Evaluation of Various Infused Cryoprotective Ingredients for Their Freeze–Thaw Stabilizing and Texture Improving Properties in Frozen Red Hake Muscle

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ABSTRACT: Various cryoprotective ingredients were evaluated for their freeze–thaw stabilizing and texture improving properties during 6-mo frozen storage when red hake (*Urophycis chuss*) fillets were injected with sorbitol–sodium tripolyphosphate (STPP) (40% to 60% : 3%), 1.5% alginate, or 0.75% alginate with soy protein isolate (SPI)–sorbitol–STPP (5% to 10% : 40% : 3%). Injection of 10% SPI, 1.5% alginate, or 0.75% alginate–5% SPI effectively improved water binding and retarded the freeze-induced texture changes when drip and cooking loss, centrifugal expressible moisture, protein extractability, SDS-PAGE profile, and Instron and sensory texture were assessed. The sorbitol and STPP combination was not as effective as alginate and SPI. Freeze-susceptible whitefish can be injected with proper cryoprotective ingredients for improved frozen storability.

Keywords: alginate, frozen storage, injection, red hake, soy protein isolate

Introduction

Prolonged storage, especially at temperatures around −18 °C, brings about significant deterioration of texture of frozen fish described as increased toughness, chewiness, rubberiness, or stringiness (Sikorski and others 1976). During extended frozen storage, ice crystals continue to grow, with increases in salt concentrations in the liquid phase leading to protein denaturation and subsequent loss of protein functionality and texture hardening (Shenouda 1980; Xiong 1997). Denaturation or insolubilization of actomyosin during frozen storage is a result of protein aggregation caused by the progressive increase in intermolecular cross-links due to the formation of hydrogen bonds, ionic bonds, hydrophobic bonds, and disulfide bonds (Matsumoto 1979; Jiang and others 1988; Ramirez and others 2000). Cross-linking may also result from increased concentrations of tissue salts such as calcium, which is known to form ionic cross-links between peptide chains (Sikorski and others 1976).

Generally cryoprotectants such as sucrose or sorbitol prevent ice crystal growth and the migration of water molecules from the protein, thus stabilizing the protein in its native form during frozen storage (Matsumoto and Noguchi 1992). Cryostabilization mechanisms of various cryoprotectants in fish muscle were further discussed in depth by MacDonald and Lanier (1997). Recently, Goeller and others (2004) compared immersion (1 h at 25%) and injection of sorbitol (60% to bring 5% uptake) for their cryoprotectability in freshwater trout chunks. Both treatments retained the original ATPase activity after 9 freeze–thaw cycles. Polyphosphates are also used as a cryoprotective agent. Sodium tripolyphosphate (STPP), when used as a dipping solution at 15%, was found to significantly improve texture of frozen mullet (*Mugil cephalus*) (English and others 1988). Krivchenia and Fennema (1988) found significantly less centrifugal drip loss and about one-third less firmness in 28-wk frozen whitefish (*Coregonous cupleaformis*) fillets with the addition of tripolyphosphates at 11.8%. This may be due to the ability of phosphate to split the actomyosin complex into myosin and actin, improving water-binding capacity and protein solubility (Shimp 1987; Gard and others 1992). When used in the washed red hake (*Urophycis chuss*) fish mince at 4% to 6%, soy protein isolate (SPI) was found to reduce the amount of free water available for ice crystallization and allow uniform ice crystal formation and fill the sarcoplasmic space between myofibrils, preventing extensive cross-linking between myofibrils (Yoon and others 1991). Examples of soy protein helping to improve textural properties of frozen fish fillets can best be seen by Crapo and others (1999), who demonstrated that injecting a 7.5% SPI solution improved the texture of giant granadier (*Albatrossia pectoralis*) fillet. In addition, sodium alginate at 0.4% was found to decrease the hardness of a frozen red hake mince (Lian and others 2000) and a cooked fish mince patty prepared from a blend of Atlantic pollock (*Pollachius virens*) and Greenland turbot (*Reinhardtius hippoglossuides*) (Rockower and others 1983). Alginate is believed to chelate calcium ions responsible for cross-linking and interfere with muscle fiber interaction through the formaton of a calcium ion bridge (Lian and others 2000). Wang and Xiong (1998) reported that addition of antioxidant (0.02% propyl gallate) together with cryoprotectants (4% sorbitol–4% sucrose) further improved the protein functionality in beef heart surimi during frozen storage.

Unlike surimi, a fish fillet cannot be mixed, making dispersion of the ingredients difficult. Currently, the seafood industry employs vacuum tumbling for marination that allows diffusion only in the outer layer of the product and leaves the center of the product unprotected from freeze-induced physical and chemical changes. On the other hand, the injection process allows relatively uniform dispersion of cryoprotectants throughout the tissue, resulting in a better cryoprotected end product. Only a few attempts have been made using injection to improve frozen seafood quality, Crapo and others (1999) in giant granadier fillets and Krivchenia and Fennema (1988)

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Fennema (1988) with modifications. Two grams of homogenized fish muscle and 30 mL of extracting solution (0.6 M KCl and 50 mM phosphate buffer adjusted to pH 7.0 with 0.1 M NaOH) were blended in a 250-mL Eberbach blending jar (VWR International, Bridgeport, N.J., U.S.A.) at a high speed for 15 s at 4 °C. The blended fish muscle was transferred to a 50-mL polyurethane centrifuge tube along with 10 mL of extracting solution used to rinse the blending jar. The mixture was then centrifuged for 30 min at 28,710 × *g* (15,500 rpm) at 4 °C in a refrigerated centrifuge (Sorvall RC02B; SS34 rotor). One milliliter aliquot of the supernatant was removed from each centrifuge tube and mixed with 9 mL of extracting solution (1:10 dilution). A 0.1 mL aliquot of diluted solution was mixed with 5 mL Bio-Rad reagent (Bio-Rad, Hercules, Calif., U.S.A.) and the absorbance was measured at 595 nm. The protein concentration was determined following the Bradford (1976) method using bovine serum albumin (Sigma Chemical Co., St. Louis, Mo., U.S.A.) as a protein standard. The protein extractability was calculated by dividing extractable protein after frozen storage by one before frozen storage and expressed as a percentage.

SDS–polyacrylamide gel electrophoresis

As an indication of the extent of freeze-induced protein-protein interaction, changes in the electrophoretic profiles of myosin and actin in the fish fillet during frozen storage were examined using SDS–polyacrylamide gel electrophoresis (SDS-PAGE). The electrophoretic profile reflects changes in the functionality and extractability of proteins during the course of frozen storage (Chang and Regenstein 1997; Huidobro and others 1998; Lian and others 2000). The present study employed electrophoresis to examine the effect of injected cryoprotectants on intermolecular interactions and to help verify the results obtained from textural measurements and protein extraction. Fillets stored at −84 °C were used as a reference where the least changes in the protein were expected to occur. Each sample was prepared according to Lian and others (2000). SDS-PAGE analysis was performed using 10% polyacrylamide gels and a 4% stacking gel overlay according to the procedure of Laemmli (1970) in a mini Protean II system (Bio-Rad). The molecular weights of the proteins in the sample were compared by estimating them with molecular weight markers SDS 6H (205,000 to 29,000 kDa) (Sigma).

Sensory analysis

Sensory evaluation was performed on cooked fillet samples by a panel of five in the sensory room under the normal lighting at the Food Science and Nutrition Research Center. The panelists consisted of graduate students and staff. Samples were prepared in the same manner as described in the cook loss determination. They were cooked at 176.6 °C (350 °F) for 15 min and served after 5 min standing at room temperature. A 9-point scale (1: least, 5: moderate, 9: very) was used to evaluate firmness, moistness, and overall texture desirability of each sample. Cooked fresh fillets were used as reference with designated scores of 4, 7, and 8 for firmness, moistness, and overall texture desirability, respectively. Each session was repeated using the same lot of samples.

Data analysis

Data analysis was conducted using a statistical software package SPSS ver. 9.0 (2001) (Chicago, Ill., U.S.A.). One-way analysis of variance (ANOVA) and Duncan's multiple range test were performed to determine significant effects of injecting cryoprotectants on textural properties of fish fillets and to detect any significant differences among treatments at $P \leq 0.05$ ($n = 4$ for physical tests; $n = 5$ for sensory analysis).

Results and Discussion

Drip loss and water binding of fish fillets injected with cryoprotectant solutions

Fillets were injected with cryoprotectant solutions and water and compared with −18 °C control and −84 °C reference (representing a fresh sample). Figure 1 indicates that among injected samples the one injected with 10% soy had the least drip loss, followed by 60% sorbitol, 40% sorbitol, and water consistently throughout frozen storage.

The relatively higher drip loss values of injected samples than uninjected ones (−18 °C control and −84 °C reference) were believed to be due to added liquid to the tissue (3 mL/30 g). However, for the centrifugal expressible moisture, the injected samples had significantly less expressible moisture than the −18 °C control throughout frozen storage even though the added moisture in the injected solutions was taken into account (Figure 2). The possible explanation is that the water remained in the frozen–thawed tissue treated with cryoprotectants tended to be bound more than that in the untreated control. As expected, the −84 °C reference displaced the least amount of moisture. This was followed by 60% sorbitol, 10% soy, and 40% sorbitol injections. The −18 °C control and the water-injected fillets both showed the least amount of moisture expressed.

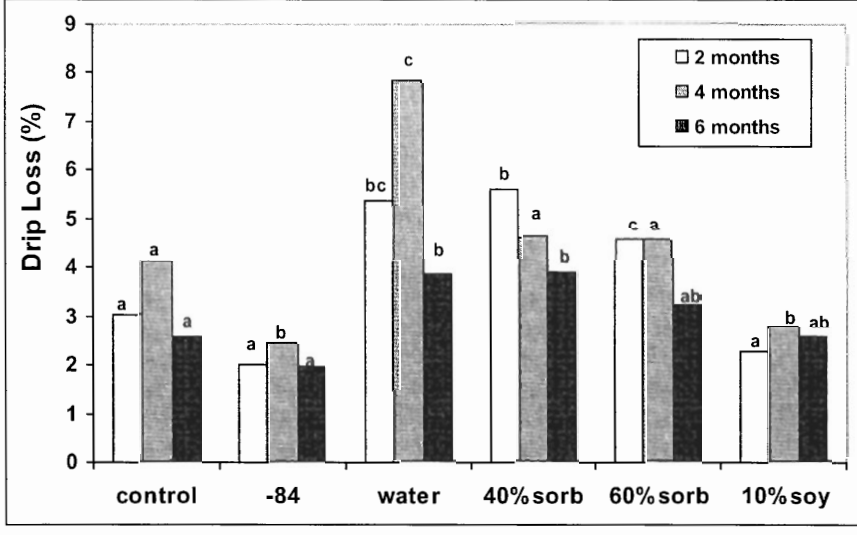

Figure 1—Drip losses of fish fillets injected with various cryoprotectants after frozen stored at −18 °C. Means within the same storage period with different letters are significantly different ($n = 4$). 40% sorb: 40% sorbitol + 3% STPP; 60% sorb: 60% sorbitol + 3% STPP; 10% soy: 10% SPI + 40% sorbitol + 3% STPP.

When alginate was tried, the 5% soy–0.75% alginate mix and 1.5% alginate showed significantly less amount of expressible moisture than the control (Figure 2). The improved water binding demonstrated by alginate could be explained by its ability to chelate calcium ions and reduce protein cross-linking with increased water holding within the myofibrillar network (Lian and others 2000). Shimp (1987) pointed out that tripolyphosphate can sequester calcium in the fish fillet. It is not clear how effective the calcium sequestering ability of phosphate is in texture control of frozen fish relative to alginate. The calcium content in red hake (29.4 mg/100 g) was found to be significantly higher than Alaska pollock (16.5) and Pacific whiting (8.7), suggesting that it plays a key role in texture hardening of fish mince since uncooked red hake mince (400 *g* penetration force) became significantly harder than Alaska pollock mince (150 g) (Lee and Lian 2002). The added effect of the water binding by SPI when injected with alginate brought about the least amount of expressible moisture in the fillets injected with the soy–alginate mix. In addition to calcium-chelating ability, alginate forms colloidal dispersion that effectively holds the water in the muscle matrix. In our previous study on red hake mince, 0.4% alginate with 4% sorbitol and 0.1% STPP kept the frozen mince from hardening and improved its dispersibility during mixing, while carrageenan was found to be ineffective (Lian and others 2000). This suggests the water binding of hydrocolloid alone will not help improve the moistness and texture of frozen mince without calcium chelation. The calcium content in red hake (29.4 mg/100 g) was found to be significantly greater than Alaska pollock (16.5) and Pacific whiting (8.7), suggesting that it plays a key role in texture hardening of fish mince since uncooked red hake mince (400 *g* penetration force) became significantly harder than Alaska pollock mince (150 *g*) after 17-wk frozen storage at −20°C (Lian and others 2000; Lee and Lian 2002).

Injection of cryoprotectants on cooking loss of fish fillets

Results of cooking loss after 0-, 2-, 4-, and 6-mo frozen storage periods are given in Figure 2. At day 0, there were less cooking losses in the injected samples compared to the control. This must be due to the increase in solids from the cryoprotectants injected into the fish fillets. The −84 °C had more cooking loss than the rest throughout the frozen storage periods due to the release of the greater amount of moisture that was retained under cryogenic storage accompanied by minimal ice crystal growth and protein denaturation. These cooking loss values were based on the weights of fillets minus freeze–thaw drip. Those which had more drip losses would have had less retained moisture to release on cooking. At 2- and 4-mo storage, the 10% soy solution had the lowest cooking losses. After 6-mo frozen storage, both 60% sorbitol and 10% soy samples exhibited lower cooking losses than the rest, where the cooking loss of 10% soy (17.48 ± 1.69; variation coefficient 9.67%) appeared more consistent than that of 60% sorbitol (16.60 ± 4.72; 28%) on the basis of the magnitude of variation coefficient. Soy protein isolate as a water-binding agent in cooked haddock mince was investigated by Karmas and Turk (1976). They found that soy protein isolate was a significantly better water binder than other proteins added (whey protein concentrate and caseinate) at 2% and 5%. Lian and others (2000) also found that red hake mince samples with added soy protein concentrate had significantly lower cooking loss than other cryoprotective additives. It is believed that soy protein has the ability to form a strong matrix in which water is held resulting in less cooking loss.

Sorbitol, on the other hand, was found to be ineffective in reducing cooking loss (Yoon and Lee 1990; Yoon and others 1991). When the solid contents of cooking drip from cryoprotected fish mince was measured against the control with no added sorbitol, Lian and others (2000) concluded that the increased solid contents in the cooking drip was due to released sorbitol which contributed to increased cooking loss.

Injection of cryoprotectants on textural properties of raw and cooked fillets

As shown in Figure 3, after 2 mo of frozen storage, all groups of raw fillets injected with cryoprotectant solutions except for water demonstrated significantly less shear firmness when subjected to textural measurements and compared to the −18 °C control. After 4 and 6 mo of frozen storage, the fillets injected with 10% soy solution remained significantly less firm than the −18 °C control and other treatments. These results are supported by Yoon and others (1991), who observed that the addition of 6% soy protein isolate kept the firmness of frozen washed red hake mince significantly lower than the untreated control. Crapo and others (1999) found that the injection of a 7.5% soy protein isolate solution produced the highest textural and desirability scores in cooked grenadier fillets. Lian and others (2000) demonstrated that red hake fish mince with 4% soy protein concentrate had significantly lower shear force than the untreated control. When alginate was evaluated in uncooked fillets, the 1.5% alginate samples were the least firm followed by the soy–alginate mix (Figure 3). This clearly followed the pattern of expressible moisture after treatments (Figure 2) and can be explained as previously on expressible moisture. The shear force values of cooked fish fillets in the present study were also found to be the lowest at month 2, but moderate at month 6 for the 10% soy group compared to other treatments (Figure 3). The addition of soy protein may improve textural properties by reducing freeze-induced shrinkage of myofibrils that results from protein intermolecular cross-linking (Yoon and others 1991; Lian and others 2000). The good water-binding ability of soy protein helped reduce the amount of free water available for ice crystallization and thus reduced protein intermolecular interaction, resulting in less freeze-induced shrinkage of muscle myofibrils during frozen storage.

Injection of cryoprotective ingredients in the right combination may have helped keep the frozen fillets from becoming harder through the cryoprotectants' ability to bind water and to reduce protein denaturation and intermolecular interactions. Protein intermolecular interactions involving the formation of disulfide and ionic bonds play an important role in textural hardening (Sikorski and others 1976; Shenouda 1980; Jiang and others 1988; Ramirez and others 2000). In addition to retarding protein intermolecular interactions, the injection of 10% soy and 60% sorbitol may have helped bind free water for ice crystal formation, reducing the concentration of tissue salts available to induce ionic bonds leading to textural hardening. As discussed earlier, alginate in a singular application, on the other hand, is the most effective in controlling protein cross-linking through chelating calcium ions, thus reducing freeze-induced texture hardening especially of the high calcium-containing fish muscle tissue.

Comparison of sensory moistness, firmness and overall texture desirability of cooked fish fillets injected with cryoprotectants

Sensory scores varied with each time period. When the moistness of cooked fish fillets was evaluated at month 2 (Figure 4), water injected samples received the highest moistness score (6.50). This was followed closely by 10% soy samples, and the 60% sorbitol and −84 °C reference, both with scores of 5.67. The −18 °C control was found to be the least moist (4.83) as expected. After 4 mo of frozen storage, the 10% soy and sorbitol samples remained more moist than the rest, with the −18 °C samples being the least (4.00). After 6 mo of

received the highest overall texture scores. The 60% sorbitol sample followed and the −18 °C sample was rated the least desirable (4.00). The lower score for 60% sorbitol may have been due to the sweetness associated with sorbitol at a high level. After 6 mo of storage, the −18 °C sample was again rated the least desirable. The −84 °C was rated the most desirable sample even though it had received a poor score on moistness, followed by the 60% sorbitol samples (5.67). The 40% sorbitol and 10% soy samples received moderate desirability scores. The high level of sorbitol at 60% may have helped bind water and retard the ice crystal growth over the duration of the 6-mo frozen storage, making it more desirable than the other treatments. Increased sweetness was noticed in the 60% sorbitol samples when compared to all other samples. This could decrease consumer acceptability, while the less sweet 10% soy or 40% sorbitol is considered more applicable. The inconsistent pattern observed in 6-mo samples could be due to sampling variations such as thickness and location of the cut fillet.

When injected with alginate, the sensory evaluation indicated a significant decrease in the firmness of the 1.5% alginate samples compared to the control (Figure 5). The 10% soy and the soy–alginate mix were also rated less firm than the control, supporting results obtained from the Instron textural analysis. Although there were no significant differences, moistness scores were higher for all 3 treated groups compared to the control. This indicates that the injection of cryoprotectants binds water and helps retain it during cooking, improving the fillet texture. Overall texture desirability was significantly

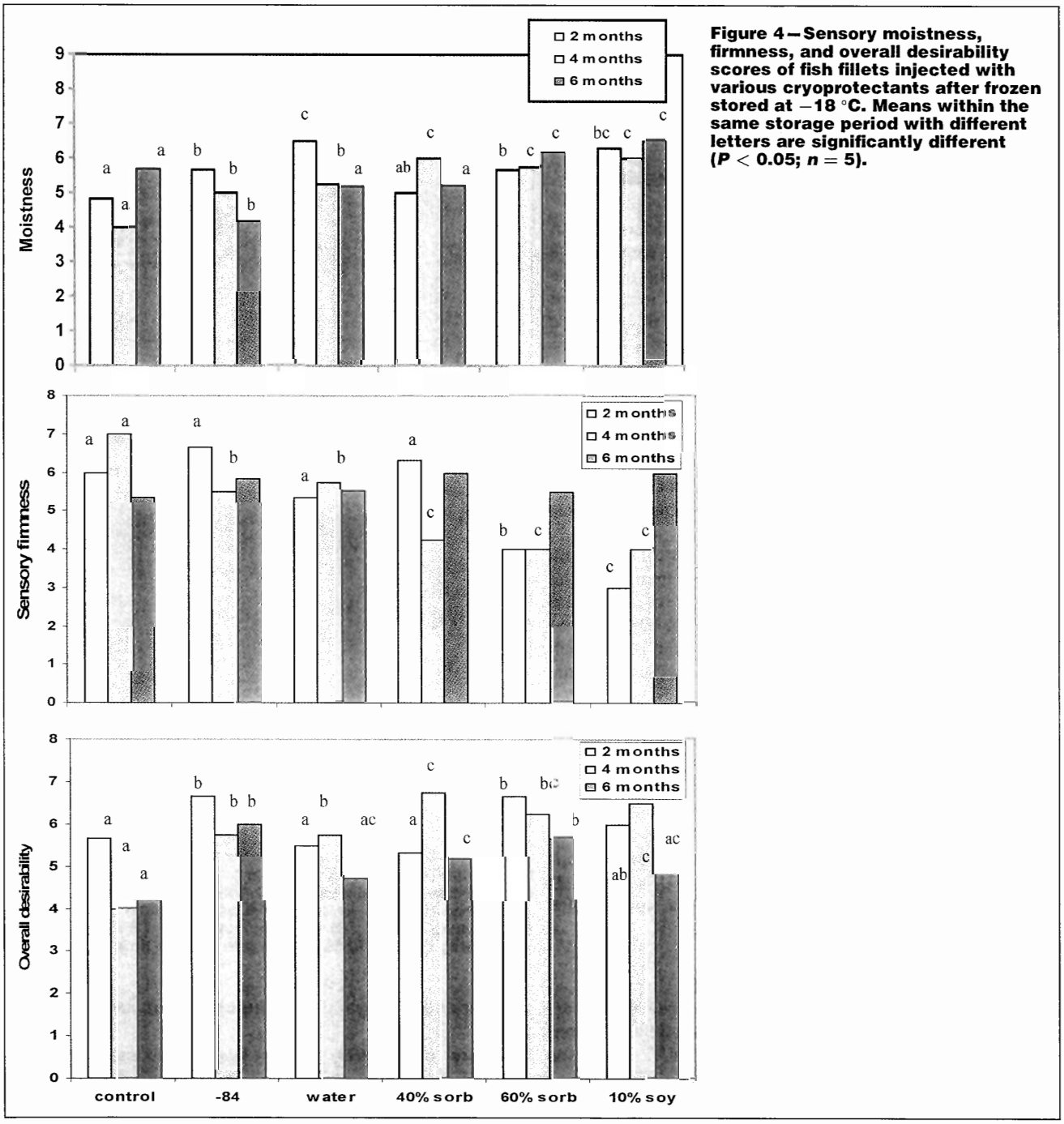

Figure 4—Sensory moistness, firmness, and overall desirability scores of fish fillets injected with various cryoprotectants after frozen stored at −18 °C. Means within the same storage period with different letters are significantly different ($P < 0.05$; $n = 5$).

found that the addition of 0.4% alginate–4% sorbitol–0.3% STPP to red hake fish mince helped prevent S–S cross-linking of structural proteins with no discernible differences in electrophoretic protein profile between the −84 °C reference and alginate-added fish mince. Chang and Regenstein (1997) found that the addition of polyphosphates increased the intensity of the MHC band and thought it was due to increased water uptake ability. Cross-linking may result from increased concentrations of tissue salts such as calcium, which is known to form ionic cross-links between peptide chains, changing protein structure (Sikorski and others 1976). The injection of sorbitol and polyphosphate into the fish tissue may have reduced large ice crystal formation and increased water uptake ability, thus decreasing tissue salt concentrations, which are known to induce intermolecular cross-linking (Sikorski and others 1976; Matsumoto 1980). The addition of a water-soluble soy protein may have prevented protein intermolecular cross-linking not only by binding water to prevent large ice crystal formation but also by filling the sarcoplasmic space between myofibrils (Yoon and others 1991).

Conclusions

The injection of cryoprotectants can significantly improve the quality of freeze-susceptible white fish fillets. The injection of water did not decrease texture hardening of the fish fillets, but improved moistness. Fillets injected with 10% SPI, 1.5% alginate, or 0.75% alginate–5% SPI with 40% sorbitol and 3% STPP displayed the best water-binding ability, texture softening, and overall liking. This was confirmed by protein extractability and SDS-PAGE profile indicating that the injection of cryoprotectants improved water binding and retarded protein denaturation and intermolecular interactions. Among cryoprotectants evaluated, alginate was found to be the most effective in keeping the frozen fish muscle from hardening by chelating calcium ions responsible for muscle protein cross-linking via a calcium ion bridge. The sorbitol and STPP combination was not as effective as alginate and SPI. Some inconsistencies noticed in the data may be attributed to sample variability in the thickness of the fillets and sampling from the fillets of different fish.

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References

Awad A, Powrie WD, Fennema O. 1969. Deterioration of fresh water whitefish muscle during frozen storage at −10 °C. J Food Sci 34(1):1–9.

Bradford M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–54.

Chang CC, Regenstein JM. 1997. Water uptake, protein solubility, and protein changes of codmince stored on ice as affected by polyphosphates. J Food Sci 62(2):305–9.

Crapo C, Himelbloom B, Pfutzenreuter R, Lee CM. 1999. Texture modification processes for giant grenadier (*Albatrossia pectoralis*) fillets. J Aquatic Food Product Technol 8(4):27–40.

English PM, Gerdes DL, Finerty MW, Grodner RM. 1988. Effects of tripolyphosphate dips on the quality of thermally processed mullet (*Mugil cephalus*). J Food Sci 53(5):1319–21.

Gard DR, Heidolph BB, Teicher H, Unland ML. 1992. Phosphates and food processing. In: Hui YH, editor. Encyclopedia of food science and technology, Vol 3. New York: John Wiley and Sons. p 2061–5.

Gill TA, Keith RA, Smith B. 1979. Textural deteroration of red hake and haddock muscle in frozen storage as related to chemical paprameters and changes in the myofibrillar proteins. J Food Sci 44(3):661–7.

Goeller LM, Amato PM, Farkas BE, Green DP, Lanier TC, Kong CS. 2004. Optimization of incorporation of low-molecular-weight cryoprotectants into intact fish muscle. J Food Sci 69(4):FEP164–71.

Huidobro A, Alvarez C, Tejada M. 1998. Hake muscle altered by frozen storage as affected by added ingredients. J Food Sci 63(4):638–43.

Jauregui CA, Regenstein JM, Baker RC. 1981. A simple centrifugal method for measuring expressible moisture, a water-binding property of muscle foods. J Food Sci 46(4):1271–3.

Jiang ST, Hwang DC, Chen CS. 1988. Effect of storage temperature on the formation of disulfides and denaturation of milkfish actomyosin (*Chanos chanos*). J Food Sci 53(5):1333–5.

Karmas E, Turk K. 1976. Water binding of cooked fish in combination with various proteins. J Food Sci 41(5):999–1003.

Krivechenia M, Fennema OR. 1988. Effect of cryoprotectants on frozen whitefish fillets. J Food Sci 53(4):999–1003.

Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–5.

Lee CM, Lian PZ. 2002. Cryostabilization of unwashed fish mince. Proc. int. commemorative symp. 70th anniversary of the Japanese Society of Fisheries Science II. Fisheries Sci 68(Suppl II):1355–8.

Lian PZ, Lee CM, Hufnagel L. 2000. Physicochemical properties of frozen red hake (*Urophycis chuss*) mince as affected by cryoprotective ingredients. J Food Sci 65(7):1117–23.

MacDonald GA, Lanier TC. 1991. Carbohydrates as cryoprotectants for meats and surimi. Food Tech 45(3):150–9.

MacDonald GA, Lanier TC. 1997. Cryoprotectants for improving frozen food quality. In: Erickson MC, Hung YC, editors. Quality in frozen foods. New York: Chapman and Hall. p 197–232.

Matsumoto JJ. 1979. Denaturation of fish muscle proteins during frozen storage. In: Fennema OR, editor. Proteins at low temperatures. Advances in Chemistry Ser No 180. Washington, D.C.: The American Chemical Society. p 205–24.

Matsumoto JJ. 1980. Chemical deterioration of muscle proteins during frozen storage. In: Whitaker JR, Fugimaki M, editors. Chemical deterioration of proteins. ACS Symposium Ser No 123. Washington, D.C.: The American Chemical Society. p 95–124.

Matsumoto JJ, Noguchi SF. 1992. Cryostabilization of protein in surimi. In: Lanier TC, Lee CM, editors. Surimi technology. New York: Marcel Dekker. p 357–88.

Ramirez JA, Martin-Polo MO, Bandman E. 2000. Fish myosin aggregation as affected by freezing and initial physical state. J Food Sci 65(4):556–60.

Rockower RK, Deng JC, Otwell WS, Cornell JA. 1983. Effect of soy flour, soy protein concentrate and sodium alginate on textural attributes of minced fish patties. J Food Sci 48(4):1048–52.

Shenouda SY. 1980. Theories of protein denaturation during frozen storage of fish flesh. Adv Food Res 26:275–311.

Shimp LA. 1987. Food phosphates in seafood. Bulletin No 6. Philadelphia, Pa.: FMC Corp.

Sikorski ZE, Olley J, Kostuch S. 1976. Protein changes in frozen fish. CRC Cric Rev Food Sci Nutr 8(1):97–129.

Wang B, Xiong YL. 1998. Functional stability of antioxidant-washed, cryoprotectant-treated beef heart surimi during frozen storage. J Food Sci 63(2):293–8.

Xiong YL. 1997. Protein denaturation and functionality losses. In: Erickson MC, Hung YC, editors. Quality in frozen foods. New York: Chapman and Hall. p 111–40.

Yoon KS, Lee CM. 1990. Cryoprotectants effects in surimi and surimi/mince-based extruded products. J Food Sci 55(6):1210–6.

Yoon KS, Lee CM, Hufnagel LA. 1991. Textural and microstructural properties of frozen fish mince as affected by the addition of nonfish proteins and sorbitol. Food Struct 10(3):255–65.