier, 1986).

This property is primarily dependent on the quantity and quality of myosin in surimi (Nishioka et al., 1990).

Cold setting enhances gelation of surimi (Niwa, 1992) by creating covalent bonds which affects the elastic and cohesive nature of gels (Lanier, 1986). It is believed to be an effect of a native transglutaminase enzyme (Kishi et al., 1991; Kimura et al., 1991). The proper setting temperatures are most likely dependent on the fish inhabitant temperature (Kamath et al., 1992). The optimum setting temperatures were suggested to be 25°C for Alaska pollock surimi (Howe et al., 1994), 35-40°C for Atlantic croaker surimi (Lanier et al., 1982; Wu et al., 1985), and 25°C for Pacific whiting surimi (Park et al., 1994).

Greenberg et al. (1991) suggested that the addition of transglutaminase from other sources can enhance the activity of transglutaminase in surimi. These include beef plasma (Kurth and Rogers, 1984) and enzyme from a microbial source, *Streptoverticillium* species (Knight, 1990). Sakamoto et al. (1995) and Seguro et al. (1995) reported that microbial transglutaminase increased the gel strength of pollock surimi gels by increasing cross linking of myosin heavy chains.

Yongsawatdigul and Park (1995) demonstrated, by ohmic heating, that slow heating improved shear stress but did not affect shear strain of pollock gels. The increasing shear stress was found to be correlated with the enhancement of myosin heavy chain cross-linking through covalent bonds. They also found that slow heating reduced both shear stress and shear strain of Pacific whiting gels, while fast heating improved shear stress and strain significantly. This could be the effects of both endogenous proteolytic enzymes in whiting and the nature of whiting which may have minimal covalent cross-linking of myosin.

Because many fish species suffered gel weakening during the conventional heating process due to proteolytic enzymes (Niwa, 1992), ohmic heating and high-pressure treatment at low temperatures were introduced for surimi gelation. Ohmic heating increased more than twofold in shear stress and shear strain of Pacific whiting surimi gels without protease inhibitor compared with those cooked by a conventional water bath (Yongsawatdigul et al., 1995). The rapid heating rate of ohmic heating minimized the degradation of actin and myosin resulting in a continuous network structure. As a result, the gel forming ability was improved.

High hydrostatic pressure (HHP) has shown its ability to decrease microorganisms, reduce enzyme activity, and improve textural changes in many foods (Farr, 1990; Okamoto et al., 1990; Hoover, 1993). Surimi gels were formed at 0°C in 10 min under 2.0-4.0 kbars of pressure (Shoji et al., 1990). Okazaki and Nakamura (1992) reported a gelation of sarcoplasmic proteins from different fish depending on fish species, pH, protein concentration, and pressure treatment. Recently Chung et al. (1994) reported increased strain values at all pressure/temperature combinations compared with heat-set controls in Pacific whiting. They also found that shear strain and stress of HHP-treated whiting gels, without inhibitors, were 3 times higher than gels prepared in a conventional water bath.

Ice Crystal Formation

Nucleation

When sufficient heat is removed from a liquid or a food system, freezing may occur. Freezing implies the phase change from water to ice and the freezing point is the

temperature at which solid and liquid phases coexist in equilibrium (Blond and Colas, 1991). However, before freezing takes place, nuclei needs to be generated. This process, nucleation, is required and necessary to initiate ice crystals before crystallization occurs (Mehl, 1989; 1990; 1992).

Supercooling is defined as a state when temperature decreases but there is no corresponding change in the system (Reid, 1983). This state will last as long as nucleation does not occur in the liquid phase (Ryder, 1987). However, once supercooling is reached, nuclei can be generated. The presence of solute and impurity greatly affect the degree of supercooling and the nucleation process (Franks et al., 1984). The nucleation rate also highly dependent on viscosity and dynamic interactions which include vibration, physical shock, and friction (Fennema et al., 1973). It was observed that in aqueous systems the nucleation rate increased as supercooling increased resulting in smaller and more numerous ice crystals (Franks 1985). This causes less damage to biological cells and minimizes the quality loss of frozen products.

Efforts to increase nucleation rate have been widely made in order to minimize the damage and quality loss of frozen products. Numerous ice nucleating agents, both non-biological and biological, have been studied. Non-biological sources were mineral particles, i.e., silver iodide or dust. Biological resources were proteins, amino acids, steroids, etc. (Lindow, 1983). These compounds, however, can induce ice nucleation only when they are in a crystalline form (Parungo and Lodge, 1965). Recently, many species of bacteria have been studied for their use as biological ice nucleators.

IN-active cells are suggested to be the most reactive heterogeneous nuclei (Lindow, 1983). IN-active bacteria included *Psudomonas* strain, *Erwinia* strain, and

Xanthomonas strain (Watanabe and Arai, 1994). Ryder (1987) successfully applied the use of *P. syringae* as an ice nucleating agent in frozen Atlantic salmon. He found that *P. syringae* treated samples had higher numbers of nuclei (or crystals) and smaller crystal sizes at -5, -10, and -18°C. Watanabe and Arai (1994) isolated *Xanthomonas campestris* INXC-1 from tea shoots and used as a primary nucleation agent in freeze concentration of fresh foods: raw egg white, fresh milk, and fruit juice.

Macromolecules or food grade polymers also demonstrated their nucleation ability in water (Reid,1983). Food gums (such as sodium alginates) and emulsified starch granules increased heterogeneous nuclei population in water (Blond and Colas, 1991). However, methylcellulose and xanthan gum were found to have slightly inhibitory effects (Muhr et al., 1986).

Crystallization

After nucleation is accomplished, crystallization is the next process in freezing. The formation of ice crystals raises the temperature of the system due to the latent heat released from crystallization (Bevilacqua et al., 1979; Reid et al., 1994). Thus, no further nucleation is generated. Mehl (1990) reported that crystal growth occurs at a higher temperature than nucleation. A high nucleation rate affiliated with a limited rate for the crystal growth creates a smooth crystalline texture in frozen foods (Blond and Colas, 1991).

Once ice formation occurs, liquid is separated to form ice crystals. This causes the concentration of the unfrozen liquid in contact with ice to increase. At each temperature, there is a specific equilibrium of ice content at the interphase as ice moves forward (Reid

et al., 1994). However, supercooling can happen at the interphase which can produce an irregular interphase. At this irregular interphase, crystalline grew in dendrites or columns from the interphase toward liquid (Bevilacqua et al., 1979). Ice crystals tend to grow in columnar structure in the direction of heat flow, away from the refrigerated border but do not grow in their sides (Bevilacqua et al., 1979).

Ice crystals grow by adhering water molecules to the already formed crystals (Mogens, 1984). Therefore, the rate and direction of crystal growth will also depend on free water molecules available. All dissolved substances retard the growth rate of ice crystals in solutions (Blond and Colas, 1991). This may be because of less available free water in the higher concentration solution. Budiaman and Fennema (1987) reported the ability of various hydrocolloids to inhibit crystal growth depending on the nature of the polymer. They found that locust bean and guar gums were most effective. Concentration of solutes was also observed to affect the rate of ice crystal growth; growth rate decreased as concentration increased (Blond , 1988).

Mehl (1990) reported that the crystals grew from the nuclei generated during nucleation and the number of growing nuclei was constant throughout the freezing period. However, recrystallization or ripening often occurs in food systems (Sutton et al., 1994). This is the phenomena that small ice crystals reduce in number and size and join together to create larger ice crystals. The rate of recrystallization was suggested to be dependent on the temperature and solute concentration gradients. The crystal growth was found to be more pronounced at higher temperatures (Bevilacqua and Zaritzky, 1980) and be numerously accelerated by temperature fluctuation (Mogens, 1984). Donhowe et al.

(1991) observed a significantly increased ice crystal size during the first 7 weeks of storage at -20°C but no significant changes for further storage.

Ice crystal formations can be measured by histological method, by visual observation using microscope and video camera, or by calorimetric method. Histological study of ice crystal formations can be carried out by fixing a frozen sample in 10% formaldehyde or Carnoy fluid , dehydrating in alcohol, embedding in paraffin, sectioning, staining (with heamotoxylin-eosin for protein samples), and viewing with a microscope (Ryder, 1987). The holes that appear in the sample are interpreted as ice crystals.

The crystal growth can also be observed by a cryomicroscope connected to a video camera. The crystal growth can be recorded on video tape and viewed on a TV screen at which crystallization can be visualized (Mehl, 1993). This was extensively used for observation of ice formation in liquid samples and ice cream (Donhowe et al., 1991). Then, image analysis was used for quantitative determination of crystal size, ice volume, or frozen fraction.

The calorimetric method can be used to observe nucleation and crystallization of ice. DSC is extensively used as calorimeter in this study. DSC thermogram shows energy changes during freezing of samples. The beginning of crystallization is observed once the exothermic peak started. As crystallization ends, the system begins to absorb energy. Upon continuous warming of frozen sample, the temperatures at the beginning and the end of crystallization can be observed (Mehl, 1990).

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II. FUNCTIONAL PROPERTY AND SHELF LIFE OF FRESH SURIMI FROM PACIFIC WHITING

Abstract

Total aerobic plate count (APC), shear stress, shear strain, and color of fresh Pacific whiting surimi stored at 5°C were determined at day 0, 1, 3, 5, and 7. Frozen surimi was prepared with four levels of cryoprotectants (0, 3, 6, and 9%) and was compared with fresh surimi for gel-forming ability. Fresh Pacific whiting surimi had a shelf life of 5 days. The gel functionality remained unchanged throughout the storage time. Strain values of fresh surimi were not different from those of frozen surimi with 9% cryoprotectants, but stress values of fresh surimi were almost 3 times higher than those of frozen surimi.

Key Words: fresh surimi, shelf life, Pacific whiting

Introduction

Pacific whiting (*Merluccius productus*) is the most abundant species off the coast of Washington, Oregon, and Northern California with a total allowable catch of 140,000 to 250,000 metric tons a year (NMFS, 1993). Processing of surimi from Pacific whiting was not commercialized in the U.S. until 1991 for at-sea operations and 1992 for shore-side operations. Surimi is stabilized fish myofibrillar proteins. It is produced by continuous processing steps of heading, deboning, mincing, washing, dewatering, and freezing with cryoprotectants. The washing and dewatering processes free fish meat from sarcoplasmic proteins, fat, and undesirable materials such as skin and fine bones. High-quality surimi is naturally odorless and colorless.

All commercial surimi is kept frozen. Cryoprotectants are required to minimize protein denaturation during frozen storage. Sucrose (4%) and sorbitol (4-5%) are commonly used along with 0.3% sodium phosphate. Several studies of different cryoprotectants to measure changes of protein functionality during frozen storage have been conducted (Arai et al., 1970; Noguchi et al., 1975, 1976; Park et al., 1987a, b; Park and Lanier, 1990; Sych et al., 1990a, b). A higher level of cryoprotectants contributes a notable degree of sweetness to surimi-based foods. Consumers often consider high sugar or sweeter products as undesirable (Baute, 1994). Search for non-sweet or low-sweet carbohydrates as effective cryoprotectants has been reported by Arai et al. (1984), Watanabe et al. (1985), Sase et al. (1987), Park et al. (1988), Sych et al. (1990a, b), and Lanier (1993). Polyalcohols, polydextrose, maltodextrin, and starch hydrolysates have been investigated. Though their cryoprotective effects were comparable to sucrose or

sorbitol, these alternatives have not been used due to legal issues and the lack of commercial tests.

During frozen storage, several changes occur in fish muscle proteins. These include denaturation, ice crystallization, dehydration, and changes in intramolecular conformation, such as salt-soluble protein, pH, ionic strength (Park, 1994b). Many proteins have exhibited instability as measured by the partial loss of functionality at subfreezing temperatures. The deterioration of proteins during frozen storage is reflected by their sharp decrease in gel-forming ability, water-holding capacity, and fat-emulsifying capacity (Iwata and Okada, 1971; Cheng et al., 1979; Holmquist et al., 1984; LeBlanc et al., 1988; Park et al., 1988; Hsu, 1990; Yoon and Lee, 1990; Sych et al., 1991). Quality changes due to freeze-denaturation can be minimized during storage with cryoprotectants. However, losses of salt-soluble proteins and gel-forming ability still occurred in cryoprotected frozen surimi. Park et al. (1988) and Sych et al. (1991) reported a loss of salt-soluble proteins in cryoprotected surimi during frozen storage. Furthermore, gel functionalities, stress and strain, of surimi mixed with cryoprotectants decreased notably due to freezing (Hsu, 1990; Sych et al., 1991; Simpson et al., 1994) and continued to decrease over prolonged storage (Park et al., 1988; Sych et al., 1990b).

Before 1960, all surimi used in the Japanese kamaboko industry was fresh surimi (Okada, 1990). There was no method available to control freeze-denaturation until Nishiya developed a technique by which freeze denaturation of proteins in Alaska pollock surimi could be prevented (Matsumoto, 1979). They added cryoprotectants such as sucrose and sorbitol to stabilize myofibrillar proteins. The use of fresh surimi in the U.S. was almost impossible due to the geographical distance of Alaska pollock surimi plants

(Dutch Harbor and Kodiak, AK) until shore-side operations were established in Oregon. Benefits of using fresh surimi over frozen surimi are: fresh surimi can be produced at a lower cost without additives and freezing process; it can be used for no-sugar or low-sugar products; it can add value to some products like 'never-frozen' surimi-based crabmeat; it can show better gel functionality and be used further at a reduced level.

The objectives of our study were to investigate gel functionality and shelf life of fresh Pacific whiting surimi and compare it with currently available frozen surimi.

Materials & Methods

Fresh Pacific whiting surimi was collected from Point Adams Packing Co. (Hammond, OR) immediately after the dewatering process and before mixing with cryoprotectants and other ingredients. Fresh surimi was placed in a polyethylene bag, kept in ice, and transported to the OSU Seafood Laboratory within 30 min. The moisture content of fresh surimi was 81±0.7% by a rapid microwave method (Morrissey et al., 1993). The testing consisted of 3 parts: 1) shelf life and functional quality of fresh surimi; 2) functional quality of frozen surimi at different levels of cryoprotectants during storage; and 3) comparison of fresh surimi with frozen surimi.

Fresh Surimi

Fresh Pacific whiting surimi was packed in food-grade plastic bags, 1.00±0.05 kg each. All samples were sealed without vacuum and kept at 3-5°C. Fresh surimi samples

were evaluated for total aerobic plate count (APC), shear stress, shear strain, and color at day 0, 1, 3, 5, and 7 during storage. All experiments were repeated twice.

Frozen Surimi

Frozen surimi was prepared by mixing fresh surimi with 0, 3, 6, and 9% cryoprotectants, a mixture (1:1) of sucrose and sorbitol (ICI Specialties, New Castle, DE), and 1% beef plasma protein (BPP; AMPC, Inc., Ames, IA) based on total weight. Mixing was conducted for 6 min in a cold room (5°C) using a Hobart mixer (model S301, Hobart Mfg Co., Troy, OH). Sub-samples of 1.00±0.05 kg were packed in food-grade bags, sealed without vacuum, frozen in a blast freezer (-27°C), and kept in the freezer at -18°C. Frozen surimi samples were evaluated for textural properties and color before freezing (day 0), and after 1 day, 2 wk, and 2 mo of frozen storage. Each treatment, with different levels of cryoprotectants, was prepared separately from different batches of fresh surimi. All experiments were repeated twice.

Freezing Effects on Surimi with Different Levels of Cryoprotectants

Fresh Pacific whiting surimi was mixed with 0, 3, 6, and 9% cryoprotectants, a mixture (1:1) of sucrose and sorbitol, and 1% BPP (w/w) for 6 min in a refrigerated room. We studied changes of shear stress and shear stain of surimi gels due to freezing. All treatments were prepared from the same batch of fresh surimi. Experiments were

conducted before freezing and after 20-24 hr of frozen storage. The experiment was repeated 4 times.

Total Aerobic Plate Count

A 10-g surimi sample was homogenized for 2 min with 90 mL of 1% sterile bacto-peptone water (Bacto-Peptone; Difco Laboratories, Detroit, MI). Higher dilutions were made, poured and spread on Petrifilms (3M, St. Paul, MN) for aerobic plate count. Petrifilm samples were incubated at 37°C for 48±3 hr. The total number of colonies were counted and reported as APC/g sample.

Gel Preparation

Moisture content of each sample was determined before gel preparation using a microwave method described by Morrissey et al. (1993). All gel formulations (with salt and BPP) were adjusted to equal moisture at 80.8-80.9%, using ice if necessary. Protein content was calculated based on moisture, cryoprotectants, and surimi content in each gel formulation. Surimi samples were comminuted with 2% salt, 1% BPP (based on total weight), and ice water in a Stephan vertical vacuum cutter (model UM 5 Universal; Stephan Machinery Corp., Columbus, OH.). Gels were prepared as described by Park et al. (1994) and cooked immediately at 90°C for 15 min. Gels removed from the water bath were chilled in ice water and stored overnight in a refrigerator.

Shear Stress and Shear Strain by Torsion Method

Gel samples were removed from the refrigerator and kept at room temperature (≈23°C) for 2 hr prior to the torsion test. Gels were cut into 2.9 cm length and both ends glued to plastic discs with glue (Krazy Glue Inc., Itasa, IL). Twelve sample specimens were used for each test. All gels were milled into an hourglass shape with a minimum diameter of 1.0 cm in the center. Then, each gel was placed in a Torsion Gelometer (Gel Consultant, Raleigh, NC). By twisting samples, shear stress and shear strain were calculated by the methods of Hamann (1983) and recorded on a strip chart. Shear stress indicates the strength and hardness, while shear strain denotes the cohesive nature.

Color Test

A Minolta Chroma Meter CR-300 (Minolta Camera Co. Ltd., Osaka, Japan) was used to measure the color of gels (5 subsamples each). CIE L*a*b* values were measured, where L* represented lightness, a* for greeness, and b* for blueness. Whiteness was calculated as whiteness index, L*-3b*, as suggested by Park (1994a).

Statistical Analysis

Analysis of variance (STATGRAPHICS, 1992) was conducted on torsion and color data to determine the significance of treatments. Least significant difference (LSD) at $p \leq 0.05$ was used to determine significant differences between mean values.

Results & Discussion

Shelf Life and Gel Functionality of Fresh Surimi

Shear stress and shear strain of gels made from fresh Pacific whiting surimi during storage (3-5°C) for 7 days were compared (Fig. II.1). During storage, the stress slightly decreased but strain value remained virtually unchanged. Stress values were between ~29 and ~36 kPa and strain values were 1.89 to 2.12. In general, strain values of gels were somewhat low. Hamann et al. (1990) suggested that a good commercial product could be made from surimi with a strain value >2.0. The slightly low strain value we observed was probably due to high moisture content of the finished gels (80.8-80.9%) compared with normal gels (78% moisture). However, Morrissey et al. (1992) suggested that the lower limit of strain of surimi gel to make a quality product was 1.9 which correlates with the traditional Japanese double-fold test for surimi.

Total aerobic plate count (APC) increased logarithmically with continued storage days (Fig. II.2). APC was 10^3 at day 0, 10^5 at day 3 and day 5, and exceeded 10^6 at day 7. Microbial quantity of fish mince $\leq 10^6$ APC/g is acceptable according to Dymsza et al. (1990). They recommended that the quality cut-off for fish mince be 10^6 APC/g. Day 5 appeared to be the last day of storage before fresh surimi exceeded 10^6 APC/g. Also, at day 7, fresh surimi exhibited a mushy flesh and strong undesirable smell. This confirmed studies by Lin et al. (1993) which showed that levels of TMA and DMA in fresh surimi did not increase until day 7.

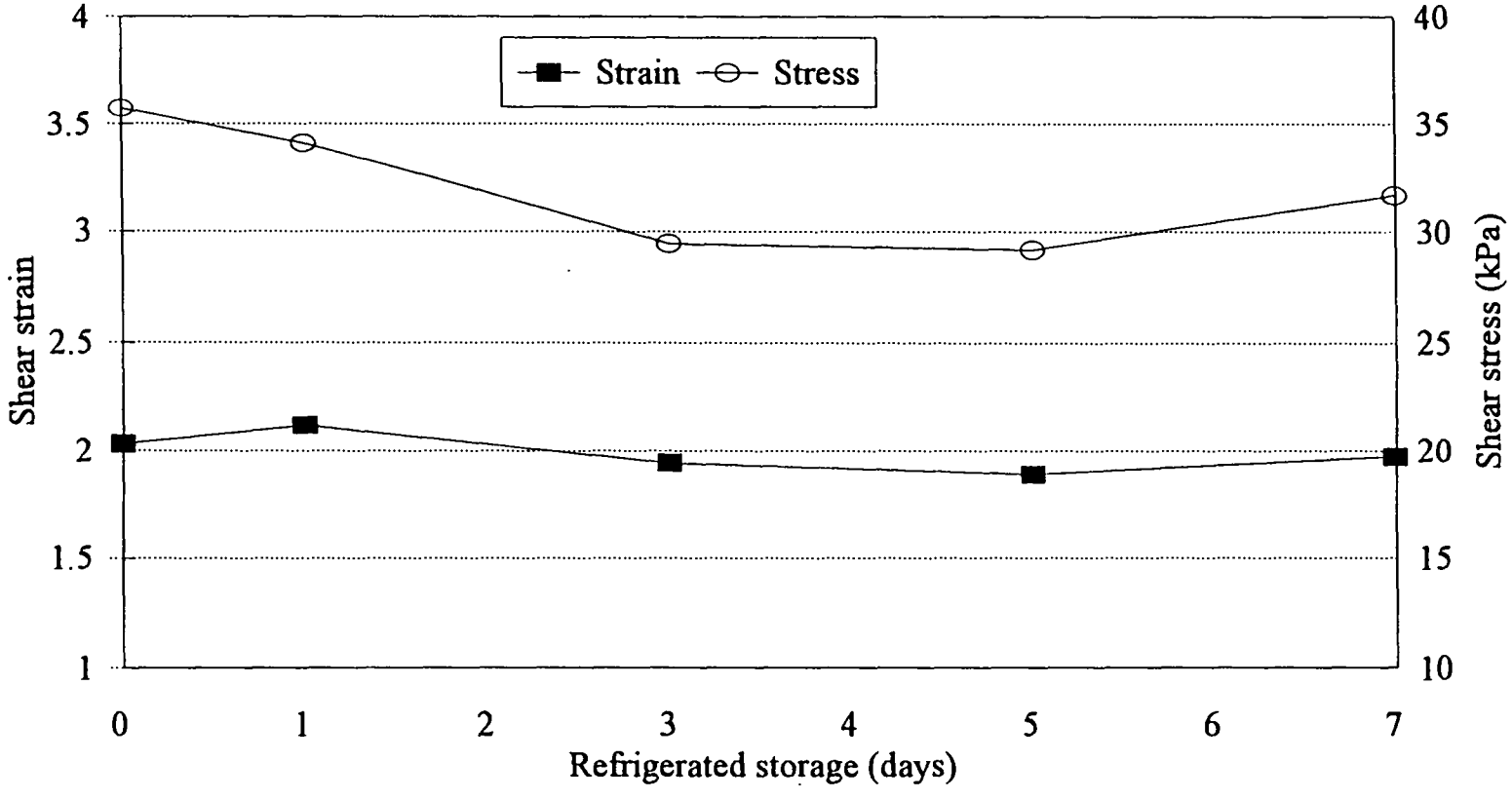

Fig. II.1 Gel Functionality (Shear Strain and Shear Stress) of Fresh Pacific Whiting Surimi as Related to Storage Time

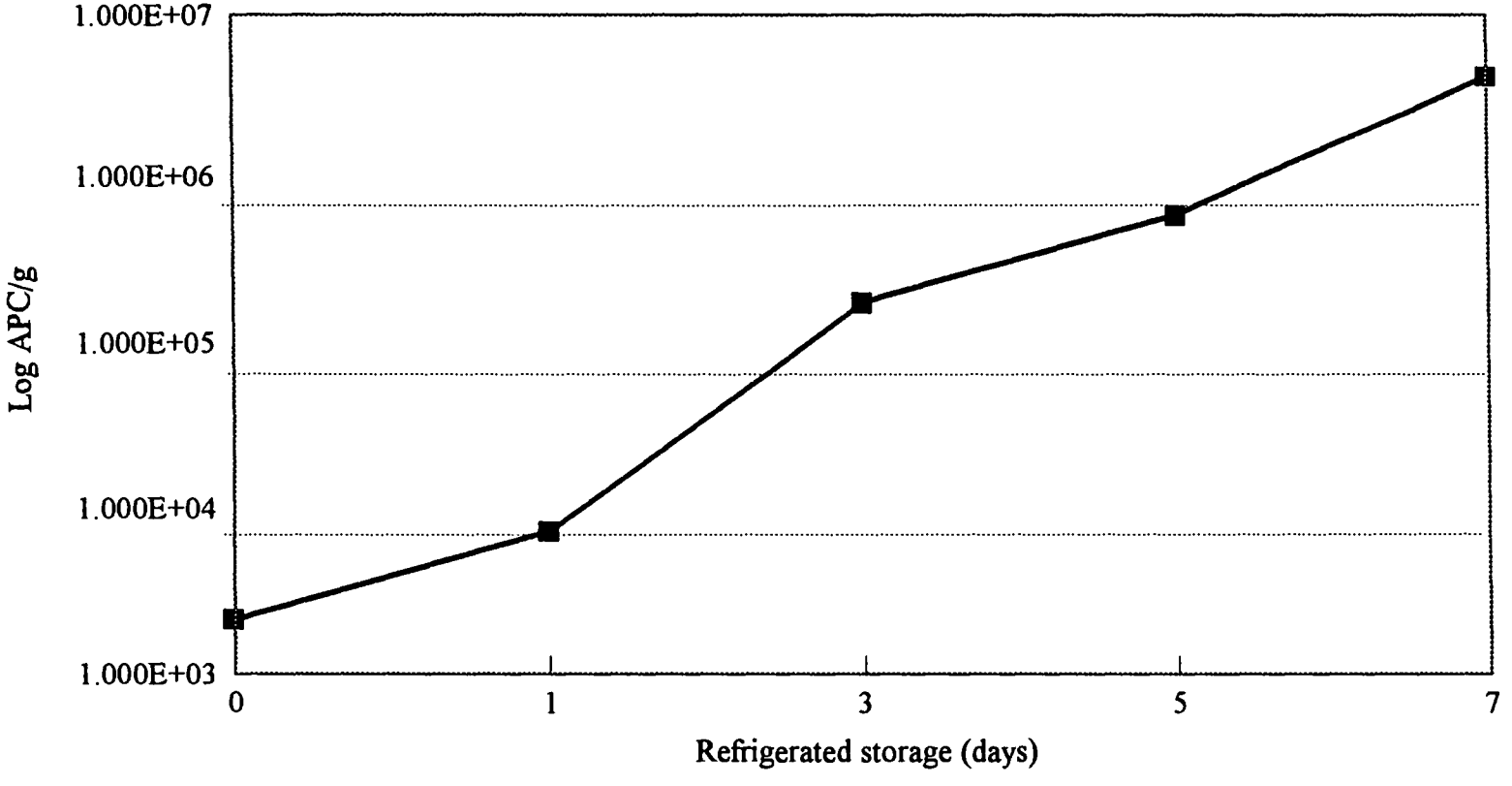

Fig. II.2 Total Aerobic Plate Count (APC) of Fresh Surimi during Refrigerated Storage.

Table II.1. Effects of storage time on gel color of fresh surimi.

Day	L*	a*	b*	Whiteness
0	82.15[a]	-2.40[a]	5.65[a]	65.19[a]
1	82.39[a]	-2.48[a]	5.61[a]	65.57[a]
3	82.15[a]	-2.29[a]	5.55[a]	65.51[a]
5	81.49[ab]	-2.34[a]	5.58[a]	64.77[a]
7	80.46[b]	-2.42[a]	6.29[b]	61.59[b]

[ab] Means in the same column followed by different superscripts are significantly ($P<0.05$) different within the same color parameter.

Both L* values and +b* values of fresh surimi did not change for the first 5 days of refrigerated storage, but decreased ($P<0.05$) at day 7. This was a result of protein decomposition and denaturation that led to the loss of translucency of fish meat (Ledward, 1992). However, a* values did not change throughout storage. As a result, whiteness (L*-3b*) of gels made from fresh Pacific whiting surimi during day 0 to day 5 of storage, (64.77-65.57), did not change (Table II.1). After 7 days storage, however, it decreased to 61.59 ($P<0.05$).

Frozen Surimi at Different Levels of Cryoprotectants

Shear stress and shear strain of surimi gels without cryoprotectants markedly decreased after 1 day freezing (Fig. II.3a and b). This was due to freeze-denaturation which caused changes in functional properties (Sikorski et al., 1976) and ice crystal growth which substantially damaged fish tissue (Lawrence et al. 1986). Texture

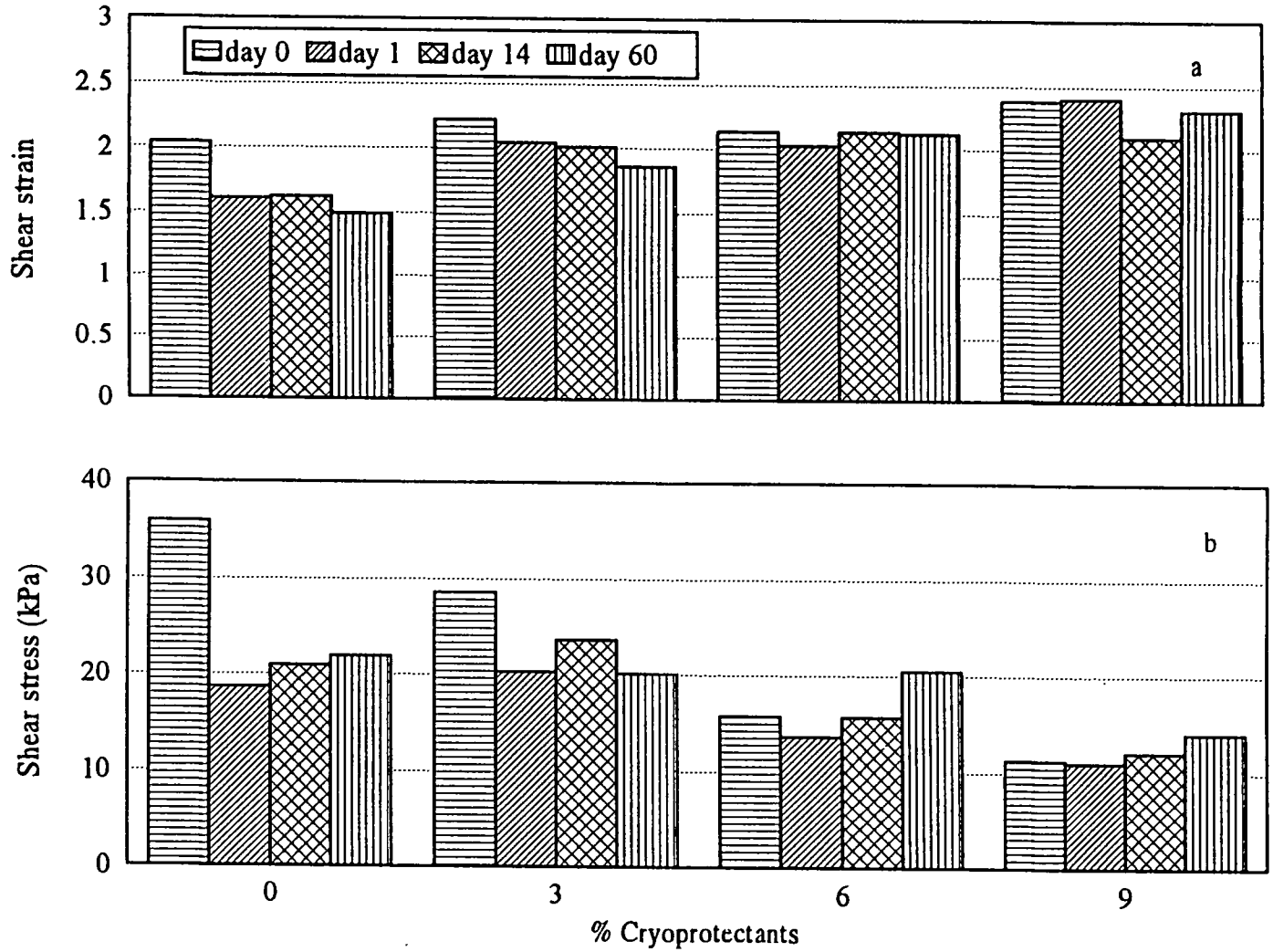

Fig. II.3 Shear Strain and Shear Stress of Frozen Surimi with Different Levels of Cryoprotectants Measured as Related to Storage Time

deterioration caused by protein denaturation, upon freezing, appeared to be minimized as levels of cryoprotectants increased (Fig.II. 3a and b): stress values decreased 48.2%, 28.8%, 13.37%, and 0% in samples with 0%, 3%, 6%, and 9% cryoprotectants, respectively.

During frozen storage, shear strains of surimi with 0% and 3% cryoprotectants markedly decreased (21.8% and 7.9%, respectively) and continued to decrease as storage time increased. However, shear strains of surimi with 6% and 9% cryoprotectants decreased only slightly with storage time. This confirmed a previous study by Yoon and Lee (1990) that reported higher levels of cryoprotectants produced more cohesive gels due to the better protected proteins in frozen surimi. Shear stress values of surimi gels with higher levels of cryoprotectants also did not decrease during frozen storage. However, shear stress seemed to increase slightly with extended frozen storage (day 14 and 60).

Unexpectedly, we observed a slight rising trend of shear strain values of gels made from fresh surimi (day 0) as level of cryoprotectants increased ($P<0.05$) (Fig. II.3a). A decreasing trend was observed in shear stress values (Fig. II.3b). The rising trend of shear strain values was probably due to a quality difference in each batch of fresh surimi used. To verify this, two additional batches of fresh surimi were evaluated.

All surimi samples showed a slight change in L* values and a* values upon freezing and remained stable during 2 mo of frozen storage (Table II.2). Surimi gels with 9% cryoprotectants had lower ($P<0.05$) b* values but higher whiteness than those with 0, 3, and 6% cryoprotectants. This may have been due to sugar and sorbitol, additives which decreased the level of protein content and subsequently decreased yellowness.

Table II.2. Color of Pacific whiting surimi gels prepared before and after freezing as affected by level of cryoprotectants.

%Cryoprotec-tants	Days of freezing	L*	a*	b*	Whiteness
0	0(Fresh)	82.04^{abc}	-2.45^{a}	5.54^{a}	65.42^{a}
	1	82.93^{a}	-2.48^{a}	5.49^{a}	66.46^{ab}
	14	82.97^{a}	-2.42^{a}	5.49^{a}	66.50^{ab}
	60	81.72^{abc}	-2.34^{a}	5.27^{ab}	66.43^{ab}
3	0(Fresh)	82.69^{abc}	-2.39^{a}	4.84^{bc}	68.17^{cd}
	1	81.97^{abc}	-2.63^{ab}	4.56^{cd}	68.29^{cdef}
	14	82.49^{ab}	-2.44^{a}	5.02^{b}	67.43^{bc}
	60	81.97^{bc}	-2.51^{a}	4.58^{cd}	68.24^{cde}
6	0(Fresh)	82.37^{abc}	-2.61^{a}	4.59^{cd}	68.62^{cdef}
	1	81.54^{bc}	-2.73^{ab}	4.43^{cde}	68.25^{cde}
	14	81.54^{bc}	-2.56^{a}	4.31^{def}	68.61^{cdef}
	60	81.28^{c}	-2.68^{ab}	4.45^{cd}	67.93^{d}
9	0(Fresh)	82.32^{abc}	-2.80^{ab}	3.99^{f}	70.35^{g}
	1	82.35^{abc}	-3.14^{b}	4.00^{ef}	70.35^{g}
	14	82.04^{abc}	-2.76^{ab}	4.00^{ef}	70.04^{fg}
	60	81.99^{abc}	-2.67^{ab}	4.00^{ef}	69.99^{defg}

^{abcdefg} Means in the same column followed by different superscripts are significantly ($P<0.05$) different within the same color parameter.
0 (Fresh) indicates that gels were prepared before freezing.

Park (1995b) also reported that higher protein contents in surimi gels resulted in less lightness and more yellowness: L* values decreased by 2.5 units, while b* values increased by 2 units as protein concentration increased by 7.5%.

Comparison between Fresh and Frozen Surimi

No significant differences in shear strain values of fresh surimi gels (day 0) were observed among different levels of cryoprotectants (Fig. II.4a). These results (repeated four times) supported our assumption that a rising trend of shear strain values of fresh surimi as cryoprotectants increased was due to a quality difference in each batch of fresh surimi. After freezing, less difference between strain values of fresh and frozen surimi were observed as level of cryoprotectants increased. The strain value of surimi gels with 0, 3, and 6% cryoprotectants decreased by 22%, 7%, and 3%, respectively, upon freezing from day 0 to day 1.

Shear stress of both (fresh and frozen) surimi gels decreased ($P<0.05$) as level of cryoprotectants increased (Fig. II.4b). Shear stress of fresh surimi without cryoprotectants (17.5% protein) was 35.84 kPa. Fresh surimi with cryoprotectants at 3% (14.9% protein), 6% (13.1% protein), and 9% (11.6% protein) had shear stress of 25.35, 18.22, 13.42 kPa, respectively. This was due to a decrease in protein content of finished gels. Stress value and rigidity were strongly influenced by the concentration of proteins or other solids in surimi and processing conditions. Strain however is affected mainly by protein quality, such as type (sarcoplasmic, myofibril, stroma), pH, and the degree of denaturation of proteins (Park, 1994b; Lanier, 1986; Howe et al., 1994).

Freezing reduced shear stress values of surimi gels at all 4 levels of cryoprotectants (Fig. II.4b). Without cryoprotectants, the stress values of frozen surimi was reduced by 45%. A reduced stress value of 21-25% was observed in frozen surimi gels with 3 and 6% cryoprotectants, and a 19% decrease in stress value was found in frozen surimi gels with 9% cryoprotectants. The most striking difference between fresh and frozen

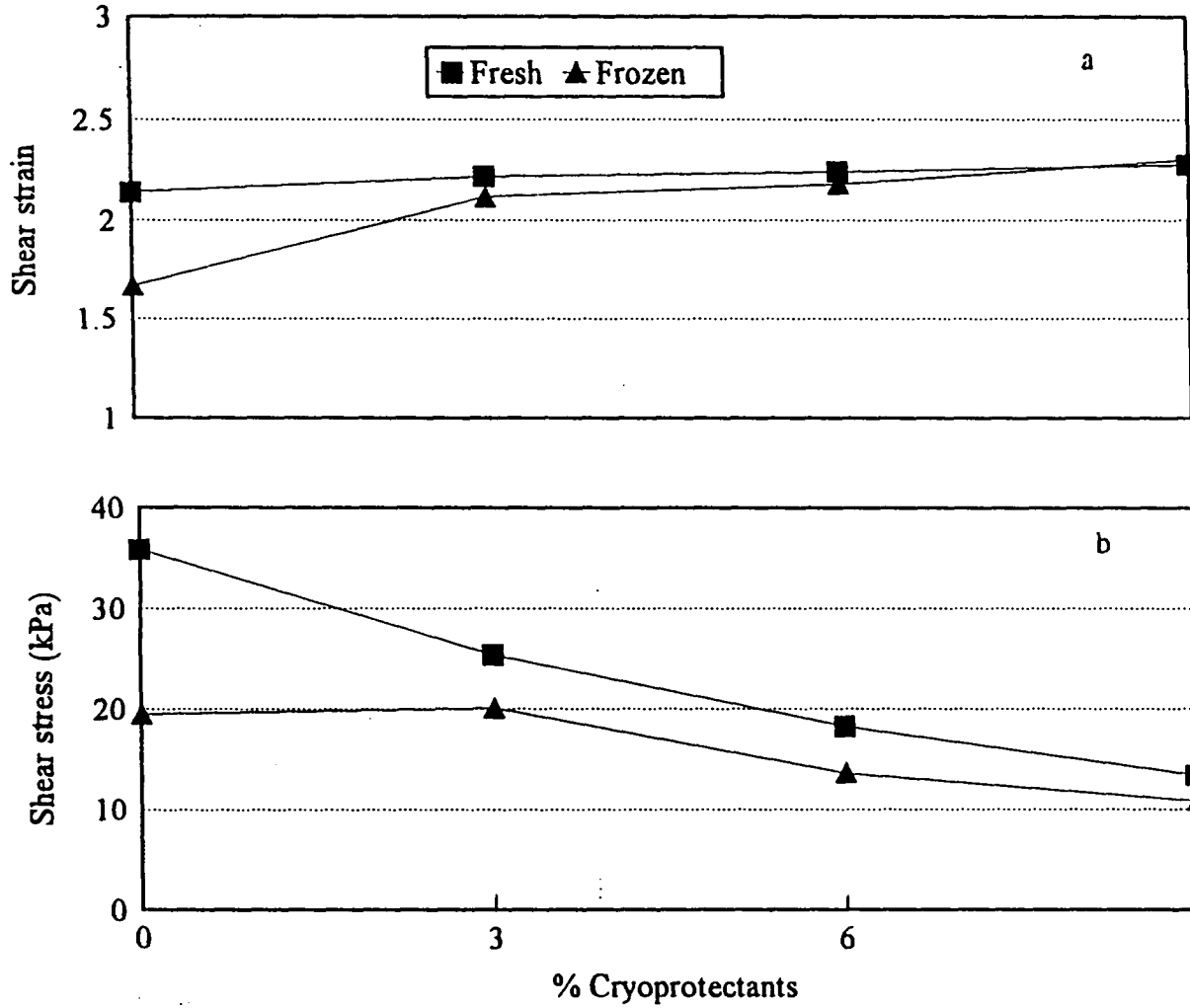

Fig. II.4 Shear Strain and Shear Stress of Fresh Pacific Whiting Surimi Gels at Various Level of Cryoprotectants. Before (Day 0) snd After Freezing for 20-24 Hr (Day 1).

surimi was noted in stress values. The stress value of fresh surimi with no cryoprotectants (35.84 kPa) was almost 3 times higher than frozen surimi with 9% cryoprotectants (11 kPa) at the same moisture level (Fig. II.4b). This clearly showed the advantage of fresh surimi over commercial frozen surimi.

Conclusion

From the results of APC, shear stress, shear strain, and color values, the use of fresh Pacific whiting surimi can be feasible for up to 5 days shelf-life at refrigerated temperature (5°C). Fresh Pacific whiting surimi also exhibited high gel-forming ability and maintained quality up to 5 days of storage.

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III. TEXTURIZED MEAT FROM PACIFIC WHITING MINCE

Abstract

Texturized meat was prepared from 4 different conditions of 6-8 mo old frozen Pacific whiting (PW) mince: unwashed mince with/without 6% cryoprotectants and 1-washed mince with/without 6% cryoprotectants. Each mince was comminuted with 1% salt and 1% BPP, formed into patties, and frozen at -7, -18, and -50°C. Microstructure formation, hardness, cook loss, color, and water holding capacity of products were observed during frozen storage (1, 5, and 20 days). Meat-like products with parallel layers were made from 1-washed mince of all temperature treatments. Unwashed mince generated a sponge-like structure and rapid quality changes during storage, thus it was not appropriate for this product. Cryoprotectants did not affect the texture formation of the product and are not required to store mince for a raw material. The optimum freeze-texturized temperature for this product was -18°C or lower, at which the quality changes can be minimized during storage depending on the desirable texture. The lower temperature (higher freezing rate) created finer layers in the product.

Introduction

Freezing processes have been used as a preservation method for agricultural products and foods since 1100 B.C. By the 18th century, the first commercial freezing industry in the U.S. had originated. Chicken and fish were the first commercially frozen products followed by eggs, red meats, small fruits, and berries in the early part of the 19th century (Enachian and Woolrich, 1977). Not only has the freezing process been recognized as a food preservation technique, but it has also been utilized as a technique to create a desirable texture in protein food products for several centuries. In Japan, 'kori-tofu' was produced by freezing and storing the tofu frozen until a porous, sponge-like structure had developed (Lawrence et al., 1986).

More recently, this technique has been of interest and developed to create a fibrous or meat-like texture called "freeze texturization", "freeze alignment", or "fabrication". This method is carried out by controllably cooling the surface of a protein slurry so that ice crystals are allowed to grow aligned and perpendicular to that surface. This creates crystalline-molded layers to the protein slurry. Consequently, after the ice crystals are removed and proteins are set by heat, a fibrous structure with thin parallel sheets is formed.

This innovative concept enabled less desirable or inexpensive protein sources to be processed to a more desirable and more valuable product. Several studies have been done on texturizing various type of protein sources including animal proteins, fish proteins, and vegetable proteins (Middendorf et al., 1975; Kim and Lugay, 1977; Boyer and Middendorf, 1975; Okumura and Wilkinson, 1970; Noguchi et al.,1979). Diverse vegetable proteins such as soybeans (Boyer et al., 1975; Okumura and Wilkinson, 1970;

Middendorf et al., 1975), soy protein isolate (Boyer et al., 1975; Middendorf et al., 1975; Kim et al., 1974), wheat protein concentrate (Kim et al., 1974), bean flake, sesame seeds, cotton seeds, alfalfa plant (Okumura and Wilkinson, 1970), and sunflower seed (Middendorf et al., 1975) were used to imitate animal meat. Animal proteins such as pork by-products or pork trimmings (Middendorf et al., 1975), beef muscle (Kim and Lugay, 1977), low grade chuck roast meat (Boyer et al., 1975; Middendorf et al. 1975), chicken, turkey (Middendorf et al., 1975), fish, egg white (Kim and Lugay, 1977), and milk (Kim and Lugay, 1977; Okumura and Wilkinson, 1970) were suggested to be individually used or specifically combined depending on the desired flavor and texture.

Pacific whiting is a nutritious and inexpensive fish protein source. Pacific whiting is not normally used as a whole fish or fillet due to its extreme softening and mushiness caused by a proteolytic enzyme in the flesh (Nelson et al., 1985: Chang-Lee et al., 1989). The major portion of Pacific whiting is used for surimi production of which only 15-20% yield was obtained (Toyoda et al., 1992). The development of alternative products is needed for more effective utilization of Pacific whiting. Freeze texturization was thought to be a technique that could be appropriately applied to utilize Pacific whiting mince, which has nearly a 32% yield (Toyoda et al., 1992).

The objectives of this study were to establish an optimum freeze texturization method for Pacific whiting mince and to investigate effects of washing, storage condition of mince, freezing temperature, freezing time, and cooking method on texture formation and quality of freeze-texturized meat made from Pacific whiting.

Materials & Methods

Frozen Mince Preparation

Pacific whiting mince samples were obtained from Point Adams Packing Co., Hammond, OR. They were collected from two different points of the surimi processing line: from a mincer output prior to washing (mince without washing, 0W) and from a tank after the first dewatering chamber (1 time washed mince, 1W). The 0W mince was directly collected from the mincer before water was added. The 1W mince was pressed through a screw press (with sieve size of 0.06 cm diameter) to have a moisture content of 81-82%. Both samples were put in polyethylene bags, kept on ice and transported to the OSU Seafood Lab within 30 min.

Each mince sample was divided into 2 portions. One portion was mixed with 6% cryoprotectants; a mixture (1:1) of sucrose and sorbitol (ICI Specialties, New Castle, DE) based on its total weight. The other was packed without any additives. All samples were packed in food-grade plastic bags, 2.00±0.05 kg each, sealed under a vacuum, and frozen using a plate freezer (Model No. 12, Amerio Plate Freezer, APV Crepaco Inc., Rosemont, Il) for 3 hr 30 min. Then all samples were transferred to a storage freezer (-18°C).

Patty Preparation

6-8 mo old frozen mince samples (unwashed mince without cryoprotectants (0W), unwashed mince with 6% cryoprotectants (0W+C), 1-washed mince without cryoprotectants (1W+C), and 1-washed mince with 6% cryoprotectants (1W+C)) were

thawed overnight at 5°C. Each mince sample was cut into cubes and comminuted into small particles at low speed for 1.5 min in a vertical vacuum cutter (model UM 5 Universal; Stephan Machinery Corp., Columbus, OH). Salt (1% w/w) was added and the mince was further comminuted at low speed for an additional 1.5 min or until the mince formed a thick paste. 1% BPP was added and the fish paste was mixed at high speed for 3 min until a homogeneous paste was obtained.

The fish paste was formed using a forming plate into patties of approximately 120 g with 11.5 cm in diameter and 1 cm thick. Patties were covered on both sides with wax paper and placed in plastic bags with not more than 3 patties per stack. The patties were freeze-texturized by freezing at -7°C, -18°C, and -50°C using chest freezers. Samples were kept in the chest freezers until they were tested. The frozen mince patties were evaluated for ice formation, texture, cook loss, and color at 1 day, 5 days, and 20 days of frozen storage. Temperature during freezing was also recorded at 10 min intervals using a Data Logger (Electronic Controls Design, Inc.) with a multiplexer (series 3000). Thermocouples were placed in the center of the sample patties.

Ice Formation Evaluation

Ice formation and structure of mince patties were determined by a histological method. Frozen water fraction was determined using "area of ice/total area" (A_T). An image analysis program (UCFImage, University of Central Florida, Orlando, FL) was used to determine A_T from the cross-sectional pictures taken through a low magnification (20X) light microscope (Nikon, SMZ-U, Type 104, Japan). The holes left by ice in the

tissue were observed as ice crystals. The low magnification (20x) microscope was used to overview ice crystal formation, which contributes to the product texture.

Sample specimens were prepared by randomly cutting 2 frozen patties of each treatment into small pieces (0.5x1x1cm), 5 pieces per patty, and immediately immersing them into a chilled fixing fluid (0.1N phosphate buffered 10% formalin). The specimens were left in the fixative for at least 24 hr. Five specimens were randomly selected from the fixative, cut into pieces of 0.25x0.5x1 cm, and placed in a tissue processing dish covered with a stainless steel cover plate. The formalin fixed samples were dehydrated in gradient alcohol and infiltrated by a tissue processor (LX 300 Tissue processor, Fisher Scientific, Pittsburgh, PA). The methods of alcohol dehydration and infiltration are shown in Appendix A.

The specimens were then embedded in 100% paraffin. Paraffin blocks were vertically sectioned with 5 μm thickness using a microtome (Spencer Model 820, American Optical Co., NY). Wrinkles were removed by floating sections on a water bath containing a small amount of gelatin. Sections were mounted onto clean glass slides and stained with haemotoxylin and eosin (as described in appendix A) using a slide stainer (S/P Slide stainer GLX, Scientific Product, McGaw Park, IL). Stained slides were covered with a cover slip using Permount (Toluene solution UN 1294, Fisher Chemical, Pittsburgh, PA).

Texture Evaluation

Frozen mince patties were individually put in a ziplog bag and steam cooked at 90°C for 30 min (inner temperature ≥80°C), then immediately cooled in cold running water (12-15°C). After determining cook loss, patties (2 patties per sample) were cut into pieces of 2×4 cm. Eight sub-samples were cut by a Warner-Bratzler shear device connected to a Sintech 1G machine (MTS/Sintech, Research Triangle Park, NC) with the cross head speed of 20 cm/min. Results were reported as averages of shear force (kg) of 8 sub-samples.

Color Measurements

After steamed patties were cut into 2x4 cm pieces, 8 sub-samples were measured for color using a Minolta CR300 colorimeter (Osaka, Japan). CIE L*a*b* values were measured, where lightness was represented by L*, red to green hue by a*, and blue to yellow hue by b*. Whiteness was calculated as whiteness= L*-3b* (Park, 1994).

Cook Loss

Frozen samples were weighed before cooking. Cook losses were determined after steam cooking and microwave cooking. For steam cooking, each sample was put in a ziplog bag, and steamed at 90°C for 30 min without thawing. For microwave cooking, each sample was defrosted for 2 min using a microwave (800 W) and continued cooking

for 2 min. The cooked samples were wrapped with 2 pieces of paper towels, left in room temperature to cool down (about 10 min), blotted dry and reweighed to determine the cooking loss. The cooking loss values were reported as an average of two or more sub-samples.

Water Holding Capacity

Water holding capacity (WHC) was defined as the percentage ratio of moisture content in meat after centrifuging (expressible moisture) to the original moisture content (Jiang et al., 1985). Expressible moisture (EM) was determined according to the method of Jauregui et al. (1981) as modified by Lin (1992). Approximately 1.5 g of cooked sample was wrapped in a polyester mesh (PE 105, type 502, 690 mesh per cm^2, Henry Simon Limited, Stockport, Cheshire, England) with a #50 filter paper used as an inner surface. Then, the sample was wrapped with three #3 filter papers and centrifuged at 16,000 rpm (Sorvall, DuPont Co., Newtown, CT) for 15 min at 2°C. The total moisture contents of cooked samples were determined using a microwave method according to Morrissey et al. (1993).

Salt Extractable Proteins

The method for determining salt extractable proteins (SEP) was modified from the method of Park et al. (1988). Twenty grams of mince sample was homogenized in 180 mL of 0.6 N KCl solution (5°C) for 2 min using a Polytron homogenizer (Brinkmann

Instruments, Westbury, NY) at speed 3-4. The homogenate was centrifuged at 5,000 x g for 30 min. Protein concentration of the supernatant was measured (Lowry et al., 1951).

SEPs were reported as a percentage ratio of the salt extractable proteins to the total proteins of the sample. Both SEP and total protein concentrations in this determination were determined using BSA as a standard in order to calculate the percentage of SEP. Proteins in the sample were solubilized in 10% SDS (An et al., 1994) and the protein concentrations were determined by the method of Lowry et al. (1951). Both salt extractable and total protein determinations were done in triplicate.

pH

Three grams of sample were homogenized in 27 mL distilled water at speed 3-4 for 2 min using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY). The pH was measured by a Corning pH meter 340 (Corning, New York, NY) in duplicates.

Proximate Analysis

Proximate analysis was performed on frozen mince samples used as raw material for texturized meat. The proximate analyses of total protein, fat, and moisture were determined according to AOAC procedures (AOAC, 1990). Total protein (TP) was determined by the Kjeldahl method and was calculated as Nx6.25. Crude fat determination was carried out by an acid hydrolysis method and moisture was determined by drying about 2 g of sample at 105-110°C for at least 16 hr.

Statistical Analysis

Significances between treatments of hardness, water holding capacity, color, cooking loss, and A_T were analyzed using the analysis of variance (STATGRAPHICS, 1992). Least significant difference (LSD) at 5% was used to determine significant differences between mean values.

Results & Discussion

Treatment Conditions

Chemical and physical properties of mince after 6 mo storage at -25°C are listed in Table III.1. Moisture of all samples, regardless of cryoprotectants, decreased after 6 mo frozen storage. The reduction of moisture was greater in unwashed mince (2.34-2.91%) than in washed mince (0.17-0.7%). This could indicate a greater drip loss during thawing of unwashed mince than washed mince. Drip loss is the amount of liquid lost from a protein system by the effect of gravity without external force (Jauregue et al., 1981) and is inversely related to WHC.

WHC is defined as the ability of proteins to hold water under the influence of an external force (Jauregue et al., 1981). WHC was calculated by subtracting EM from moisture content (MC) of samples after freezing. Washed mince had higher WHC than unwashed mince. The addition of cryoprotectants slightly decreased WHC of unwashed mince but slightly increased WHC of washed mince.

Table III.1 Physico-chemical Propeties of Frozen Pacific Whiting Mince as Raw Material.

Properties	0W	0W+C	1W	1W+C
Moisture before freezing(%)	83.15	80.05	81.54	78.53
Moisture after freezing (%)	80.24	77.71	81.37	77.83
Water Holding Capacity (%)	22.57	20.14	33.00	35.19
Total Proteins (%) (Kjeldhal)	11.36	10.19	11.02	10.36
Salt Extractable Proteins (mg/mL)	2.70	5.03	3.11	12.89
Total Proteins (Lowry)(mg/mL)	17.70	16.16	20.11	19.49
% Salt Extractable Proteins	15.25	31.13	15.44	66.01
Lipids (%)	1.97	2.08	1.67	1.40
pH	7.10	7.16	7.10	7.03

0W=unwashed mince, 0W+C=unwashed mince + 6% cryoprotectants, 1W=1-washed mince, 1W+C=1-washed mince + 6% cryoprotectants

Total proteins (TP) determined by the Kjeldahl method were 11.36%, 10.19%, 11.02%, and 10.36% in 0W, 0W+C, 1W, and 1W+C, respectively. Washed and unwashed mince had similar protein contents. However, SEP, which mainly indicated myofibrillar protein content of mince, was different between cryoprotected washed and unwashed mince (0W+C and 1W+C) when cryoprotectants were added. SEP increased about 2 times in unwashed mince (15.25% in 0W and 31.13% in 0W+C) and more than 3 times in washed mince (15.44% in 1W, 66.01% in 1W+C). This is probably due to the cryoprotective effects of sugar and sorbitol added to the mince. Cryoprotectants had less effect on unwashed mince than washed mince since unwashed mince contained a greater amount of lipids, enzymes, and other substances which might have accelerated the denaturation of myofibrillar proteins. Oxidized products of lipids and free fatty acids formed during frozen storage may interact hydrophilically or hydrophobically with proper sites of proteins, and create a hydrophobic environment. This hydrophobicity of proteins can increase aggregation and as a result, decrease protein extractability (Kussi et al., 1975; Sikorski et al., 1976; Srikar et al., 1989). It is widely accepted that the loss of SEP during frozen storage can be reduced by the addition of cryoprotectants (Sych et al., 1990a, b; Park et al., 1987b, 1988, 1993; Magnusdottir, 1995).

Lipid contents of 0W, 0W+C, 1W, and 1W+C were 1.97, 2.08, 1.67, and 1.40%, respectively. Washed mince had a 0.30-0.68% lower lipid content than unwashed mince. This was due to the removal of fats and lipids by washing. The pH of all samples was in the neutral range (7.03-7.16).

The temperature profiles of frozen mince patties showed different freezing rates between treatments (Fig. III.1). Freezing rate is reversely related to the time required to

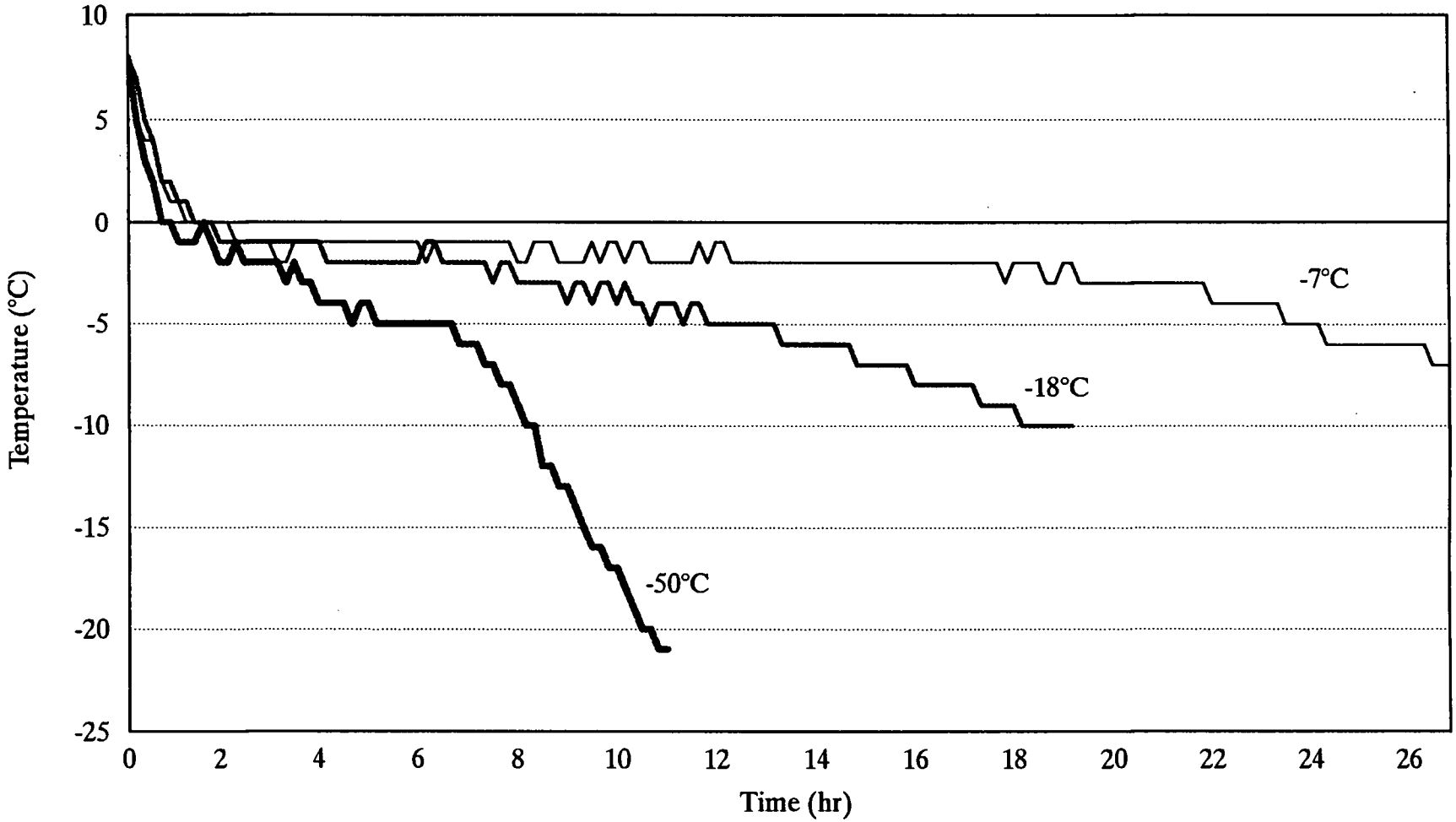

Fig. III.1 Inner Temperature of Texturized Meat Patties

change the internal temperature of samples from +5°C to -5°C (Chou, 1993). The freezing rate of -7°C was about 2 times slower than that of -18°C and approximately 4 times slower than that of -50°C (Fig. III.1).

Effects of Treatment Conditions on Ice Area and Texture Formation

A_T, area of ice/total area, was determined by image analysis, indicating the fraction of water frozen. A high A_T value implies a high fraction of water that is converted to ice and is inversely proportional to unfrozen water. A_T values also indicate the degree of ice which compresses protein to form thin layers; the higher the A_T value, the bigger the crystals and the more compact protein layers. A_T was in the range of 50-78% for -7°C treatment, 52-82% for -18°C treatment, and 41-75% for -50°C treatment (Fig. III.2). Freezing temperatures showed no significant impact on A_T. This was in agreement with Bevilacqua (1979) who reported that fractions of frozen water are not affected by temperature. Extended freezing time, however, showed an increase of A_T for -7°C treatment but showed no effects on -18°C and -50°C treatments (except 0W mince patties at -18°C). This implies that more water was converted to ice as freezing time was prolonged at -7°C.

An indirect technique was used to evaluate the structure of freeze texturized meat using the microscope. At -7°C, patties made from 0W showed a nondirectional spread of relatively large ice crystals after being frozen for 1 day (Fig. III.3a). The crystals appeared to grow in the same direction and were almost parallel to each other after 5 days of freezing (Fig. III.3b). At 20 days of frozen storage, ice crystals formed a clear columnar

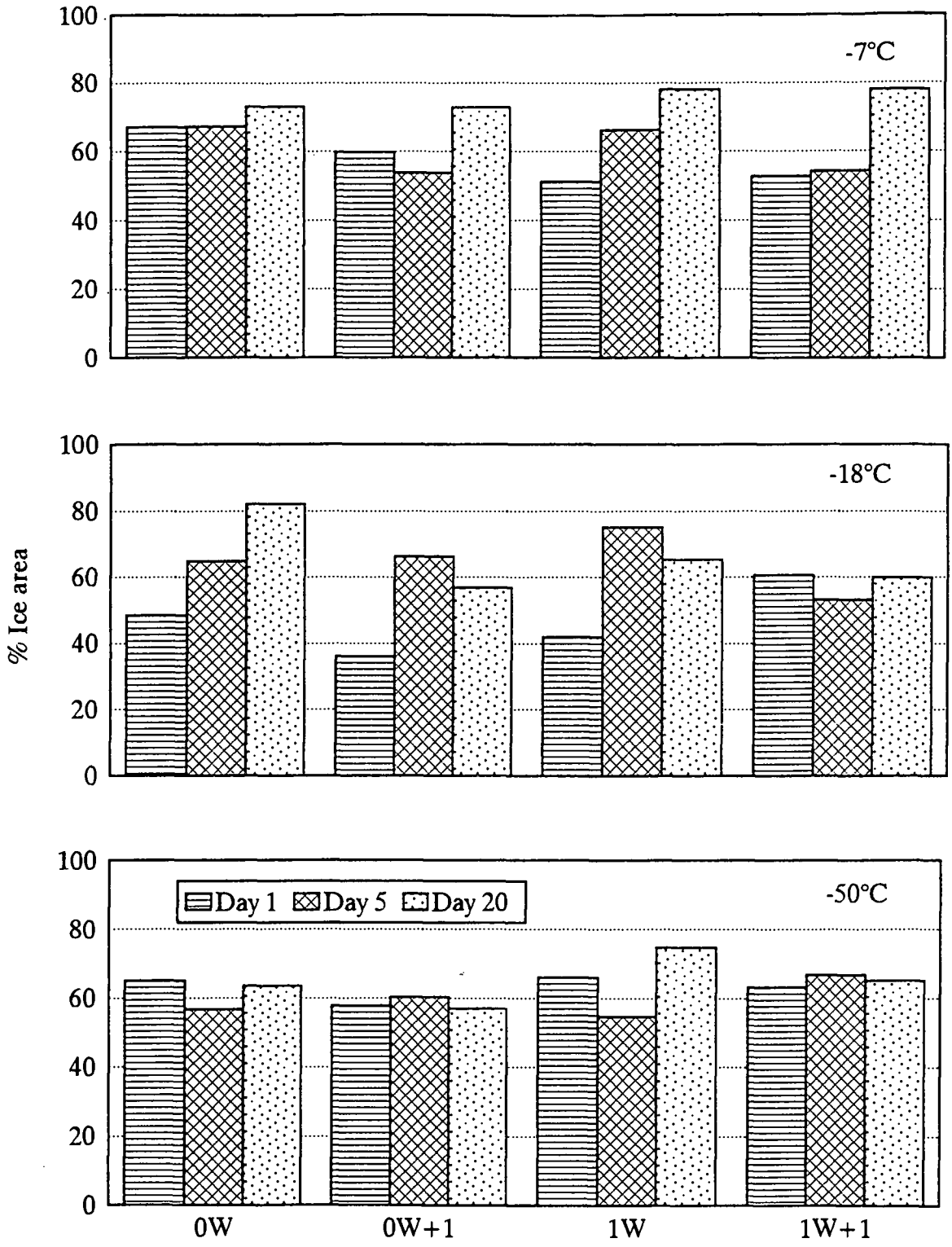

Fig. III.2 A_T (% Ice Area)of Texturized Meat Patties

Fig. III.3 Ice Formation of Texturized Meat Patties Made from 0W Mince Frozen at -7°C: (a) Day 1, (b) Day 5, (c) Day 20.

Fig. III.4 Ice Formation of Texturized Meat Patties Made from 0W+C Frozen at -7°C: (a) Day1, (b) Day 5, (c) Day 20.

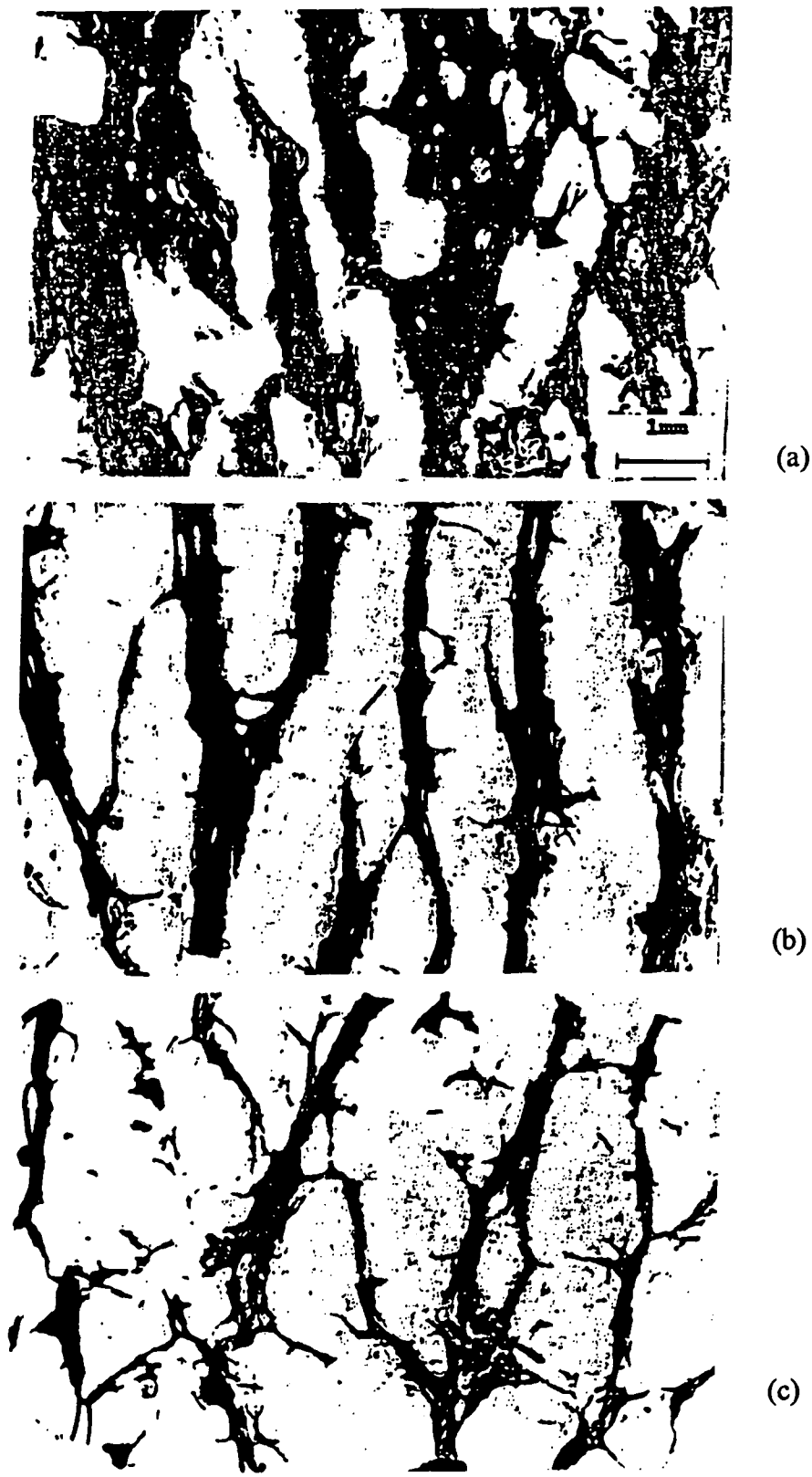

Fig. III.5 Ice Formation of Texturized Meat Patties Made from 1W Mince Frozen at -7°C: (a) Day 1, (b) Day 5, (c) Day 20.

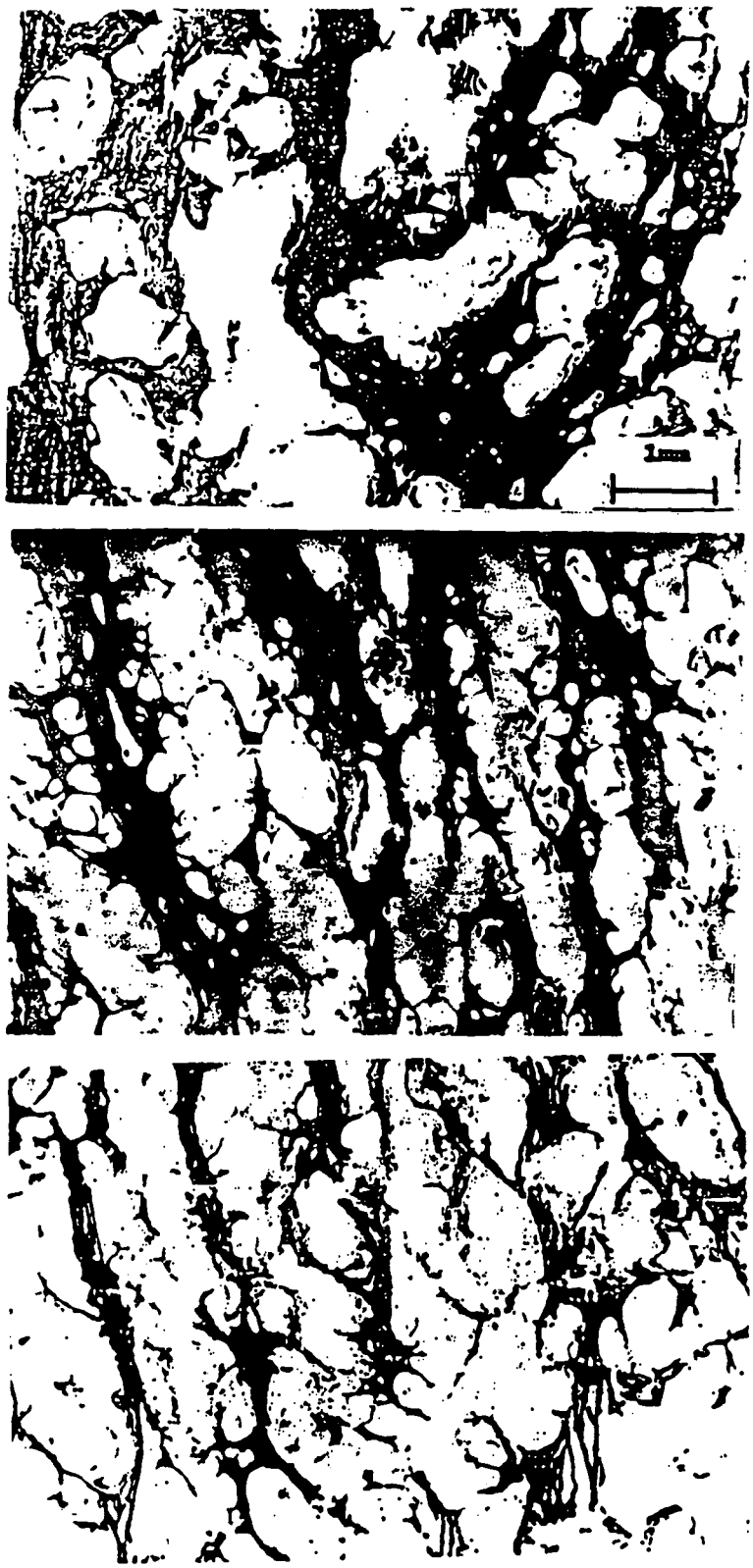

Fig. III.6 Ice Formation of Texturized Meat Patties Made from 1W+C Frozen at -7°C: (a) Day1, (b) Day 5, (c) Day 20.

structure parallel each other (Fig. III.3c). These ice columns grew in length and width. As a result, they compressed the meat between ice columns into thin layers and showed a clear layer structure. This may be the reason for the increased A_T values of frozen patties at 20 days compared with those at 1 and 5 days (Fig. III.2).

Frozen patties made from 0W+C and frozen at -7°C showed more numerous yet smaller, ice crystals compared with those of 0W mince patties (Fig. III.4a). This could be due to the higher concentration of liquid phase of mince patties caused by added cryoprotectants and soluble materials in unwashed mince. This implies that greater nucleation occurred in a higher concentration of liquid. The higher number of nuclei produced the more numerous, but smaller in size, of ice crystals (Ryder, 1987). The results at day 5 and day 20 of frozen storage (Fig. III.4b, III.4c) were similar to those observed in 0W patties; ice columns grew bigger and became parallel to each other with extended time and showed a significant decrease in thickness of meat between ice columns. Furthermore, meat layers appeared to be thinner and less continuous (more broken fragments) than those from 0W mince.

Frozen patties from washed mince, both 1W and 1W+C, contained fewer ice crystals (white area) and thicker meat layers (dark area) after 1 day of frozen storage (Fig. III.4a, III.5a) compared to those of unwashed mince (Fig. III.3a, III.4a). As freezing time increased, both patties from 1W and 1W+C showed an increase in ice column size with thinner meat layers. It was also described by Bevilecqua et al. (1979) that as ice columns grew in their length, their cross sectional areas increased as well. The ice showed a more column-like structure as freezing time was extended. This could be due to the growth of ice crystals as time was prolonged by drawing water from neighboring areas,

thus compressing protein layers and finally breaking those layers which were in the direction of crystal growth. More broken fragments were also observed at day 20 when cryoprotectants were added to the mince (Fig. III.5c, III.6c).

As temperature decreased to -18°C, ice crystals in patties made from 0W mince after being frozen for 1 day (Fig. III.7a) were not much different in size and number from 0W+C mince (Fig. III.8a). At day 5 and day 20 of frozen storage, samples made from 0W+C mince showed the growth of ice crystals in the form of individual ice crystal growth (Fig. III.7b, III.7c) while those made from 0W showed ice existing in the form of column-like crystals (Fig. III.8c).

Frozen patties made from 1W at -18°C (Fig. III.9a) showed long ice crystal columns with smaller column width compared to those frozen at -7°C on day 1. Patties made from 1W+C mince, as observed in -7°C treatment, showed ice crystal formation in the form of a porous or sponge-like structure (Fig. III.10a). After freezing for 5 days, ice crystals in both samples appeared to be more columnar in structure and meat layers were more compact (Fig. III.9b, III.10b). However, prolonging frozen storage to 20 days did not significantly affect structure of the patties made from washed mince (Fig. III.9c, III.10c).

At -50°C, frozen patties made from unwashed mince (both 0W and 0W+C) developed a porous structure in which ice crystals existed as individual crystals at day 1 and day 5 of frozen storage (Fig. III.11a, b, III.12a, b). Once storage time was prolonged to 20 days, ice crystals tended to form some degree of columnar structure, but not parallel to each other (Fig. III.11c, III.12c). The meat was compressed into thin layers at day 20 but the layers were broken into small fragments. These broken fragments may be due to

Fig. III.7 Ice Formation of Texturized Meat Patties Made from 0W Mince Frozen at -18°C: (a) Day 1, (b) Day 5, (c) Day 20.

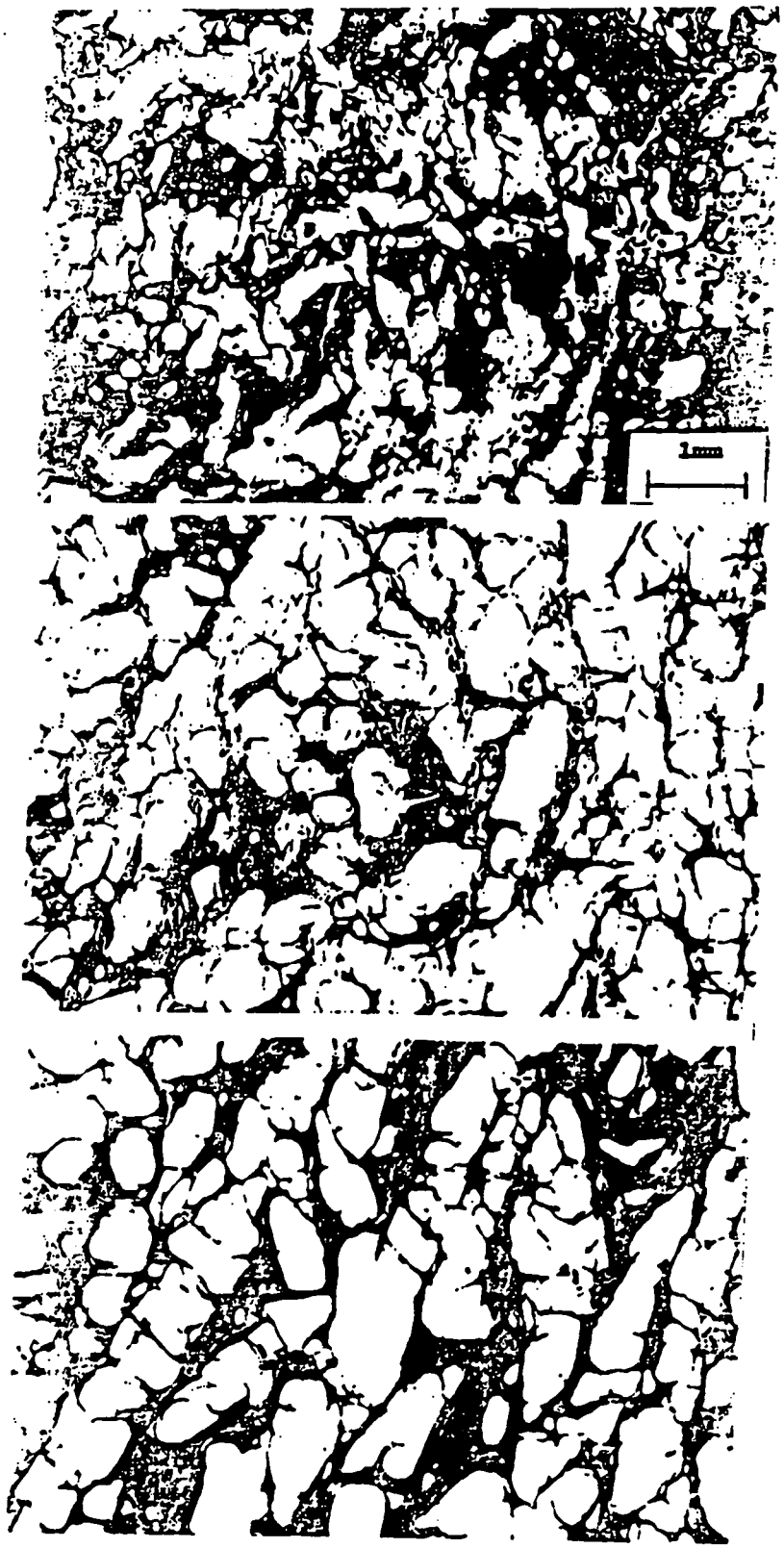

Fig. III.8 Ice Formation of Texturized Meat Patties Made from 0W+C Frozen at -18°C: (a) Day1, (b) Day 5, (c) Day 20.

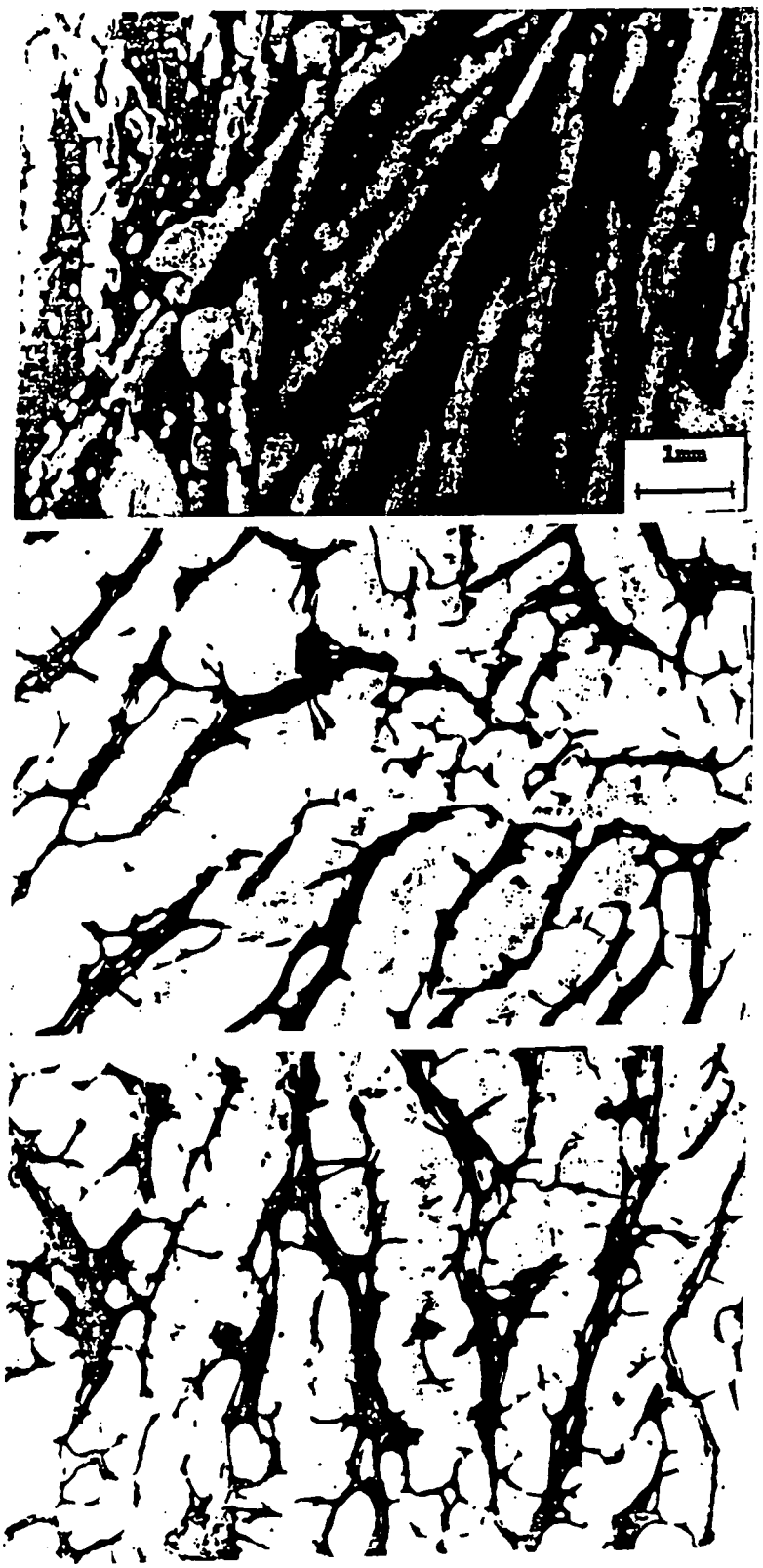

Fig. III.9 Ice Formation of Texturized Meat Patties Made from 1W Mince Frozen at -18°C: (a) Day 1, (b) Day 5, (c) Day 20.

Fig. III.10 Ice Formation of Texturized Meat Patties Made from 1W+C Frozen at -18°C: (a) Day1, (b) Day 5, (c) Day 20.

Fig. III.11 Ice Formation of Texturized Meat Patties Made from 0W Mince Frozen at -50°C: (a) Day 1, (b) Day 5, (c) Day 20.

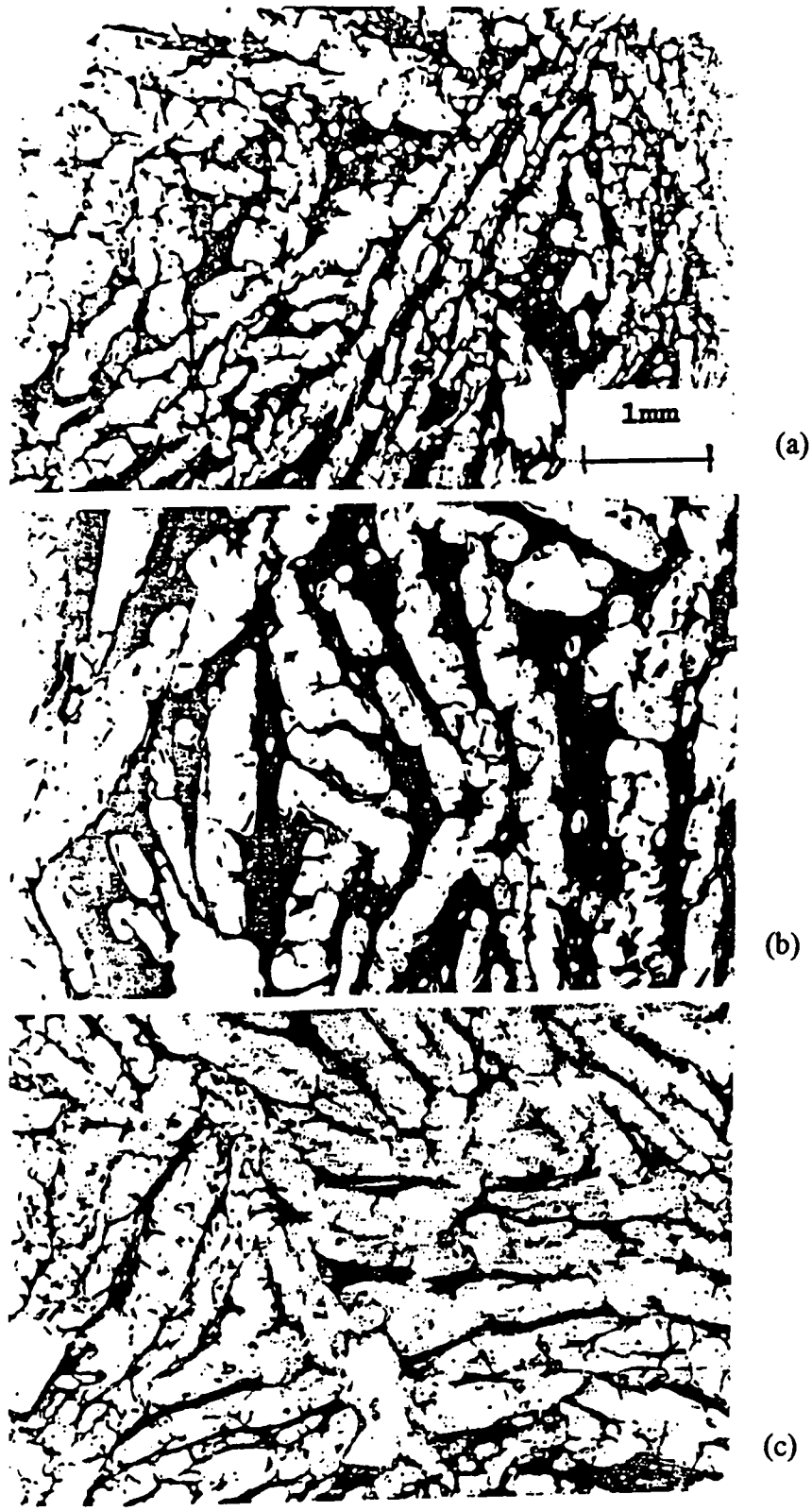

Fig. III.12 Ice Formation of Texturized Meat Patties Made from 0W+C Frozen at -50°C: (a) Day1, (b) Day 5, (c) Day 20.

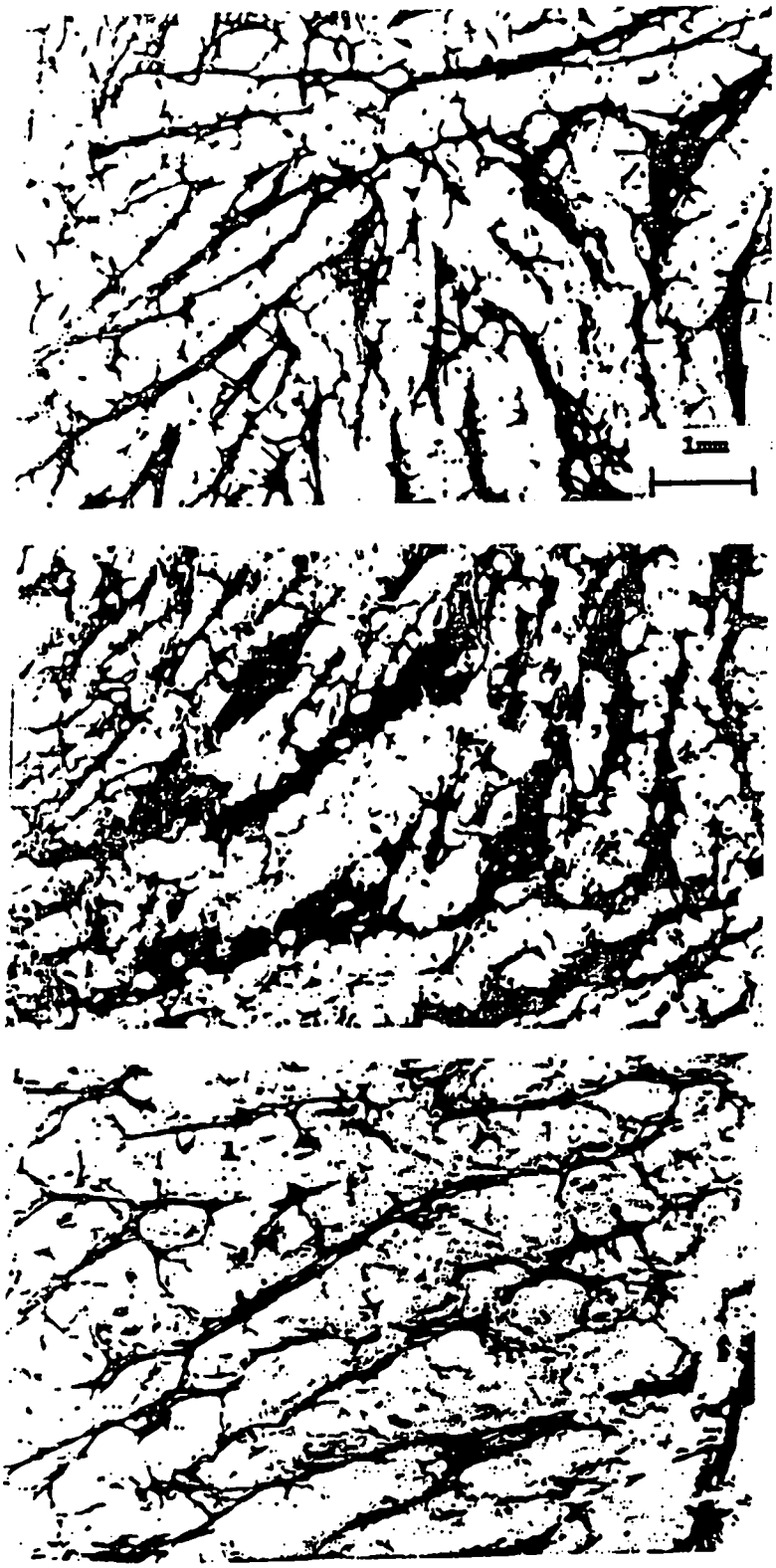

Fig. III.13 Ice Formation of Texturized Meat Patties Made from 1W Mince Frozen at -50°C: (a) Day 1, (b) Day 5, (c) Day 20.

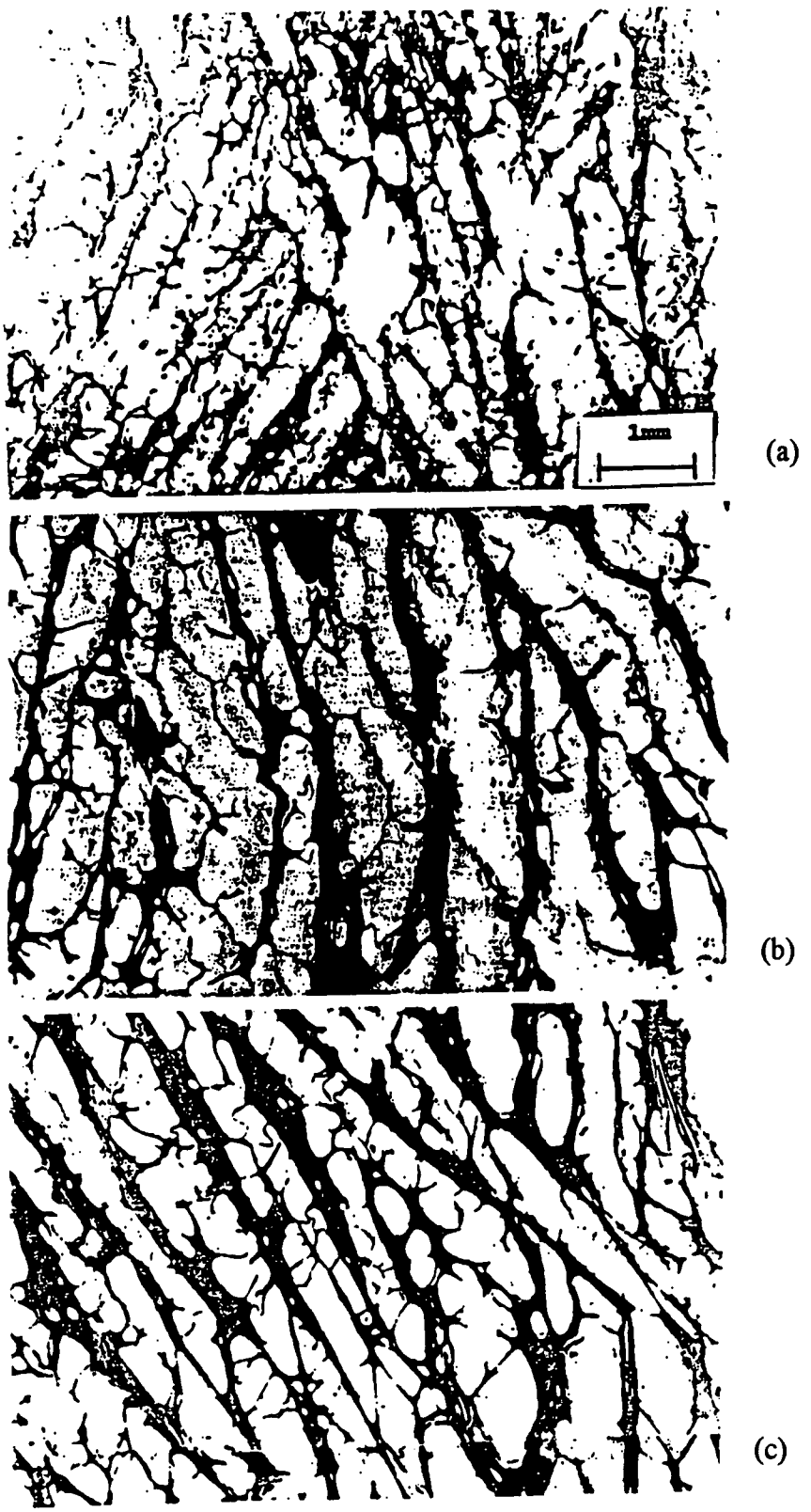

Fig. III.14 Ice Formation of Texturized Meat Patties Made from 1W+C Frozen at -50°C: (a) Day1, (b) Day 5, (c) Day 20.

the damage caused by ice crystals. Since the number of nuclei created is proportional to freezing rate (Bevilacqua et al., 1979), patties at -50°C, with faster freezing rates, had a higher number and more distribution of nuclei. Once ice crystals grew, meat layers were broken into small fragments.

Ice crystals in frozen patties made from washed mince (both 1W and 1W+C) treated at -50°C were bigger and looked more column-like at day 1 and 5 than those made from unwashed mince. At day 20, parallel meat sheets were created. Small amounts of broken fragments were found in patties made from 1W mince but not in those made from 1W+C mince (Fig. III.13c, III.14c). This could be due to higher functional properties in 1W+C mince as indicated by SEP (Table III.1) contributing to a stronger gel which could resist the damage of ice crystals.

For all temperature treatments, samples with cryoprotectants showed smaller ice columns than those without cryoprotectants after 20 days of frozen storage. This may be due to the effects of cryoprotectants which increased the solid concentration and thus, decreased the freezing point of the liquid phase of the samples. All dissolved solutes were suggested to retard the growth of ice crystals (Blond and Colas, 1991). Sugars and soluble proteins slowed down the propagation of ice crystals (Lusena, 1955). This could explain the why the relatively smaller ice crystals and the sponge-like structure were observed in samples made from unwashed mince with cryoprotectants (with higher content of sugars and soluble proteins) in all temperature treatments.

Effects of Treatment Conditions on Shear Force

Shear force indicates the hardness of cooked mince patties. At -7°C, the shear force of texturized mince patties prepared from unwashed mince (0W) increased significantly as storage time was prolonged (Fig. III.15). Shear force of all samples subjected to this temperature was between 0.2-0.7 kg. This trend was also observed by Hsieh and Regenstein (1989) in frozen mince from cod and ocean perch. They reported a gradual increase in hardness during 24 weeks of storage at -7°C. Shear force of patties prepared from 1W mince increased in the first 5 days of frozen storage and remained stable up to 20 days. This implied that one washing cycle could retard the development of shear force caused by freeze denaturation in a long term storage.

At -18°C, all samples had shear forces between 0.20-0.44 kg (Fig. III.15b). Shear forces of all samples at -18°C were lower than those of samples at -7°C at day 5 and day 20. Storage time (20 days) seemed to have no effect on shear force of all samples at -18°C. However, patties prepared from 1W mince had slightly higher shear force than samples prepared from 0W mince. Shear force of samples at -50°C was between 0.19-0.32 kg. As observed in samples at -18°C, storage time had no significant effect on shear force of patties at -50°C. This was in agreement with Hsieh and Regenstein (1989) who reported no significant changes in hardness and cohesiveness of mince stored at -20°C and -40°C.

Shear force of patties made from washed mince (1W and 1W+C) was statistically higher ($p<0.05$) than that of patties made from unwashed mince at day 1 of frozen storage for all temperature treatments. This may be due to the higher content of myofibrillar

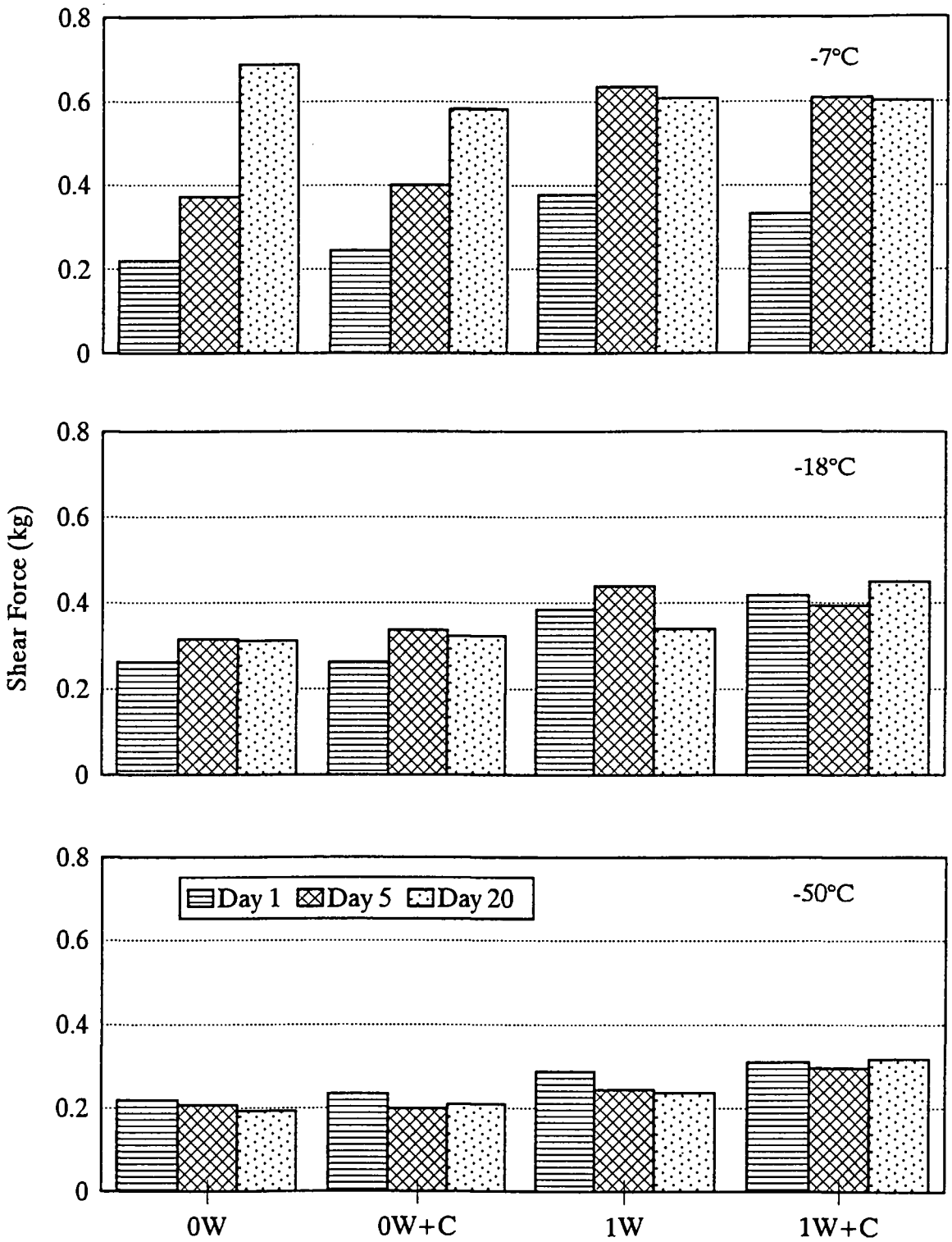

Fig. III.15 Shear Force of Texturized Meat Patties

proteins in washed mince. MacDonald et al. (1992) reported that gel strength of washed mince was higher than that of unwashed mince. Sadowska and Sikorski (1976) and Okada (1964) also found that the removal of fish albumin or water soluble proteins increased gel strength by 30% and 74%, respectively.

Shear force of the texturized patties decreased as temperature decreased (Fig. III.15) after 5 days frozen storage. This could be due to the development of hardness caused by freeze-induced contraction in muscle protein units which was suggested to be a result of loss of water molecules and the following protein cross-linking (Yoon et al., 1991). Slow freezing created ice crystals in the form of extracellular ice which grew as the water diffused out of the cells, while fast freezing created both extracellular and intracellular ice, thus less water was lost from the cells (Connell, 1964). Samples at -7°C tended to lose more water from protein cells than samples at -50°C. Therefore, samples at -7°C may produce harder textures. Hsieh and Regenstein (1989) and Yoon et al. (1991) suggested that the development of toughness in frozen mince was from the crosslinks formed in the muscle which caused the tissue to become more insoluble and tougher.

Nevertheless, at lower temperature (-18°C and -50°C) where the formations of cross-linking and formaldehyde was minimized (especially at -50°C), patties made from washed mince had a higher shear force than those made from unwashed mince. This could be a result of higher SEP (Table III.1), and a higher content of insoluble myofibrillar proteins in washed mince than unwashed mince.

Shear force was found to increase with extended frozen storage only in the -7°C treatment, but frozen storage up to 20 days had no effect on hardness for the -18°C and

-50°C treatments. The shear force of samples made from 1W mince was higher ($p<0.05$) than those made from 0W mince. This could be due to a higher content of water soluble proteins in 0W mince, which might have interfered with freeze-induced cross linking of myofibrillar proteins and prevented texture hardening. Lin and Park (1995) confirmed that washing removed sarcoplasmic proteins in the initial washing step and subsequently, concentrated myofibrillar proteins. It was suggested by Yoon et al. (1991) that sarcoplasmic proteins acted as a blocker which may prevent protein cross-linking and freeze-induced contraction of myofibril. Although -7°C seemed to create a good layer texture in this product, it is not an appropriate treatment for a long term storage due to the development of hardness. In addition, cryoprotectants added to the mince before freezing did not significantly affect hardness of either 0W and 1W mince.

Effects of Treatment Conditions on Cook Loss

Mince conditions, such as washed/unwashed and with/without 6% cryoprotectants, had significant effects ($p<0.05$) on cook loss after steaming. Mince patties made from 0W+C mince had the highest cook loss after steaming (Fig. III.16), followed by those made from 0W, 1W, and 1W+C mince ($p<0.05$) in all temperature treatments. Cook losses of patties made from 0W mince were between 17.85-24.35%, from 0W+C mince were 26.14-31.97%, from 1W mince were 13.53-21.54%, and from 1W+C were 12.03-22.26%. The increase cook loss in unwashed mince could be partly because of the higher fat content (1.97-2.08%) of unwashed than washed mince (1.4-1.67%) (Table III.1). Bhattachaya et al. (1988) and Bhattachaya and Hanna (1989) also

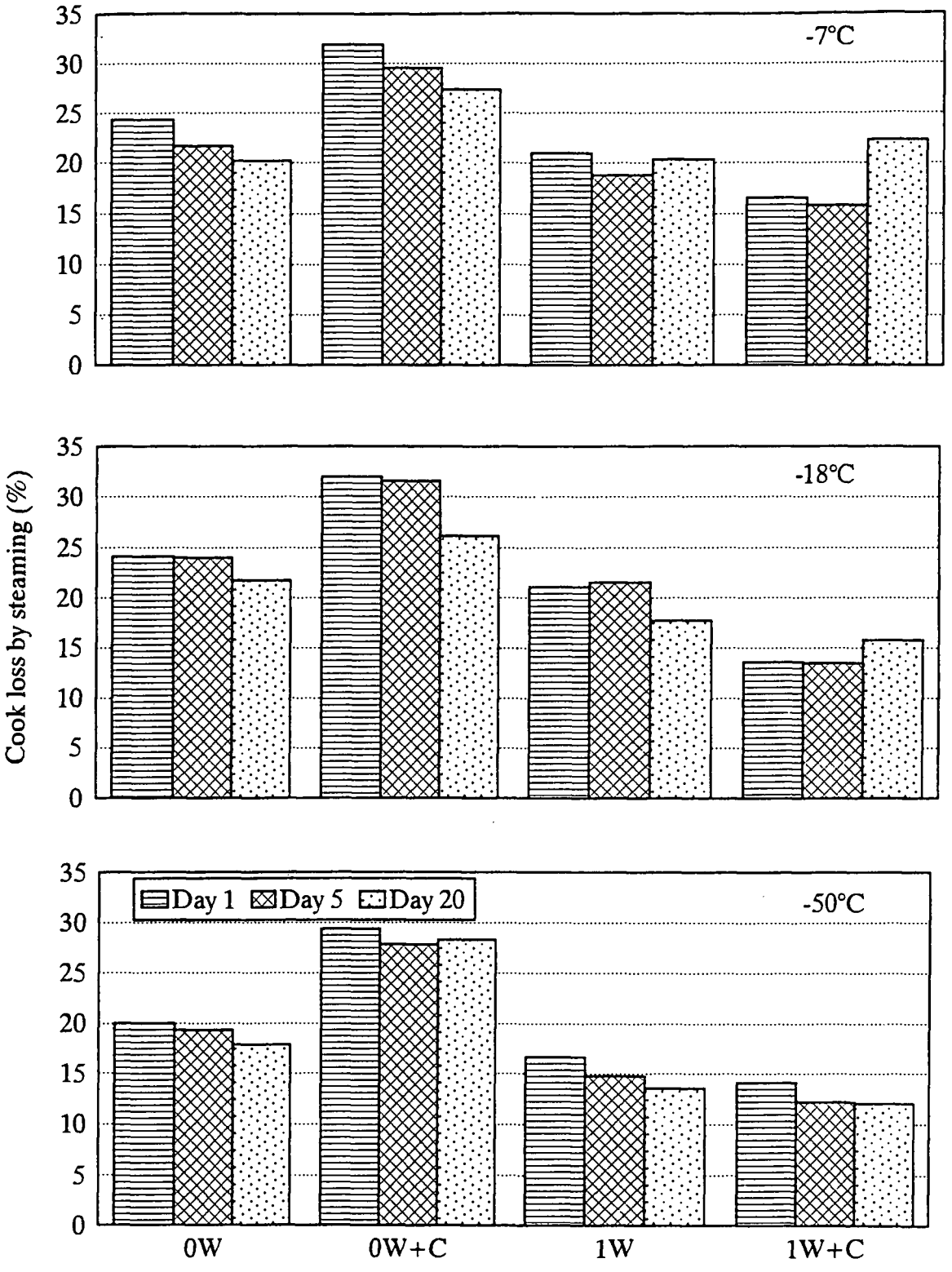

Fig. III.16 Cook Loss of Texturized Meat Patties Determined by Steaming

observed an increase in cook loss in samples with higher fat content. In addition, the lower myofibrillar protein content in unwashed mince might have contributed to the increased cook loss.

The cook loss determined by steam cooking was significantly affected by the frozen storage. Cook loss of 1 day storage was not different from 5 days, but higher ($p<0.05$) than that after 20 days storage. This disagreed with LeBlanc et al. (1988) in cod fillets and Winger and Fennema (1976) in beef muscle who observed the increased cook loss as storage time increased. The reversed results observed in this study could be a result of surface dehydration of long period freezing which caused a dry surface and subsequently created a dry skin on the surface of the product. This skin could act as a wrapping film which prevented water from getting out of the patties and present as a cook loss. This phenomena did not occur in frozen mince or fillet because mince patties were formed from a homogeneous paste in which all particles bound to each other. Once it was dehydrated by freezing the surface became dry and a skin was formed. Unlike meat patties, the surface of frozen mince or fillets has cracks or pores to allow exudate to drain out freely during cooking.

The use of the microwave (800W) to determine cook loss showed a significant difference ($p<0.05$) in mince condition and freezing temperature, but not in frozen storage (Fig. III.17). The patties made from 0W+C mince had highest cook loss in all temperature treatments, as observed by steam cooking. Cook loss of patties treated at -7°C was higher ($p<0.05$) than those at -50°C, but not different from those at -18°C.

Microwave cooking caused higher cook loss than steam cooking in all treatments. This phenomena was also observed in chicken breast by Mallikarjunan and Hung (1995)

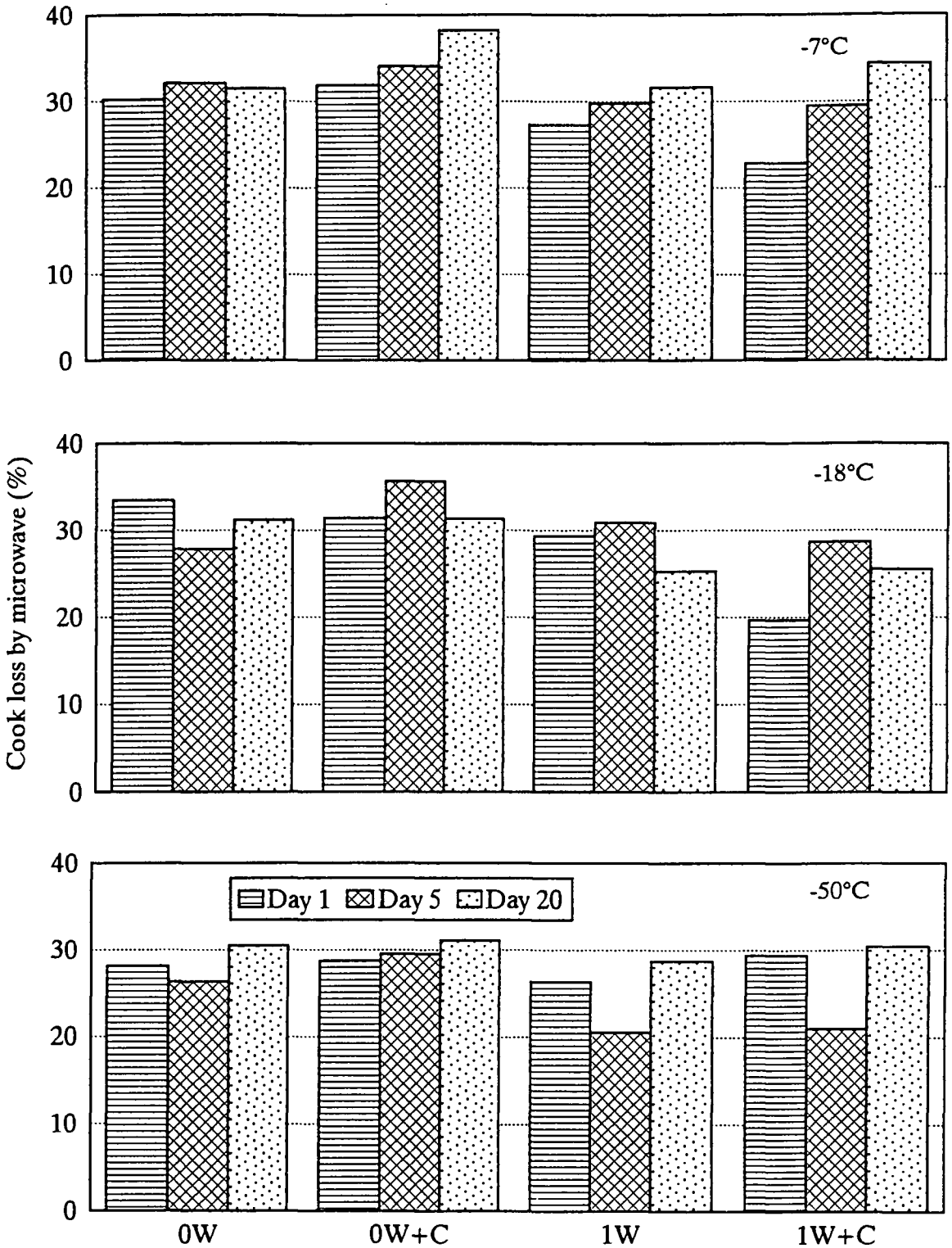

Fig. III.17 Cook Loss of Texturized Meat Patties Determined by Microwave

that microwave cooking at 800W power caused higher cook loss than boiling and baking. This could be because microwave cooking caused some level of dryness and burnt at the edge of the samples in order for the middle spot to reach the desired temperature.

Effects of Treatment Conditions on Whiteness

Whiteness, calculated as L*-3b*, decreased ($p<0.05$) as frozen storage was prolonged for samples treated at -7°C (Fig. III.18). Samples made from 0W and 0W+C mince showed a large decrease in whiteness while those made from 1W and 1W+C mince showed only a slight decrease with extended storage at -7°C. Bhattachaya et al. (1988) and Scott et al. (1988) also observed a loss of color as the frozen storage time increased. This may be partly due to the higher fat content of unwashed mince which affected the color of the sample during freezing, as suggested by Bhattachaya et al. (1988). However, -18°C and -50°C treatments did not change the whiteness of samples with extended frozen storage including samples from unwashed mince. These temperatures could be cold enough to retard or stop the effects of fats or other active compounds on color changes.

Different temperature treatments did not have significant effects on samples made from washed mince (1W and 1W+C) but had effects on unwashed mince (0W and 0W+C). After 5 days frozen storage, whiteness of samples made from 0W mince increased ($p<0.05$) as temperature decreased from -7°C to -18°C, but remained stable as temperature decreased to -50°C. On the other hand, samples made from 0W+C mince showed a constant increase in whiteness with decreasing temperature.

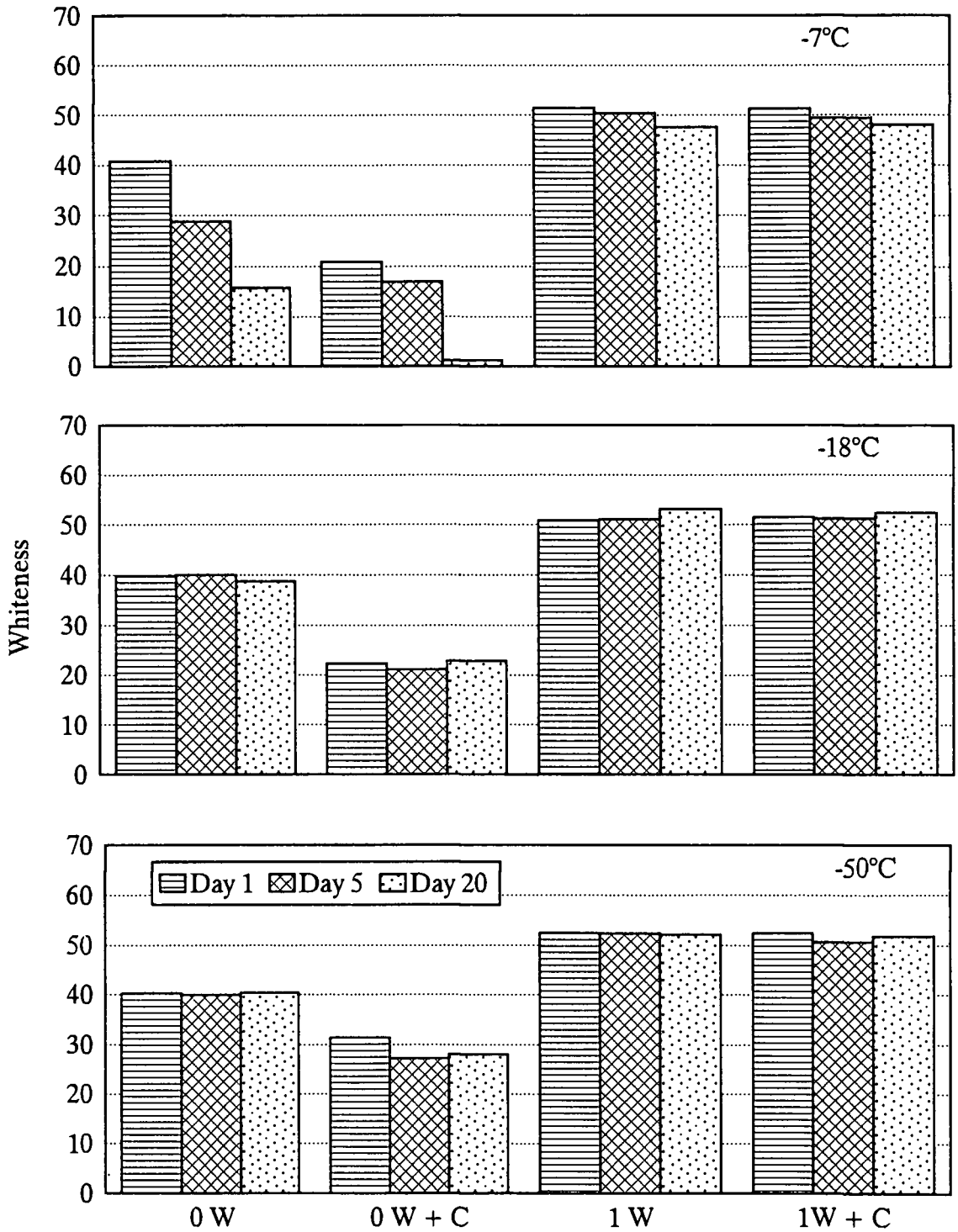

Fig. III.18 Whiteness of Texturized Meat Patties.

Interestingly, cryoprotectants showed an adverse effect on whiteness of unwashed mince but showed no effect on whiteness of washed mince. Mince patties made from 0W+C had lowest whiteness values in all temperature treatments. This could be due to cryoprotectants which, added into the unwashed mince before freezing, contributed to browning during the cooking process. Although cryoprotectants (sucrose and sorbitol) are non-reducing sugars and do not undergo browning reaction, they may be converted to reducing sugars during freezing storage by enzymes or other reactive compounds presented in unwashed mince. However, after mince was washed, cryoprotectants did not affect the whiteness of washed mince because most sarcoplasmic proteins and enzymes were washed out.

Effects of Treatment Conditions on Water Holding Capacity

Water holding capacity (WHC) was calculated by subtracting expressible moisture from total moisture of samples. The type of mince has significant effects on WHC in all temperature and time treatments (Fig. III.19). Mince patties made from 1W mince had highest WHC followed by patties made from 1W+C, 0W, and 0W+C ($p<0.05$). This is similar to the WHC results of the frozen mince (as raw material) (Table III.1) which showed that washed mince had higher WHC than unwashed mince. Cryoprotectants seemed to decrease WHC of both washed and unwashed mince after they were freeze-texturized. WHC of all samples tended to decrease slightly ($p<0.05$) with storage time for the -18°C treatment but it showed no trend for -7°C and -50°C treatments.

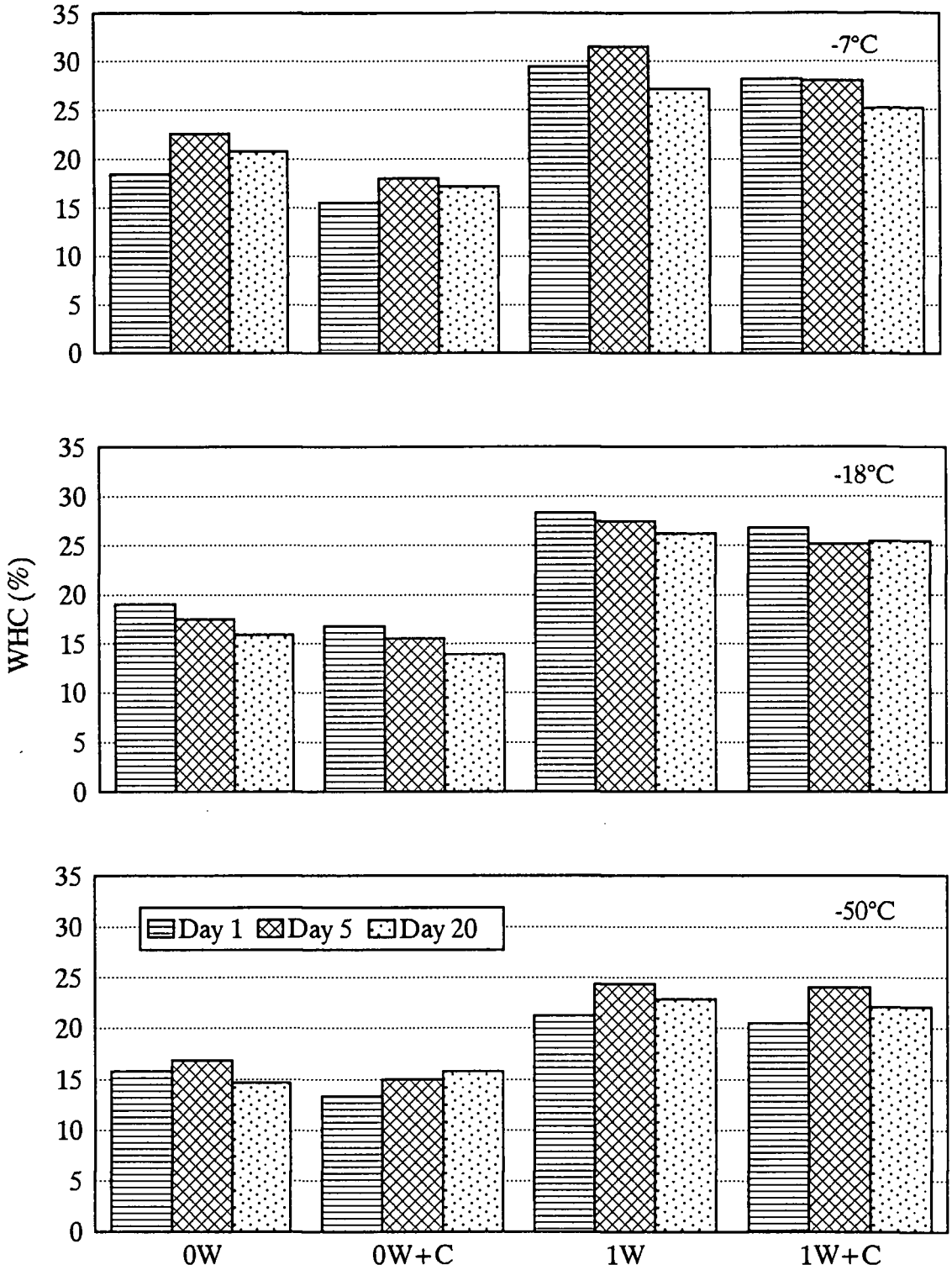

Fig. III.19 Water Holding Capacity of Texturized Meat Patties

Hsieh and Regenstein (1989) also reported a gradual loss of WHC in minced cod and minced ocean perch during 24 weeks frozen storage at -7°C but WHC remained unchanged at -40°C. However, they found that the WHC curves were unstable in the first 10 weeks of frozen storage. They suggested, according to Weinberg et al. (1984), that the variations might be due to several factors (i.e., anions and pH) which affected properties of WHC. This could be a reason for the unstable results of WHC in this study.

Conclusion

Texturized meat was developed by the application of a freeze texturization technique. Unwashed Pacific whiting mince was not suitable as raw material for texturized meat due to its rapid degradation of physical and chemical properties. Pacific whiting mince with 1 washing cycle yielded a much more stable product throughout 20 days storage.

Temperature treatment (or freezing rate) was found to strongly affect layer formations in the meat. Freezing at -7°C was found to create clearly separated layers with bigger spaces between sheets as a result of large ice columns. However, the storage at -7°C created diverse effects on the texturized meat such as development of hardness and deterioration of color. The storage at -18°C was found to be appropriate for freeze texturization of Pacific whiting mince. This condition significantly retarded changes in quality of frozen patties and created parallel layers or meat-liked texture in the patties. An extremely low temperature (-50°C) was found to minimize quality deterioration and create very fine sheets in the product.

Moreover, cryoprotectants seemed to have no significant effect on freeze-induced texture formation or other quality attributes of washed mince. Therefore, cryoprotectants are not needed to store fish mince as a raw material for meat patties.

One important thing that needs to be emphasized is that all frozen patties were made from frozen mince which had been stored frozen for 6-8 mo. All chemical and physical changes which might have occurred during frozen storage were already present. If fresh mince samples were used, obvious changes in quality could be observed. This study was a preliminary study focusing on the feasibility and the practical conditions for the concept of this product. Further study is needed for shelf life and market acceptability.

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IV. SUMMARY

The utilization of Pacific whiting (*Merluccius productus*) can be extended in the form of fresh surimi and the use of minced flesh for texturized meat production instead of only commercially frozen surimi. Fresh surimi had a shelf life of 5 days at 5°C and remained its gel forming ability throughout the storage time. Loss of gel functionality during frozen storage was minimized as the level of cryoprotectants increased. Shear strain of fresh surimi was not different from that of frozen surimi with 9% cryoprotectants but shear stress was almost 3 times higher than frozen surimi.

Texturized meat could be made from Pacific whiting mince by comminuting the mince into a protein slurry and controllably freezing the slurry to create a texturized protein layers which give a meat-like structure. Pacific whiting mince with 1-washing cycle was found to be appropriate for making this product. Unwashed mince showed a rapid quality changes during storage, especially at higher temperature, and created a sponge-like texture. A freezing temperature of -18°C or lower was found to minimize the quality changes during storage. The texture formation of the product could be controlled by adjusting freezing rate during texturization. Faster freezing rate created finer and thinner layers to the product.

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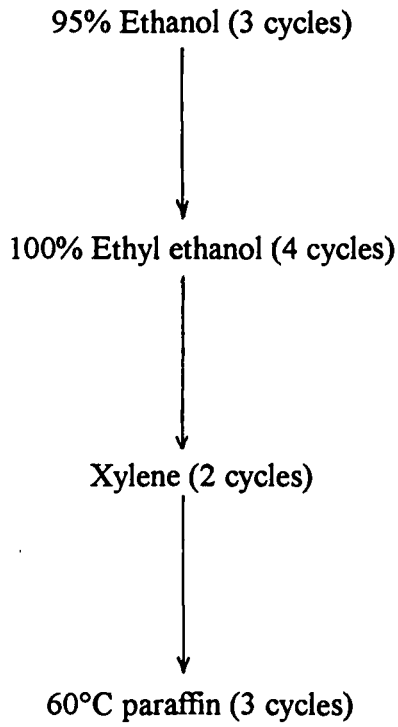
APPENDICES

Appendix A

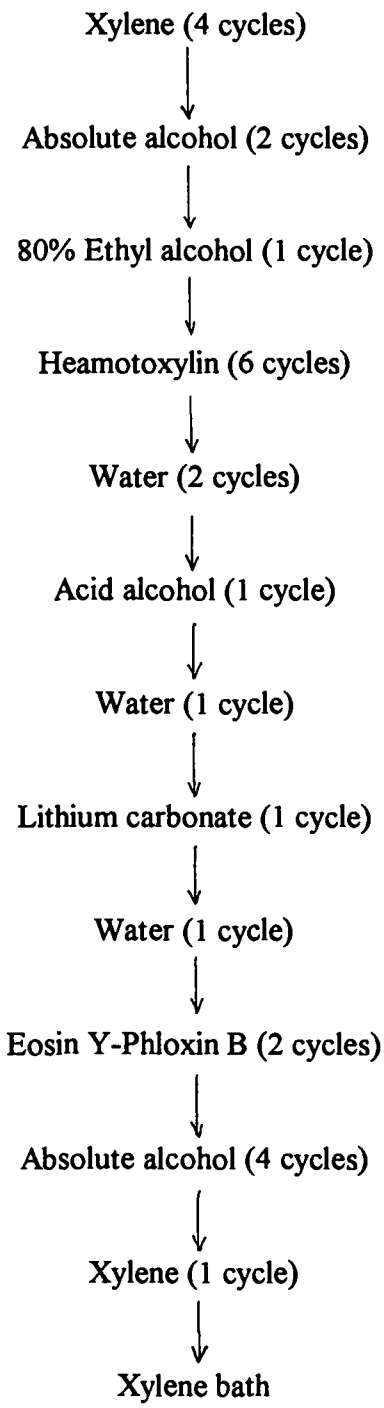
Sample Preparation for Microscopic Study

Alcohol Dehydration and Infiltration



One cycle means washing for 1 hr.

Heamotoxylin and Eosin Staining Method



One cycle means immersing for 30 sec.

Appendix B

Textural Properties of Fresh surimi at Reduced Level Comparing with Frozen Surimi

The results observed in part II of this thesis (Functional Properties and Shelf Life of Fresh Surimi from Pacific Whiting) demonstrated that fresh surimi had higher gel functionality than frozen surimi. It implied that surimi based products could be made of fresh surimi with reduced surimi content without changing functional properties. This experiment was conducted to confirm the investigation previously mentioned.

Materials & Methods

Fresh surimi was obtained from Point Adams Packing Co. (Hammond, OR) and transferred to the OSU Seafood Laboratory within 30 min. One portion of fresh surimi was mixed with 8% cryoprotectants (1:1 sucrose:sorbitol) at low speed for 3 min using a vertical vacuum cutter (Stephan Machinery Corp., Columbus, OH.). Then it was frozen for 2 days before being used to make gels.

The other portion, fresh surimi, was used to make gel with 3 different levels of surimi content. Gels were made using commercial seafood analog formulation which contain 50% surimi. Fresh surimi content was used at 50%, 46% (having the same protein content as with using 50% frozen surimi containing cryoprotectants), and 42.2% (8.3% reduced protein level compared to frozen surimi). The frozen surimi was used at 50% surimi content. The formulation for gel preparation were shown in Table B1.

Table B.1 Gel formulations

Ingredients(%)	0% Fresh	46% Fresh	42.2% Fresh	50% Frozen
Surimi	50	46	42.2	50
Sugar	2	2	2	0
Sorbitol	2	2	2	0
Corn starch	3	3	3	3
Wheat starch	5	5	5	5
Modified starch	1	1	1	1
Salt	2	2	2	2
BPP	1	1	1	1
Water	34	38	41.8	38
TOTAL	100	100	100	100

Gels were prepared by comminuting surimi with all the above ingredients in the Stephan vertical vacuum cutter, as described by Park et al. (1994) and cooked immediately at 90°C for 15 min.

The textural properties (shear stress and shear strain) of the gels were measured by a torsion test as previously described in part II of this thesis. Data were analyzed using the ANOVA test (STATGRAPHICS, 1992).

Results & Discussion

Shear strain of fresh surimi in all levels was between 2.47-2.56 and was not significantly different in either level of surimi content (Fig. B.1a). Shear strain of frozen surimi was 2.37 and was statistically lower ($p<0.05$) than that of frozen fresh surimi of all 3 levels. This was due to freeze denaturation of proteins as described in part II of this thesis.

Shear stress of fresh surimi was 31.29, 37.56, and 45.15 kPa at surimi levels of 42.2%, 46%, and 50%, respectively (Fig. B.1b). Shear stress of frozen surimi was 34.93 kPa. Shear stress of frozen surimi was higher than ($p<0.05$) that of 42.2% fresh surimi, lower ($p<0.05$) than that of 50% fresh surimi, but not different from that of 46% fresh surimi. This could be explained by the level of solid content which contributes to shear stress of gels. Frozen surimi had a protein content of 8.28%, which was equal to that of fresh surimi at 46%. Therefore, frozen surimi had shear stress equal to fresh surimi at 46%. Once the level of surimi content changed, shear stress values changed according to the protein content.

These results implied that fresh surimi can be used at the reduced level of at least 8.3% due to its higher functional property than frozen surimi. Although shear stress of fresh surimi at an 8.3% reduced level (42.2% fresh surimi) was lower than that of frozen surimi, it is still recommended for use in surimi analog production. Shear stress is generally known to be affected by solid content of gels. Therefore, this lower shear stress value of gels made from fresh surimi at a reduced level could be overcome by substituting surimi content with other solids which have lower cost. Shear strain, which is mainly

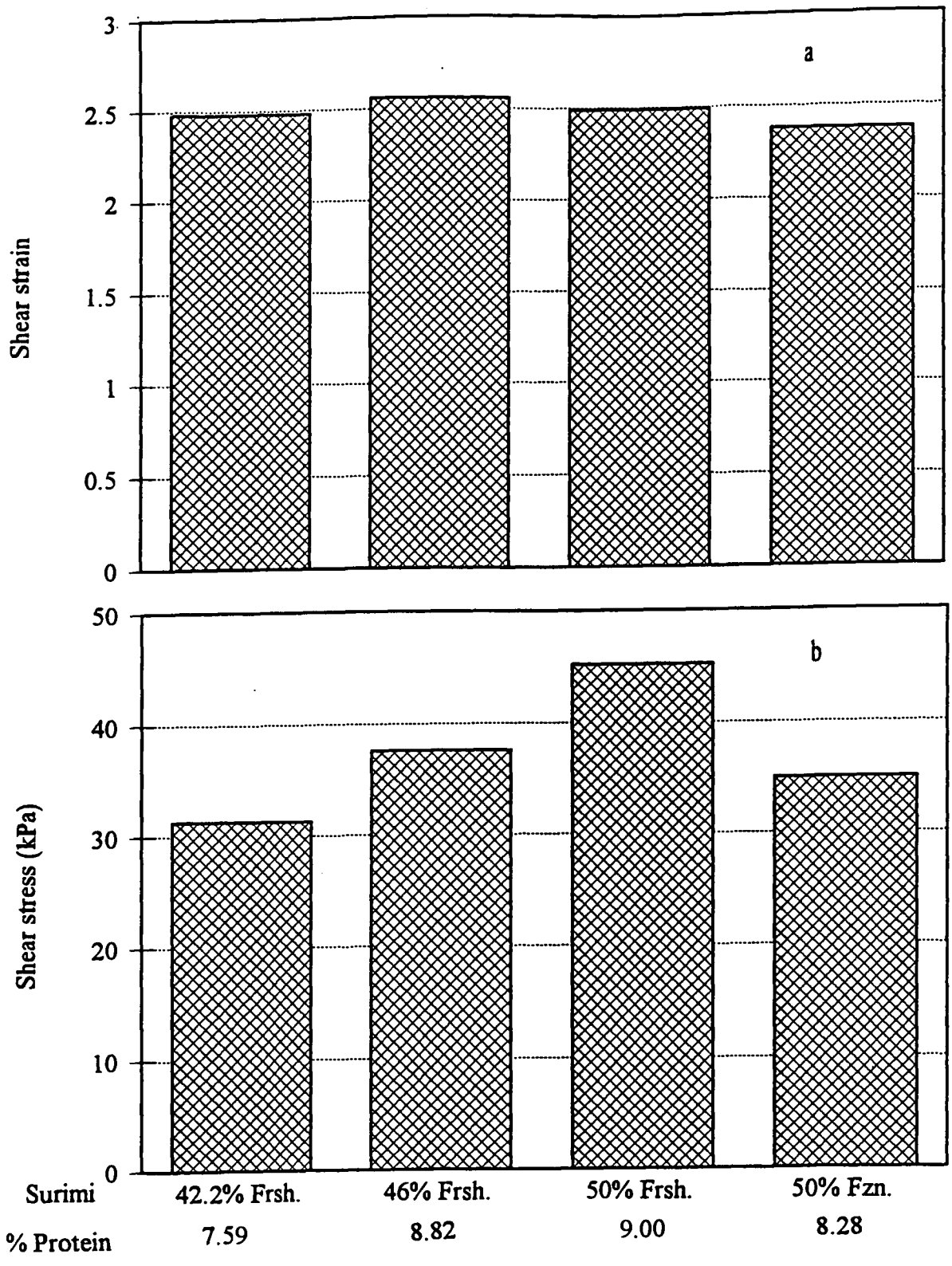


Fig. B.1 Shear Strain (a) and Shear Stress (b) of Fresh Surimi Gels at Reduced Levels Comparing with Frozen Surimi

affected by the protein functionality, obviously showed that fresh surimi can be used at a reduced level of at least 8.2%.

Appendix C

Effects of Cooking Conditions on Quality of Texturized Meat

Fish patties made from Pacific whiting mince without BPP exhibited a firm and elastic texture if cooked by microwave with 2 min defrosting followed by 2 min cooking. This is due to the rapid heat transfer of microwaving which inhibited proteolytic enzymes from causing mushiness. However, this was true only with thin patties (1 cm or thinner). Moreover, due to the non-uniformity of microwave heating, patties cooked by microwave showed a small degree of mushiness in the middle of the patties and some degree of dried skin at the edge. Mince patties with mushiness still showed the parallel sheet characteristic after cooking, but could not hold that texture when subjected to a force (i.e., by pressing with a finger) because of softness of the meat.

After 1% BPP was added to the patties, the softening problem was eliminated, but the non-uniform cooking characteristic still existed after cooking by microwave. Patties with 1% BPP could be cooked by microwave without defrosting but the cooking time was dependent on the starting temperature of the patties (3 min for -7°C, 4 min for -18°C, and 4.5 min for -50°C). Patties cooked this way seemed to have higher degrees of juiciness than those cooked after defrosting.

When a grilling method was used to cook mince patties, the patties with 1% BPP exhibited a firm texture while the patties without BPP were soft and mushy, although they were cooked after thawing. Patties should be cooked with high heat for a short time to prevent dryness and formation of skin. By heating the griller with the lid closed for about

10 min on high heat before cooking, and cooking the patties without thawing for 7-10 min (flip at 4-5 min), firm, tender, and juicy patties can be obtained.

Mince patties cooked by steaming showed a firm texture with 1% BPP but a soft texture without BPP. Mince patties were cooked (reaching inner temperatures of 80 C) by steaming frozen patties at 90°C for 30 min. Longer cooking times (2 hr) caused the patties to be more yellow or lower whiteness but did not change hardness or cook loss of the patties.

It is important to note that thawing patties before cooking greatly affected layer formation of the patties. Patties thawed at 5°C over night and patties thawed at room temperature for 3 hr did not show layer texture after cooking. However, thawing for 2 min by microwave followed by microwave cooking conserved the layer characteristic of the patties. This could be due to the melting of ice crystals between the layers during long thawing periods allowing protein layers which were separated by ice crystals to combine with each other. This led to the loss of layer characteristics of the patties after cooking. Thawing by microwave did not cause this because it took a very short time to melt the ice crystals, and protein layers were fixed by further heating, immediately after ice crystals melted. This did not leave enough time for protein layers to move and compress back with each other.

Quality and texture changes during storage is the major concern for the product to be commercialized. If coarse texture is desired, high temperature treatment needs to be applied which may show rapid quality changes during storage. This could be overcome by moving the texturized meat to store at lower temperature once the desired texture is obtained. However, to minimize the quality and texture changes during storage, product

should be cooked after the desired texture is created then, stored as a frozen cooked product.