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The Effect of Cryoprotectants on the Properties of Pacific Sand Lance *Ammodytes personatus Girard* Surimi During Frozen Storage

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Abstract

We investigate the effects of cryoprotectant mixtures on the quality of sand lance surimi (SLS) during storage at -30°C. We monitored freeze-induced denaturation of myofibrillar protein in SLS and examined the texture profile of SLS gel. Freeze-induced denaturation was assessed by evaluating SLS Ca^{+2}-ATPase activity. SLS gels prepared with sorbitol or sucrose and a mixture of both as cryoprotectant. Higher concentrations of cryoprotectants resulted in significantly higher residual SLS Ca^{+2}-ATPase activity at the same storage time ($P < 0.05$). Residual Ca^{2+}-ATPase activity of SLS prepared with sorbitol was higher than that of sucrose when cryoprotectant concentration and storage period were same. A blend of sorbitol and sucrose resulted in a stronger cryoprptective effect of SLS myofibrillar protein than did sorbitol or sucrose alone. The presence of a phosphate compound in SOP (3% sorbitol + 0.2% phosphate compound) resulted in higher SLS Ca^{2+}-ATPase activity than that of did 5% sorbitol. The hardness, brittleness, and elasticity values and a folding test of the SLS gels were significantly affected by cryoprotectant concentrations and the storage time. Preference scores and acceptance for texture in a sensory evaluation of the SLS gels increased with increasing sorbitol or sucrose concentration.

Key words: Sand lance surimi, Cryoprotectant, Ca2 -ATPase activity, Texture, Sorbitol, Sucrose

Introduction

Frozen surimi is thawed, chopped, and mixed with water to control the protein concentration manufacturing of surimi-based products such as kamaboko. The mixture is ground with salt to solubilize myofibrillar proteins (Sano et al., 1988). This minced paste is heated to facilitate transformation of the viscous sol into an elastic gel (Numakura et al., 1990). Therefore surimi is the wet concentrate of myofibrillar protein prepared from fish mince in which most of the undesirable substances including sarcoplasmic protein, blood, fat, pigments and odorous materials have been removed by leaching with potable water. The surimi protein must be stabilized during frozen storage to produce high quality, good texture kamaboko. The fish muscle myofibrillar proteins, particularly myosin or actomyosin, which possess enhanced gel-forming, water holding and other functional properties, are very important for determining surimi gel strength. The surimi gelation related to the formation of cross-links between the myosin heavy chain induced by endogenous transglutaninase (Seki et al., 1990; Kumazawa et al., 1995) and thermal formation of non-covalent and disulfide bonds (Hossain et al., 1998). Therefore surimi gel strength is directly related to the degree of myofibrillar protein denaturation in fish muscle (Benjakul et al., 1997; 2003a; 2003b).

Surimi may lose its functional properties during frozen stor-

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age due to unfolding of the myofibrillar protein (mainly myosin), which exposes nonpolar amino acids and that become available for hydrophobic bond formation with same groups nearby. This process leads to protein aggregation (Shenouda, 1980), loss of gelling and water holding capacity (Carvajal et al., 1999) and decrease myosin ATPase activity (Suzuki, 1981). Cryoprotectants such as sucrose or sorbitol are required to prevent these undesirable changes in the myofibrillar protein and to preserve maximum functionality of surimi. It is well documented that these sugars work by stabilizing actomyosin, increasing surface tension (Arakawa and Timasheff, 1982) and the amount of bound water, preventing protein water loss (Buttkus, 1970), and maintaining protein solubility (Lim and Reid, 1991; Herrera and Sampedro, 2002). A mixture of sucrose and sorbitol is the preferred as cryoprotectant for fish surimi as both have excellent cryoprotective effects on fish myofibrillar protein (Lee, 1984; Yoon and Lee, 1990).

Phosphates are widely added to surimi with cryoprotectants such as sugar or sorbitol (Sultanbawa and Li-Chan, 2001) because phosphates improve the functional properties of the products by increasing water holding capacity of fresh fish and decreasing thaw loss of frozen fish (Chang and Regenstein, 1997). Adding phosphate to surimi increases pH, hence, it not only improves water holding capacity of the gel but also increases solubility of myofibrillar (Park, 2000). In contrast, phosphate slows down and inhibits setting of the surimi gel, as it chelates the Ca^{2+} ions (Julavittayanukul et al., 2006). Studies on the effects of phosphate on surimi have been mostly conducted with white muscle fish surimi, and information on dark fleshed fish is scarce.

Sand lance is a dark muscled fish species and is the main species caught off the Gangwon coast of Korea. This area has yielded >9,000 ton/year since 1993. Due to a lack of product innovation for sand lance, this fish mostly prepared as a plain dried product called gwuamegi. The best method for preparing surimi from this fish should be established to enhance the value of this fish and optimize it as fishery resource.

The aim of this study was to evaluate the effects of various cryoprotectant mixtures on the quality of sand lance surimi (SLS) during frozen storage. We investigated freeze-induced denaturation of SLS by determining Ca^{2+}-ATPase activity and assessed the influence of denaturation on the textural properties of surimi by measuring the textural profile.

Materials and Methods

Surimi and surimi gel preparation

The SLS was prepared according to the method of Park et al. (1985) with a slight modification. Sand lance mince was suspended in cold (5°C) 0.5% $NaHCO_3$ solution at a 1:5 ratio (w/v). This mixture was stirred gently for 15 min, and then the upper solution was removed. This procedure was repeated three times. The washed mince was dewatered by centrifugation at 15,000 rpm for 10 min. A 100-g aliquot of dewatered mince was mixed with designated various cryoprotectant mixtures (sorbitol, sucrose, sodium triphosphate, or their blends) in various concentrations (3, 5 or 8%) and ground with a grinder for 10 min at <10°C. These surimi mixtures were stored in PVC film at -30°C until further experiments.

Frozen surimi was thawed at 3°C for 15 min and cut into 1-cm thick slices to prepare the gel. The slices were ground with 2.5% NaCl for 10 min. A total of 150 g ground sample was encased in Krehalone casing film (Ø 3.0 cm) and incubated at 40°C for 30 min. Then the samples were heated at 90°C for 40 min and cooled down under tap water (15°C).

Determining Ca^{+2}-ATPase activity

Freeze-denaturation of actomyosin in the SLS was carried out by measuring the Ca^{+2}-ATPase activity index of myofibrils according to the method of Katoh et al. (1977). Myofibrils from frozen surimi were prepared according to the method of Katoh et al. (1977). About 4g of the partially thawed surimi was homogenized with three volumes of chilled 0.1 M KCl-20mM Tris-maleate buffer (pH 7.0) at 18,000 rpm for 30 s (three times) using a universal homogenizer (Tokyo Nihon Seiki Seisakusho Co., Tokyo, Japan). The homogenate sample was centrifuged at 1,400 *g* for 10 min at < 5°C. The sediment was washed with five volumes of same buffer, stirred, and centrifuged. This procedure was repeated until a clear supernatant was obtained, and the solution was use as the myofibrils. Protein concentration was determined by the Biuret method (Gornall et al., 1949) using bovine serum albumin as the standard.

Myofibrils (0.2-0.5 mg) were incubated at 25°C in final concentrations of 100 mM KCl, 25 mM Tris-maleate (pH 7.0), 5 mM $CaCl_2$, and 1 mM ATP. This reaction was terminated after 5 min by adding chilled 30% TCA solution at a final concentration of 5%. Free inorganic phosphate was measured by colorimetry (Shimadzu UV-1800, Tokyo, Japan) in triplicate. The determinations of free inorganic phosphate content were made in triplicate. Ca^{+2}-ATPase activity is expressed as μmoles inorganic phosphate released per mg protein per min.

Textural analysis

The texture profile analysis (TPA) of the SLS gel was performed at ambient temperature with a rheometer (Fudoh, NRM-2010J, Tokyo, Japan) and a 1-kg cell according to the method described by Yoo (2011). The following textural parameters were calculated from the TPA curves according to the method described by Yoo (2011): hardness at 70% deformation, brittleness, elasticity and cohesiveness.

Folding test

A surimi gel-folding test was conducted using the method

described by Yoo (2011) to investigate the binding structure of the surimi gels. The folding tests were performed by slowly folding a 3-mm slice of surimi gel in half, and then in half again to examine structural rupture of the surimi gel slice. The number of folds required to crack the slice was scored from 1.00 - 5.00 and assigned to 5 classes: AA, A, B, C or D:, where AA (score 5.00) is the best quality and D (score 1.00) is poorest.

Sensory evaluation

SLS gels were evaluated for texture, taste and overall acceptance by 15 untrained panelists. A 5-point hedonic scale, in which a score of 1 = not like very much, 3 = neither like nor dislike and 5 = like extremely, was used for the sensory evaluation.

Statistical analysis

Every experiment was replicated three times. Experiments were conducted on two type of cryoprotectants (sucrose, and sorbitol) with four concentrations (0%, 3%, 5%, and 8%) of sorbitol and three concentrations (0%, 3%, and 5%) of sucrose. Mixtures of 3% sucrose and 3% sorbitol (SOS) as well as 3% sorbitol with 0.2% sodium triphosphate compound (SOP) were also tested. The least significant difference at 5% was applied to define a significant difference between mean values. All analyses were performed using SAS software V8.1 (SAS Institute, Cary, NC, USA).

Results and Discussion

Effects of various cryoprotectants on the SLS Ca^{2+}-ATPase activity

SLS Ca^{2+}-ATPase activity of the control group (without cryoprotectant) was decreased significantly after first month in frozen storage and decreased gradually until only 2.38% of initial activity (2.10 μmol/min/mg) was detected after 5 months. Ca^{2+}-ATPase activity of SLS with sorbitol (3, 5, and 8%) or sucrose (3% and 5%) as cryoprotectants declined significantly ($P < 0.05$) slower than did that of the control. SLS Ca^{2+}-ATPase activity with 8% sorbitol after 5 months of storage was 63.29% of initial activity, whereas it was 42.85 and 30.95% under the sorbitol 5% and 3% treatments, respectively (Fig. 1). SLS with 5% sucrose had 0.75 μmol/min/mg Ca^{2+}-ATPase activity, which was 35.71% of initial activity. Using 3% sucrose as a cryoprotectant resulted in 23.81% of initial activity (Fig. 2). These results reveal that SLS Ca^{2+}-ATPase activity is associated with cryoprptectant concentration in a dose-dependent manner. Higher concentrations of sorbitol or sucrose resulted in significantly ($P < 0.05$) higher residual SLS Ca^{2+}-ATPase activity whereas a longer storage period resulted

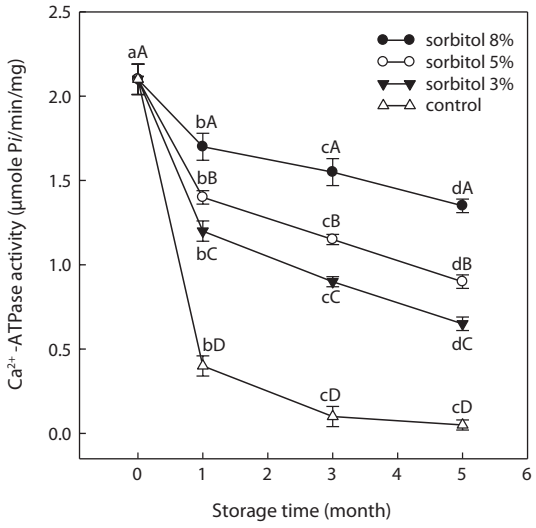

Fig. 1. Changes of Ca^{2+}-ATPase activity of SLS prepared with different levels of sorbitol during storage at -30°C.

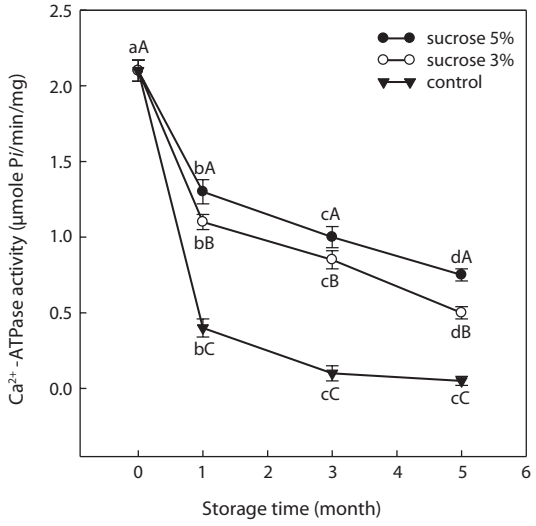

Fig. 2. Changes of Ca^{2+}-ATPase activity of SLS prepared with different levels of sucrose during storage at -30°C.

in significantly lower SLS Ca^{2+}-ATPase activity ($P < 0.05$). These results show that residual SLS Ca^{2+}-ATPase activity with sorbitol was higher than that with sucrose after a given storage time. Ca^{2+}-ATPase activity is a good indicator of the degree of the myosin molecule denaturation during cold storage (Roura and Crupkin, 1995; Benjakul et al., 1997; 2003a; 2003b; Herrera and Mackie, 2004). Myofibrillar protein Ca^{2+}-ATPase activity is due to the globular heads of myosin. A sharp decrease in activity of the SLS without cryoprotectants after 1 month of frozen storage (Figs. 1 and 2) indicated severe myosin denaturation, particularly in the head region (Zhou et al.,

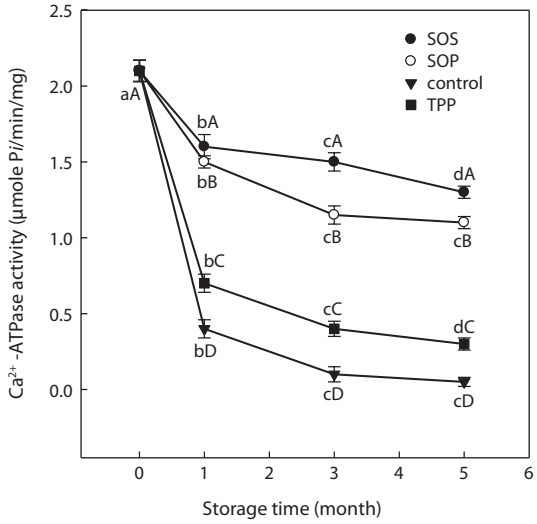

Fig. 3. Changes of Ca^{2+} -ATPase activity of frozen SLS prepared with various blends of cryoprotectants during storage at -30°C.

2006). This decrease in ATPase activity probably indicates the conformational changes in the myosin globular head as well as aggregation of this portion because myosin conformational changes are caused by ice crystals and increased ionic strength in the system (Benjakul et al., 1997). Similar results have been reported in other fish muscle systems without cryoprotectants (Benjakul et al., 2003a; Herrera and Mackie, 2004; Zhou et al., 2006). Zhou et al. (2006) reported a remarkable decrease in tilapia surimi Ca^{2+}-ATPase activity after 3 weeks of storage at -18°C, and Benjakul et al. (2003a) found a distinct decrease in Ca^{2+}-ATPase activity in lizardfish, croaker, threadfin bream, and bigeye snapper muscles after 24 weeks of frozen storage at -18°C.

These results show that sorbitol or sucrose prevented the decrease in SLS Ca^{2+}-ATPase activity and that these compounds had a cryoprotective effect on sand lance muscle protein during frozen storage.

Sorbitol and sucrose are common cryoprotectants widely used in surimi industry. The mixture of both is preferable, as it has excellent cryoprotective effects on fish myofibrillar proteins (Lee, 1984; Yoon and Lee, 1990) and is a less sweet with fewer calories in the final product compared with sucrose only as a cryoprotectant. SLS Ca^{2+}-ATPase activity with SOS and SOP was monitored to assess the effects of cryoprotectant combinations on freeze denaturation of SLS myofibrillar protein during storage at -30°C. Residual SLS Ca^{2+}-ATPase activity with added SOS decreased gradually and was 61.90% of initial activity (from 2.1 to 1.3 μmol/min/mg) after 5 months of storage (Fig. 3). This value was higher than that of the 5% sorbitol or 5% sucrose only treatments (Figs. 1 and 2). Therefore, the mixture of sorbitol and sucrose had a stronger cryoprotective effect on SLS myofibrillar protein than did sorbitol or sucrose alone. Residual SLS Ca^{2+}-ATPase activity with added SOP decreased to 1.10 μmol/min/mg after 5 months of storage. Residual SLS Ca^{2+}-ATPase activity with added SOP exhibited a significantly higher value compared with 5% sorbitol alone.

Among all blends, SOS resulted in the highest ($P < 0.05$) residual Ca^{2+}-ATPase activity. SLS Ca^{2+}-ATPase activity with added SOS was 76.2% (from 2.10 to 1.60 μmol/min/mg) of initial activity after 1 month of storage. SOP resulted in significantly ($P < 0.05$) higher Ca^{2+}-ATPase activity, compared with adding 0.2% polyphosphate alone during the entire storage period. Phosphate compounds are added to surimi to improve gel formation, as these compounds dissociate protein complexes (Matsukawa et al., 1995). SLS Ca^{2+}-ATPase activity in the 0.2% phosphate treatment showed a significantly ($P < 0.05$) higher value compared with the control. This result indicates that phosphate compounds possess some cryoprotective effect on SLS during frozen storage. Dissociation of protein complexes by adding phosphate may prevent myosin aggregation due to the conformational change in the myosin globular head caused by ice crystals (Benjakul et al., 1997; Benjakul and Bauer, 2000).

Effects of cryoprotectants on the textural properties of the SLS gel

The changes in textural parameters of SLS gels prepared with various concentrations of sorbitol during storage at -30°C are shown in Table 1. The hardness, brittleness, and elasticity values as well as the folding test in all SLS gels increased significantly ($P < 0.05$) at varying rates depending on the storage time after 1 month of storage when sorbitol concentration increased. The hardness and brittleness values of SLS gels with 8% sorbitol increased significantly ($P < 0.05$) to twice those of the control. The values of all textural parameters in SLS gels after 1 month of storage increased significantly ($P < 0.05$) in association with increasing sorbitol concentration, are consistent with the report by Lee et al. (1985) for sardine. Storage period had a significantly ($P < 0.05$) effect on the textural parameters of the SLS gels. Longer storage decreased textural parameter values. Sych et al.(1991) reported that a decrease in gel-forming ability of fish muscle during frozen storage is associated with freeze denaturation of surimi actomyosin via aggregation of the protein chains, leaving them unavailable for subsequent gel formation during heat processing of surimi. Benjakul and Bauer (2000) and Benjakul et al. (2003) reported that myosin denaturation during frozen storage results in inferior network formation, which causes a lower elasticity and poor water holding capacity of the gel matrix. In our experiment, the large decrease in the SLS textural parameters in samples without cryoprotectants was concomitant with a decrease in Ca^{2+}-ATPase activity (Fig. 1). Therefore, freeze denaturation of surimi actomyosin via Ca^{2+}-ATPase activity is an important factor to evaluate.

Table 2 shows the changes in SLS gel textural parameters in

the different sucrose concentrations treatments during storage at -30°C. The hardness, brittleness, elasticity and cohesiveness values as well as the folding test results of gels increased significantly ($P < 0.05$) in all SLS gels at varying rates when the sucrose concentration was increased, depending on storage time. After 5 months of storage, the hardness value of the control decreased significantly ($P < 0.05$) by 18.5% compared with that obtained in fresh surimi (week 0). The hardness values of SLS gels prepared with 3 and 5% of sucrose were 0.21 kg and 0.26 kg, respectively, and decreased significantly ($P < 0.05$) by 38.8% and 48.1%, compared with their corresponding initial values. The hardness value increased significantly ($P < 0.05$) at the same storage time when the sucrose concentration was increased. The cohesiveness values of SLS gels with 3 or 5% sucrose added decreased significantly ($P < 0.05$), but these gels retained significantly ($P < 0.05$) higher cohesiveness by 59.2 and 70.4%, respectively, compared with their initial values. All textural parameters values of gels with added sorbitol were higher than those with added sucrose at same concentration (3 and 5%). All textural parameter values of SLS gels with added sorbitol were significantly ($P < 0.05$) higher than those with sucrose when 8% sorbitol (Table 1) was compared with 5% sucrose (Table 2). These results indicate that adding sorbitol instead of sucrose as a cryoprotectant

Table 1. Changes of textural parameters of SLS gel prepared with various concentrations of sorbitol during storage at -30°C

Textural parameter	Storage time (month)												
	0	1				3				5			
	Control	Sorbitol concentration (%)				Sorbitol concentration (%)				Sorbitol concentration (%)			
		0	3	5	8	0	3	5	8	0	3	5	8
Hardness (kg)	0.54 ± 0.040^{A}	0.20 ± 0.071^{dB1}	0.35 ± 0.052^{cB}	0.40 ± 0.081b^{bB}	0.50 ± 0.023^{aB}	0.18 ± 0.042^{dB}	0.25 ± 0.061^{cC}	0.32 ± 0.018^{bC}	0.38 ± 0.046^{aC}	0.10 ± 0.022^{cC}	0.25 ± 0.034^{bC}	0.30 ± 0.063^{abC}	0.35 ± 0.037^{aC}
Brittleness (kg)	0.51 ± 0.010^{A}	0.19 ± 0.028^{cB}	0.29 ± 0.073^{bB}	0.35 ± 0.054^{abB}	0.47 ± 0.078^{aB}	0.17 ± 0.059^{bB}	0.24 ± 0.070^{abC}	0.31 ± 0.028^{aBC}	0.30 ± 0.047^{aC}	0.08 ± 0.076^{cC}	0.22 ± 0.060^{bC}	0.28 ± 0.063^{aC}	0.27 ± 0.055^{aC}
Elasticity (cm)	3.6 ± 0.011^{A}	2.2 ± 0.042^{dB}	3.0 ± 0.051^{cB}	3.2 ± 0.049^{bB}	3.5 ± 0.031^{aB}	2.0 ± 0.017^{cBC}	2.3 ± 0.039^{bC}	2.6 ± 0.049^{abC}	2.8 ± 0.047^{aC}	1.0 ± 0.053^{dC}	2.0 ± 0.048^{cC}	2.2 ± 0.027^{bD}	2.4 ± 0.035^{aD}
Cohesiveness	0.71 ± 0.023^{A}	0.45 ± 0.028^{dB}	0.58 ± 0.019^{cB}	0.65 ± 0.043^{bB}	0.70 ± 0.047^{aB}	0.35 ± 0.071^{cC}	0.51 ± 0.049^{bC}	0.52 ± 0.050^{bC}	0.68 ± 0.026^{aBC}	0.20 ± 0.013^{cD}	0.48 ± 0.055^{bC}	0.50 ± 0.078^{abC}	0.64 ± 0.065^{aC}
Folding test	4.98 ± 0.002^{A}	3.92 ± 0.013^{dB}	4.31 ± 0.010^{cB}	4.52 ± 0.007^{bB}	4.70 ± 0.009^{aB}	2.87 ± 0.083^{dC}	4.03 ± 0.025^{cC}	4.46 ± 0.006^{bC}	4.61 ± 0.014^{aC}	2.83 ± 0.074^{dC}	4.00 ± 0.043^{cC}	4.27 ± 0.012^{bD}	4.57 ± 0.036^{aC}

[1]The capital letter corresponding to storage time in same column indicate the significant differences ($P < 0.05$).
The small letter corresponding to concentration in same column indicate the significant differences ($P < 0.05$).

Table 2. Changes of textural parameters of SLS gel prepared with various concentrations of sucrose during storage at -30°C

Textural parameter	Storage time (month)									
	0	1			3			5		
	Control	Sucrose concentration (%)			Sucrose concentration (%)			Sucrose concentration (%)		
		0	3	5	0	3	5	0	3	5
Hardness (kg)	0.54 ± 0.040^{A}	0.20 ± 0.07^{cB1}	0.32 ± 0.030^{bB}	0.36 ± 0.029^{aB}	0.18 ± 0.042^{bB}	0.22 ± 0.061^{abC}	0.28 ± 0.051^{aC}	0.10 ± 0.078^{cC}	0.21 ± 0.027^{bC}	0.26 ± 0.050^{aC}
Brittleness (kg)	0.51 ± 0.010^{A}	0.19 ± 0.028^{bB}	0.29 ± 0.012^{aB}	0.30 ± 0.020^{aB}	0.17 ± 0.059^{dB}	0.21 ± 0.043^{cC}	0.27 ± 0.010^{bC}	0.08 ± 0.076^{dC}	0.20 ± 0.029^{cC}	0.25 ± 0.032^{bC}
Elasticity (cm)	3.6 ± 0.011^{A}	2.2 ± 0.42^{bB}	3.0 ± 0.60^{aB}	3.2 ± 0.55^{aB}	2.0 ± 0.17^{bB}	2.5 ± 0.084^{aBC}	2.6 ± 0.22^{aC}	1.0 ± 0.53^{cC}	2.0 ± 0.47^{bC}	2.2 ± 0.16^{aD}
Cohesiveness	0.71 ± 0.023^{A}	0.45 ± 0.028^{cB}	0.58 ± 0.049^{bB}	0.62 ± 0.017^{aB}	0.35 ± 0.07^{cC}	0.49 ± 0.02^{bC}	0.58 ± 0.043^{aC}	0.20 ± 0.013^{cD}	0.42 ± 0.037^{bD}	0.50 ± 0.021^{aD}
Folding test	4.98 ± 0.002^{A}	3.92 ± 0.013^{cB}	4.35 ± 0.054^{bB}	4.49 ± 0.009^{aB}	2.87 ± 0.083^{cC}	4.01 ± 0.046^{bC}	4.50 ± 0.017^{aB}	2.83 ± 0.074^{cC}	4.01 ± 0.006^{bC}	4.52 ± 0.013^{aC}

[1]refer to Table 1.

was desirable because the sweetness intensity and the calories from sorbitol are lower than those of sucrose.

The changes in the textural parameters of SLS gels prepared with various cryoprotectant blends during storage at -30°C are listed in Table 3. The control hardness parameter of SLS surimi decreased significantly ($P < 0.05$) with a longer frozen storage period, and it was 0.10 kg after 5 months of storage. This was equivalent to 18.5% of the initial value (0.54 kg). The hardness of SOS and SOP also decreased significantly ($P < 0.05$) over time. But these values after 5 months of storage were 0.32 and 0.31 kg, which was 59.2 and 57.4% of initial values. The SOS and SOP hardness values were significantly ($P < 0.05$) higher than that of the control, but the SOS and SOP treatments were not significantly different from each other. The other textural parameters (brittleness, elasticity and cohesiveness) shared the similar trends to the variations in hardness during frozen storage. These results reveal that these textural parameters are less sensitive to measure frozen denaturation of fish myofibrillar protein. The finding that SLS Ca^{2+}-ATPase activity with 0.2% polyphosphate was still measurable after 5 months (Fig. 3) shows that polyphosphate has cryoprotectant ability. This result was concomitant with the slight differences between the SOS and SOP values in all textural parameters (Table 3). The smallest number of differences in the textural parameter values was observed between SLSs prepared with 5% sorbitol and 5% sucrose (Tables 1 and 2). The SOP textural parameters (Table 3) were higher than those of SLSs prepared with sorbitol or sucrose alone. Lee et al. (1985) reported that adding polyphosphates to sardine surimi results in better gel texture after frozen storage. Torigai and Konno (1996) reported that

Table 3. Changes of textural parameters of SLS gel prepared with various cryoprotectant blends during storage at -30°C

Textural parameter	Storage time (month)									
	0	1			3			5		
	Control	NoC[1]	SOS[2]	SOP[3]	NoC[1]	SOS[2]	SOP[3]	NoC[1]	SOS[2]	SOP[3]
Hardness (kg)	0.54 ± 0.040^{A}	0.20 ± 0.071^{bB}	0.45 ± 0.084^{aB}	0.43 ± 0.050^{aB}	0.18 ± 0.042^{bB}	0.35 ± 0.028^{aC}	0.35 ± 0.043^{aC}	0.10 ± 0.022^{bC}	0.32 ± 0.064^{aC}	0.31 ± 0.051^{aC}
Brittleness (kg)	0.51 ± 0.010^{A}	0.19 ± 0.028^{aB}	0.39 ± 0.061^{aB}	0.38 ± 0.021^{aB}	0.17 ± 0.059^{bB}	0.34 ± 0.070^{aB}	0.33 ± 0.017^{aC}	0.08 ± 0.076^{bC}	0.30 ± 0.043^{aC}	0.30 ± 0.039^{aC}
Elasticity (cm)	3.6 ± 0.011^{A}	2.2 ± 0.42^{bB}	3.4 ± 0.11^{aB}	3.3 ± 0.40^{aB}	2.0 ± 0.17^{bB}	2.5 ± 0.28^{aC}	2.6 ± 0.43^{aC}	1.0 ± 0.53^{bC}	2.3 ± 0.69^{aC}	2.3 ± 0.10^{aC}
Cohesiveness	0.71 ± 0.023^{A}	0.45 ± 0.028^{bB}	0.68 ± 0.047^{aB}	0.68 ± 0.026^{aB}	0.35 ± 0.071^{bC}	0.58 ± 0.018^{aC}	0.60 ± 0.043^{aC}	0.20 ± 0.013^{bD}	0.54 ± 0.070^{aC}	0.53 ± 0.089^{aD}
Folding test	4.98 ± 0.002^{A}	3.92 ± 0.013^{cB}	4.60 ± 0.020^{bB}	4.57 ± 0.002^{aB}	2.87 ± 0.083^{bC}	4.51 ± 0.016^{aC}	4.52 ± 0.023^{aC}	2.83 ± 0.074^{cC}	4.41 ± 0.034^{bC}	4.47 ± 0.015^{aC}

[1]Do not added any cryoprotectant.
[2]Cryoprotectant blends of sorbitol (3%) and sucrose (3%).
[3]Cryoprotectant blends of sorbitol (3%) and sodium triphosphate (0.2%).
Superscript letters refer to Table 1.

Table 4. Sensory evaluation of SLS gel prepared with different concentrations of sorbitol during storage at -30°C

Sensory parameter	Storage time (month)											
	1				3				5			
	Sorbitol concentration (%)				Sorbitol concentration (%)				Sorbitol concentration (%)			
	0	3	5	8	0	3	5	8	0	3	5	8
Texture	3.0 ± 1.2^{aA1}	4.2 ± 1.8^{abA}	4.2 ± 1.0^{bA}	4.5 ± 0.9^{cA}	2.0 ± 1.8^{aA}	4.0 ± 1.2^{bA}	4.0 ± 1.6^{bA}	4.5 ± 1.9^{bA}	1.5 ± 0.8^{aB}	3.5 ± 1.3^{bA}	3.5 ± 1.5^{bA}	4.2 ± 1.0^{bA}
Taste	3.2 ± 0.4^{aA}	4.2 ± 1.0^{bA}	4.2 ± 1.0^{bA}	4.0 ± 0.7^{bA}	3.0 ± 0.8^{aA}	4.1 ± 1.0^{bA}	4.1 ± 0.3^{bA}	4.0 ± 0.6^{cA}	1.5 ± 1.2^{aB}	4.0 ± 1.1^{bA}	4.0 ± 1.2^{bA}	3.8 ± 1.8^{bA}
Overall acceptance	3.2 ± 0.2^{aA}	4.2 ± 1.7^{abA}	4.2 ± 0.9^{bA}	4.5 ± 1.2^{bA}	2.5 ± 0.1^{aB}	4.2 ± 1.0^{bA}	4.2 ± 1.3^{bA}	4.5 ± 1.4^{bA}	1.5 ± 0.3^{aC}	3.6 ± 1.3^{bA}	4.0 ± 1.5^{bA}	4.2 ± 1.1^{bA}

[1]The capital letter corresponding to storage time in same column indicate the significant differences ($P < 0.05$).
The small letter corresponding to concentration in same column indicate the significant differences ($P < 0.05$).

phosphate compounds cause the actomyosin complex to dissociate into actin and myosin. Actomyosin dissociated, and a strong gel network was formed when phosphates were added at a optimal concentration for preparing protein gel (Ellinger, 1975). Xiong and Kupski (1999) demonstrated that reducing the salt concentration produced a synergism with phosphate to dissociate actomyosin in chicken fillets. Park (2000) also reported that the increasing pH caused by the phosphate compound resulted in improved gel water holding capacity and better solubilization of myofibrillar protein. Therefore, adding a mixture of sorbitol-polyphosphates rather than sucrose or sorbitol alone when preparing SLS would produce increasing cryoprotectant efficacy as well as improve the SLS gel texture after frozen storage.

Effects of cryoprotectant on the SLS gel sensory evaluation

The preference scores for the SLS gels prepared with different quantities of sorbitol during storage at -30°C are shown in Table 4. The texture and overall acceptance scores of the SLS gels with 8% sorbitol at the same storage time tended to be the higher than those with the 3 or 5% concentrations, but the differences were not significant. The taste of SLS prepared with 8% sorbitol was less preferable compared with that of SLS with 3 and 5% sorbitol. The overall acceptance scores of the SLS gel with sorbitol did not decrease remarkably during storage. However, the scores for the SLS gels without sorbitol decreased significantly ($P < 0.05$). The differences in overall acceptance scores for the SLS gels among 3, 5, and 8% sorbitol treatments were not significant.

Table 5 shows the preference scores for the SLS gels prepared with different quantities of sucrose during storage at -30°C. After 1 or 3 months of storage, preference scores based on texture, taste, and overall acceptance of the SLS gels tended to increase in accordance with sucrose concentration, but the difference was not significantly different. Adding 3 and 5% sucrose resulted in significantly ($P < 0.05$) the higher preference values based on texture, taste, and overall acceptance of SLS gels compared with gels without sucrose.

Table 6 shows the preference scores for SLS gels prepared with various cryoprotectant mixtures during storage at −30°C. All SLS gel preference scores for texture, taste and overall acceptance prepared without cryoprotectant (NoC) decreased significantly ($P < 0.05$) depending on storage time, but visible decreases of these scores for SOS and SOP were not observed. No significant differences in these scores were observed between SOS and SOP at the same storage time.

Table 5. Sensory evaluation of SLS gel prepared with various concentrations of sucrose during storage at -30°C

Sensory parameter	Storage time (month)								
	1			3			5		
	Sucrose concentration (%)			Sucrose concentration (%)			Sucrose concentration (%)		
	0	3	5	0	3	5	0	3	5
Texture	3.0 ± 1.2^{aA1}	4.0 ± 1.3^{bA}	4.2 ± 0.7^{bA}	2.0 ± 1.8^{aA}	4.0 ± 1.6^{bA}	4.0 ± 0.7^{bA}	1.5 ± 0.8^{aB}	3.5 ± 1.3^{bA}	3.8 ± 1.9^{bA}
Taste	3.4 ± 0.4^{aA}	4.2 ± 0.6^{bA}	4.2 ± 1.0^{bA}	3.0 ± 0.8^{aA}	4.0 ± 1.0^{bA}	4.0 ± 0.2^{bA}	1.5 ± 1.2^{aB}	3.4 ± 1.1^{bA}	3.6 ± 1.7^{bA}
Overall acceptance	3.2 ± 0.2^{aA}	4.0 ± 1.0^{abA}	4.1 ± 0.3^{bA}	2.5 ± 0.1^{aB}	4.0 ± 1.1^{bA}	4.0 ± 1.0^{bA}	1.5 ± 0.3^{aC}	3.6 ± 1.4^{bA}	3.8 ± 1.2^{bA}

[1]The capital letter corresponding to storage time in same column indicate the significant differences ($P < 0.05$).
The small letter corresponding to concentration in same column indicate the significant differences ($P < 0.05$).

Table 6. Sensory evaluation of SLS gel prepared with various cryoprotectants blends during storage at -30°C

Sensory parameter	Storage time (month)								
	1			3			5		
	NoC[1]	SOS[2]	SOP[3]	NoC[1]	SOS[2]	SOP[3]	0	SOS[2]	SOP[3]
Texture	3.0 ± 1.2^{aA}	4.4 ± 1.6^{bA}	4.2 ± 1.3^{bA}	2.0 ± 1.8^{aB}	4.2 ± 1.3^{bA}	4.0 ± 1.7^{bA}	1.5 ± 0.8^{aC}	4.0 ± 0.9^{bA}	4.0 ± 1.5^{bA}
Taste	3.4 ± 0.4^{aA}	4.2 ± 0.9^{bA}	4.0 ± 1.0^{bA}	3.0 ± 0.8^{aB}	4.0 ± 1.4^{bA}	4.2 ± 1.1^{bA}	1.5 ± 1.2^{aC}	4.0 ± 1.0^{bA}	4.0 ± 1.3^{bA}
Overall acceptance	3.2 ± 0.2^{aA}	4.4 ± 1.5^{bA}	4.2 ± 1.6^{bA}	2.5 ± 0.6^{aB}	4.2 ± 1.0^{bA}	4.0 ± 1.4^{bA}	1.5 ± 0.3^{aC}	4.0 ± 1.7^{bA}	4.0 ± 1.9^{bA}

[1] Do not added any cryoprotectant.
[2] Cryoprotectant blends of sorbitol (3%) and sucrose (3%).
[3] Cryoprotectant blends of sorbitol (3%) and sodium triphosphate (0.2%).
Superscript letters refer to Table 1.

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