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Effects of Processing Conditions on Nutritional Qualities of Seafood

2. Effects of Cryoprotectants on the Protein Qualities of Pollock Surimi

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To determine the optimal level of cryoprotectant on the denaturation of pollock surimi produced in Korea, the relative cryoprotective effects of crystalline sorbitol alone and in combination with sucrose were assessed. Freeze induced protein denaturation was also studied as affected by polyphosphates and maltodextrin during frozen storage at -25℃ for 16 weeks. Variables evaluated included salt extractable protein, drip loss and *in vitro* protein quality. The best cryoprotective effect was achieved from sucrose/sorbitol 1:1(*w/w*) mixture at 8% with 0.2% sodiumpyrophosphate and sodiumtriphosphate(1:1, *w/w*) in surimi by measurement of salt extractable protein and drip loss. Those cryoprotectants had little effect on surimi protein quality during frozen storage as measured by trypsin inhibitor(TI), protein digestibility and computed protein efficiency ratio(C-PER). Protein digestibility of surimi was not changed significantly by polyphosphate and maltodextrin at various levels($p<0.05$), with the exception of 4 or 6% sorbitol and 10% sucrose alone which resulted in a higher digestibility. 8% sorbitol/sucrose (5:3, *w/w*) treatment without polyphosphates showed the highest cryoprotective effectiveness from digestibility assay.

Introduction

Frozen surimi, which is used as a basic material for kamaboko and seafood substitutes, is defined as a washed minced fish meat using white meat fish such as Alaska pollock, cod and red hake *et al.* Among those fish surimis, Alaska pollock surimi is the most widely used as a basic material for crab meat substitutes and kamaboko and it provides approximately 60% of the total surimi-based products in Korea(The Fisheries. Assoc. Korea, 1992).

Even though the consumption of surimi products is increasing year by year, the availability of pellagic Alaska pollock, which is a major source for Korean surimi, has decreased dramatically from the end of 1980's. Therefore the demand for the effective utilization of surimi appears to be growing. Freezing is usually employed to preserve surimi, and frozen storage has been proven to be an important long term storage method for surimi. But extended frozen storage can produce profound effects on structural and chemical properties such as pro-

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tein denaturation of surimi which has a high content of myofibrillar protein(actomyosin) and can produce an elastic texture in surimi products.

To improve the quality of frozen surimi, various techniques for controlling the denaturation have been studied with special attention to cryoprotectants(Noguchi and Matsumoto 1970, Suzuki 1981, Park and Lanier 1987). Freezing of surimi is done commercially by mixing sucrose, sorbitol and polyphosphate(Lee 1984, Sych *et al.* 1990). Recently, a crystalline form of sorbitol in combination with sucrose was employed in industrial manufacture of surimi(Lee 1984, Yoon and Lee 1990). Several studies have been undertaken to identify the effects of such polyphosphates on the surimi quality(Okamura *et al.* 1959, Tanikawa *et al.* 1963, Iwata *et al.* 1971, Umemoto *et al.* 1971, Kawashima *et al.* 1973, Park and Lanier 1987, Park *et al.* 1988). The effectiveness of sucrose and sorbitol in the stabilization of fish muscle protein have been assessed with respect to total ATP-ase activity(Arai *et al.* 1970), jelly strength(Noguchi and Matsumoto 1975, Noguchi *et al.* 1975), protein suspendability(Park and Lanier 1987), gel-forming ability(Park *et al.* 1988), salt-extractable protein content(Sych *et al.* 1990), and expressible moisture and drip loss(Yoon and Lee, 1990). Currently, the Alaska pollock surimi manufacturing industry in Korea uses a crystalline form of sorbitol in combination with sucrose.

The aim of this study, to establish the optimal amount and combination ratio of sorbitol as well as sucrose with the other cryoprotectants on Alaska pollock surimi produced in Korea, stemmed from an effort to reduce production costs and improve frozen stability. Our study was also designed to determine the effects of various cryoprotectants on protein quality, including digestibility, trypsin inhibitor content and protein efficiency ratio during frozen storage, because previous works have not evaluated the nutritional quality in surimi.

Materials and Methods

1. Preparation of Surimi

Fresh Alaska pollock(*Theragra chalcogramma*) fillets were obtained by courtesy of Samho Seafood Inc. in Pusan city. The fillets were run through a chopper(Hashimoto Can Co., Model SP-R) and washed twice for 5 *min.* each with 3 times of chilled water(12℃). After each wash, the slurry was strained through a fine plastic screen for 10*min.* Dewatering was carried out in a basket centrifuge(Mitsubishi Electric Co., Model SB-EV). To this dewatered minced meat(87% moisture), four kinds of cryoprotectants(8% sorbitol, 8% sucrose, 8% maltodextrin, 4% sucrose-4% sorbitol) were added together with 0.2% sodiumpyrophosphate and sodiumtriphosphate(1:1, *w/w*) on a meat weight basis. Experiments were also conducted to study the effect of different levels and combinations of those cryoprotectants on the protein quality of surimi including digestibility, PERs and trypsin inhibitor content. All additives were directly incorporated into surimi(*w/w*) by mixing at low speed for 1 *min.* in a Hobart mixer. Samples were vacuum-packed, rapidly frozen at -60 ± 2℃ and stored at -23 ± 2℃ for 16 weeks.

2. Analytical Procedures

Proximate Analysis and Amino Acid Analysis

Replicate analyses for each samples were undertaken to measure the quantity of protein(N×6.25) as well as moisture which were determined by the procedures described in AOAC(1990). As with the moisture analysis, 5 gram of surimi was dried on Infrared Moisture Balance(Kett, Model F-1A) three times to an exact weight. Amino acid profiles of samples were analyzed twice according to the acid hydrolysis method with LKB-Autoanalyzer(4150, α-type). Tryptophan content was determined by the procedure of Hugli and Moore(1972) and sulfur-containing amino acids were quantitatively oxidized using performic acid according to the method of Spencer and World(1969).

Salt Extractable Protein(SEP) and Drip Loss

SEP was determined following the method of Sych *et al.*(1990) modified from the procedure of Dyer *et al.*(1950). A 20 gram of sample was ground

with 180*ml* cold 0.6N KCl solution in a Homogenizer(Kokusan, Model H-AM) at 5,000*rpm* and SEP extracted overnight. The next day, samples were centrifuged at 5,000×*g* for 10*min*. at 4℃. The content of protein in supernatant was measured by micro Kjeldahl method, and the process duplicated. The amount of drip loss was determined using the frozen surimi blocks(30×40×10*mm*) in polyethylene film bags and calculated the loss after thawing those blocks to room temperature(22 ± 1℃) for 16 hours.

Protein Quality Determination

The *in vitro* protein digestibility of all samples was measured by the AOAC procedure(1982) using four enzymes including trypsin(Sigma, 14,000 BAEE units/*mg* solid), α-chymotrypsin(Sigma, 41 units/*g* solid), peptidase(Sigma, 50 units/*g* solid) and bacterial protease(*Streptomyces griceus*, Sigma, 58 units/*mg* solid). The reference protein used in digestibility assay was ANRC casein. The amount of trypsin inhibitor(TI) was determined using the procedure described by Ryu *et al*(1992). Results of TI were expressed in trypsin inhibitor equivalent, which equals the mg of purified soybean trypsin inhibitor per gram of samples. Computed protein efficiency ratio(C-PER), discriminant computed protein efficiency ratio(DC-PER) and predicted digestibility were calculated following the procedures of AOAC(1982).

Result and Discussion

Effect of Various Cryoprotectants

Cryoprotective properties of ingredients in pollock surimi containing 0.2% sodium pyrophosphate and sodiumtriphosphate(1:1, *w/w*) were compared at 8% levels. Drip losses of cryoprotectant treated samples ranged from 45%(C8) to 60%(D8) as decribed in Yoon and Lee(1990). All treated samples showed generally around 30% of salt extractable protein(SEP) but control showed only 20% of SEP. There were no significant differences among samples(Table 1). Sorbitol with sucrose treatment (SC), which is used in manufacturing the Korean industrial surimi, ranked second in drip loss and SEP among the samples used. This cryoprotection of SC to denaturation would be expected since preferential effect excluding from contact with the surface of protein, leading to preferential hydration of protein when compared with other cryoprotectants(Carpenter and Crowe, 1988).

Thus SC treatment seemed to be beneficial in protection against frozen denaturation of surimi made from Alaska pollock. Overall drip percentage were higher than the expressible moisture content in previous report(Yoon and Lee, 1990) and smaller SEP amounts were noted than those from frozen cod surimi(Sych *et al.*, 1990). However, those differences in drip and SEP shown in our study could be attributed to differences in fish species,

Table 1. Cryoprotective effectiveness in frozen pollock surimi[1]

Cryoprotectant[2] level(%)	Moisture (%)	Drip loss (%)	Salt extractable protein (%)
Control	83.13	13.84 ± 1.24[a]	20.14 ± 3.22[b]
SC4:4	82.29	7.42 ± 0.62[a]	33.53 ± 2.65[a]
C8	82.09	6.29 ± 0.95[b]	28.28 ± 4.34[a]
S8	82.39	8.16 ± 3.14[a]	35.41 ± 2.23[a]
D8	82.30	8.21 ± 2.41[a]	29.33 ± 1.18[a]

[1] Stored at -25℃ for 16 weeks and then thawed at 7℃ for 24 hours.

[2] SC 4:4; Sucrose 4% + Crystalline Sorbitol 4%.
C8; Crystalline Sorbitol 8%.
S8; Sucrose 8%.
D8; 10 D.E. Maltodextrin.

a, b ; Mean with the same letter are not significantly different following a Duncan's test($p<0.05$).

frozen temperature and storage period. Trypsin inhibitor(TI), measured immediately after frozen storage in unsalted control surimi was 3.62 ± 1.58 *mg%*. Equivalent amounts were up to 25% of TI in pollock fillet, indicating the water soluble TI was rinsed out through surimi processing(Ryu and Lee, 1985). Treating cryoprotectant improved the protein quality of surimi in terms of TI content and *in vitro* protein digestibility after frozen storage at 25℃ for 16 weeks(Table 2). This can be explained by hydration of protein acting to inhibit denaturation by forming a compact structure and maintaining protein quality for frozen storage(Park *et al.*, 1988). As measured by C-PER using the amino acid profiles in Table 3 and protein digestibility, a protein quality preserving effect similar to frozen surimi was noted from cryoprotectants treatment especially by SC 4:4, and a significant drop of C-PER resulted in unsalted control surimi. Predicted digestibility and DC-PER assay were not sensitive to the protein quality changes occurring in frozen storage. Salt extractable protein(SEP) from surimi samples for each treatment during frozen storage is shown in Fig. 1. The greatest decline was detected after the control surimi had been stored for 2 weeks, there was no further decline until 16 weeks later. The decreases of SEP from 39.8% to 19.2% were very close to the results of Sych *et al.*(1990) which reported cod surimi control decreased from 38.7% to 20.3% during 16 weeks storage at -20℃. Samples containing 8% crystalline(T3) or 8% maltodextrin(T5) had somewhat higher SEP concentrations than the control samples through-out frozen storage. The greatest stabilizing effect to pollock surimi was from carbohydrate/polyol treatment, 8% sucrose/crystalline sorbitol(1:1)(T2) and 8% sucrose(T4). SEP for these samples did not significantly vary as much as the previous results(Graboeska and Sikorski 1973, Noguchi *et al.* 1976, Rao 1983, Park *et al.* 1988).

Effect of Cryoprotectant Level

Additions of 0.2% polyphosphate improved protein digestibility to some degree, but no significant differences in digestibility were found among samples with polyphosphate from 0.2% to 1.0% levels (Fig. 2). Those results suggest that 0.2% polyphosphate treatment alone had no appreciable synergistic effect on protein denaturation, even though the greater level treated to control surimi (Park and Lanier 1987). The negligible effectiveness has been explained well by the study of double destabilization, a loss of protection by actin due to its dissociation and a direct destabilization of dissociated myosin by polyphosphates(Konno, 1992). Sorbitol is generally incorporated in surimi to substitute for sucrose which might make the taste too sweet or turn the finished product a brownish co-

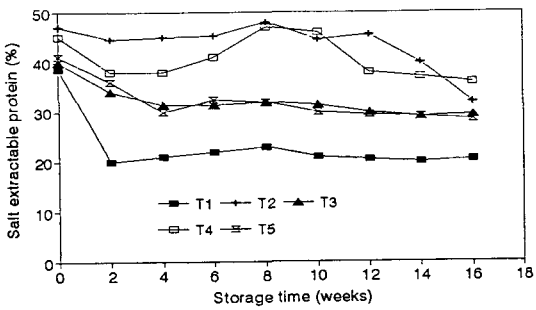

Fig. 1. Changes in salt extractable protein of pollock surimi as a function of storage time at -25℃ for the various experimental treatments.
T1, control; T2, 4% sucrose+4% crystalline sorbitol; T3, 8% crystalline sorbitol; T4, 8% sucrose; T5, 8% maltodextrin.

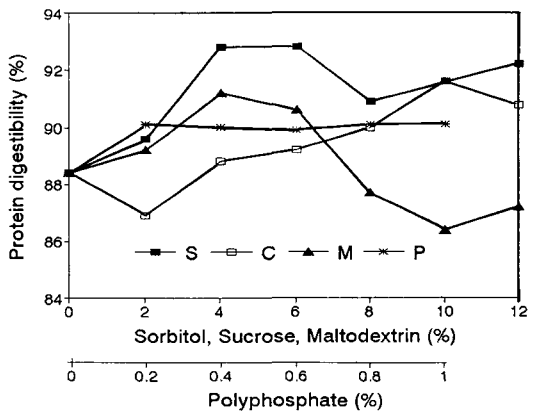

Fig. 2. Effects of cryoprotectants level on the protein digestibility of pollock surimi after 16 weeks frozen storage at -25℃.
S, crystalline sorbitol; C, sucrose; M, maltodextrin; P, polyphosphate.

2. Effects of Cryoprotectants on the Protein Qualities of Pollock Surimi

Table 2. Protein quality of frozen pollock surimi[1] stored at -25℃ for 16 weeks

Cryoprotectant[2] level	TI[3]		*In vitro* dig.(%)		C-PER		Predicted dig.(%)		DC-PER	
	0 week	16	0	16	0	16	0	16	0	16
Frozen pollock fillet	13.84 ± 0.17	17.72** ± 0.17	85.9 ± 3.54	84.0 ± 0.87						
					2.32	2.19	87.7	82.0	2.11	2.10
Salted surimi[4]	8.87 ± 0.34	7.52 ± 1.74	89.6 ± 2.08	89.0 ± 0.20	2.35	2.26	87.5	87.5	2.11	2.10
Control surimi	3.62 ± 1.58	2.21 ± 1.10	92.8 ± 1.10	88.4 ± 1.58	2.56	2.04	87.7	85.4	2.08	2.08
SC4:4	0.76 ± 0.59	ND[5]	91.6 ± 1.75	90.2 ± 0.44	2.44	2.26	87.7	83.4	2.10	2.10
C8	0.45 ± 0.16	ND	90.9 ± 1.71	88.7 ± 0.38	2.44	2.14	87.7	86.6	2.10	2.10
S8	1.26 ± 0.32	ND	90.4 ± 1.27	90.0 ± 0.11	2.56	2.14	87.7	85.8	2.10	2.10
D8	0.91 ± 0.35	ND	87.7 ± 4.61	86.3 ± 0.24	2.56	2.04	87.7	83.7	2.10	2.10

1; 0.2% of sodiumtriphosphate and sodiumpyrophosphate(1:1, w/w) added before cryoprotectant treatment.

2; Used cryoprotectants are same as in Table 1.

3; *mg*/100*g* solid.

4; 2.5% NaCl added.

5; Not detected.

**: $p < 0.01$, significantly different from 0 week.

Table 3. Amino acid profiles of pollock fillet and surimis (*g*/16*g* N)

Amino acid	Pollock fillet	Unsalted surimi	Salted surimi
Lys	9.96	9.38	8.93
His	2.40	2.30	2.30
NH_3	0.73	0.84	0.76
Arg	6.60	6.50	5.94
Asp	10.23	10.83	11.50
Thr	4.20	4.78	4.82
Ser	4.12	5.30	4.61
Glu	18.90	18.71	18.16
Pro	4.50	5.20	5.21
Gly	4.59	4.41	5.58
Ala	5.19	5.64	5.19
Cys	1.01	1.01	1.10
Val	4.50	4.57	4.80
Met	2.89	3.19	3.40
Ile	4.55	5.52	5.21
Leu	8.15	8.67	8.97
Tyr	3.55	4.10	4.10
Phe	3.59	3.31	3.58
Trp	0.90	1.21	1.40
Total	100.56	105.47	105.56

lor. Therefore, in deciding the treated level of sorbitol, care must be taken because the kamaboko made from sorbitol treated surimi tend to have harder textures compared to those with sucrose(Suzuki 1981). Cryoprotective effect on protein digestibility of pollock surimi increased with the level of sorbitol to 4% or 6% without further increasing digestibility at levels of sorbitol higher than 6%. The protein digestibility of frozen surimi rose steadily with an increased level of sucrose to 10%. On the other hand, the enhancing effect of protein digestibility could not be achieved if the surimi was treated with maltodextrin at a level higher than 8 %. Overall cryoprotectants containing surimi showed higher protein digestibility than the control samples. The pattern of cryoprotection was differed significantly from the results in Fig. 1 and previous reports(Yoon and Lee 1990, Sych *et al.* 1990). Such discrepancies could have been due to different properties measured between two tests, namely, digestibility test and salt extractability test of protein. The sample conditions also, such as combining the treatment with polyphosphate, could have contributed to the discrepancies.

Effectiveness of Combinations of Sorbitol with Sucrose

Protein digestibility as an index of frozen denaturation of pollock surimi was checked to find out the effectiveness of combinations of sorbitol with sucrose(SC)(Fig. 3). In general, surimi industries employed combinations of sorbitol/sucrose 1:1 (*w/w*) at 8% level, because it could give a good cryoprotetive qualities such as expressible moisture, rigid texture and salt soluble proteins. But in our study, samples treated with combinations of sorbitol and sucrose revealed increased protein digestibility to maxima from sorbitol/sucrose 5:3(*w/w*) mixture at 8% in surimi. With ratios of sorbitol to sucrose higher than 5:3(*w/w*), on the contrary, digestibility showed in some drop. This result suggested that stabilization of protein structure occurred effectively when the level of sorbitol reached 5:3(*w/w*) with sucrose, otherwise, the higher level of sorbitol blocked the cryoprotective effect of sucrose due to formation of a compact network through preferential hydration of protein, which forms the basis of cryoprotection.

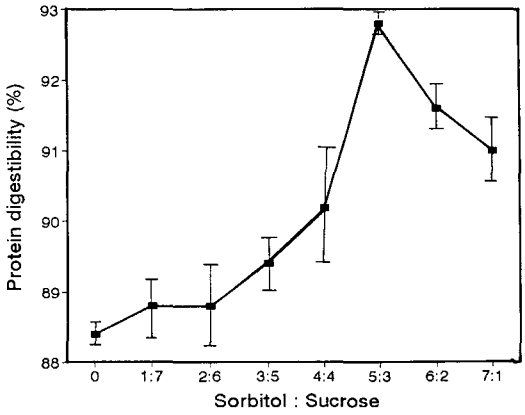

Fig. 3. Changes in protein digestibility of pollock surimi as a function of mixing ratio for crystalline sorbitol and sucrose in 8% total level;
1:7, sorbitol 1% + sucrose 7%;
2:6, sorbitol 2% + sucrose 6%;
3:5, sorbitol 3% + sucrose 5%;
4:4, sorbitol 4% + sucrose 4%;
5:3, sorbitol 5% + sucrose 3%;
6:2, sorbitol 6% + sucrose 2%;
7:1, sorbitol 7% + sucrose 1%.

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해양식량자원의 가공조건별 영양적 품질평가

2. 명태연육 단백질품질에 미치는 냉동변성방지제의 영향

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명태연육 냉동변성을 억제할 수 있는 냉동변성방지제의 적정량을 알아보기 위하여 crystalline sorbitol을 비롯한 세가지의 변성방지제 및 sorbitol/sucrose 혼합제제를 첨가하여 -25℃에서 16주간 저장했을 때의 단백질품질 변화를 실험하였다.

8% 수준의 sucrose-sorbitol 혼합제제(1:1, *w/w*)를 0.2% Na-pyrophosphate/ Na-triphosphate(1:1)와 병용하였을 때 drip loss가 가장 적었으며, 염용성단백질량은 가장 많았다. 또한 혼합제제를 사용했을 때는 trypsin inhibitor량, 단백질소화율 및 단백효율비의 변화가 거의 없어 단백질품질 보전에 효과적이었으나, 냉동변성방지제를 처리하지 않았을 경우에는 단백효율비와 소화율은 급격히 떨어졌었다. Polyphosphate나 maltodextrin의 경우에는 처리 농도가 증가하여도 소화율저하 방지에는 별효과가 없었으나, 4~6%의 sorbitol, 또는 10%수준의 sucrose는 polyphosphate를 병용하지 않아도 소화율저하는 효과적으로 막을 수 있었다. 8% 수준의 sorbitol/sucrose 혼합제제(5:3, *w/w*)처리가 소화율 보전효과로 볼 때 냉동연육의 단백질품질저하 방지에 가장 효과적이었다.