Thermoprotective Effect of Sorbitol on Proteins during Dehydration

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Thermoprotective properties of sorbitol were evaluated on freeze-dried fish mince and spray-dried egg white and skim milk proteins by measuring changes in functionality. The thermoprotective effect of sorbitol appeared to be a function of sorbitol concentration as evidenced by increased gel-forming ability, water-binding ability, emulsifying capacity, and enthalpy (ΔH) for denaturation, with no significant changes in denaturation temperature (T_d). The greater water-binding ability resulting from the sorbitol treatment before rather than after thermal hydration may be attributed to increased hydrophobic interaction and hydrophilicity from the formation of a protein–sorbitol complex.

INTRODUCTION

Freeze-drying and spray-drying are dehydration methods that are employed in drying heat-sensitive proteinaceous materials with minimum losses of functional properties intended. However, changes in the functional properties of proteins still occur due to thermal or freezing treatments involved during such dehydration processes (Baldwin et al., 1967; Zabik and Brown, 1969; Galyean and Cotterill, 1979). In an effort to reduce such thermally- or freeze-induced adverse changes in functional properties, sugars were examined as thermo- and cryoprotective agents. Proposed mechanisms by which certain sugars stabilize proteins are as follows: (1) promotion of preferential hydration which is facilitated by an increased surface tension of water during freezing (Lee and Timasheff, 1981; Arakawa and Timasheff, 1982); (2) preservation of native conformation through preferential exclusion of solutes during freezing or through a direct interaction between sugars and polar residues in the protein surface during drying (Carpenter et al., 1990; Crowe et al., 1990); (3) strengthening of hydrophobic interaction during heating (Gerlsma and Stuur, 1972, 1974; Back et al., 1979). These suggest that the mechanisms for cryoprotection are different from those for thermoprotection. Sucrose and sorbitol are shown to be highly effective in preserving the functional properties of myofibrillar proteins in refined fish mince (surimi) during frozen storage (Tamoto et al., 1961; Ooizumi et al., 1981; Matsumoto et al., 1985; Yoon and Lee, 1990) and dehydration (Niki et al., 1982; Niki and Igarashi, 1982).

However, little information is available on the protective effect of sorbitol on functionalities of various proteins during commercial dehydration processes. Examining the protective effect of sorbitol at a macromolecular level could provide an answer to the feasibility of its use in preserving functional properties of heat-sensitive commercial proteins. The objective of this study was to evaluate the thermoprotective effect of sorbitol on protein during various commercial dehydration processes.

MATERIALS AND METHODS

Material Preparations. Refined fish mince was prepared from red hake (*Urophycis chuss*) by washing the ground mince twice with 4-fold water (10 °C), refining, and dewatering (Lee, 1986). Unpasteurized cow's skim milk was obtained from a local dairy plant. Fresh egg white was obtained by separation from the whole egg purchased from the local store. Liquid sorbitol (70% solid) was added to refined fish mince (18% solid) at 0, 2.8, and 4.0%; skim milk (8.2% solid) at 0, 1.4, 2.8, 4.3, and 5.7%; and egg white (11.1% solid) at 0, 1.4, 2.8, 4.3, and 5.7%. (All percentages are on a wet weight basis.) No pH adjustment was made after addition of sorbitol.

Drying Methods. A refined fish mince block (1.5 × 23 × 29 cm) was freeze-dried using a freeze-drier (Virtis Model USM15, Gardiner, NY). Freeze-drying was done at a pressure of 100 μmHg and a platen temperature of 60 °C, which simulated drying at an elevated temperature. The drying process involved freezing at −20 °C for 12 h and drying from −20 to 30 °C for 30 h, where 30 °C was the product temperature at the completion of freeze-drying. Skim milk and egg white were spray-dried using a mini-spray drier (Buchi/Brinkman Model 190, Westbury, NY) at the feeding rate of 2.8 mL/min at 160 and 100 °C of inlet and outlet air temperature, respectively.

Gel-Forming Ability. The gel-forming ability was determined on the freeze-dried refined fish mince and the spray-dried egg white but not on the spray-dried skim milk. Skim milk did not form a gel that was firm enough for gel testing. Freeze-dried refined fish mince was hydrated to the moisture level of the raw material (82%), comminuted with 2% salt (on a surimi wet weight basis) for 10 min in a silent cutter, and subjected to gel preparation and the compression test following the procedure described by Lee and Chung (1989). For the spray-dried egg white, the penetration test (Lee and Chung, 1989) was used to determine gel-forming ability. The spray-dried egg white was hydrated to 85% moisture, of which 15 g was transferred into a 20-mL beaker and heat-set at 90 °C for 20 min in a steam cooker prior to gel testing.

Water-Binding Ability (WBA). The water-binding ability was determined after thermal gelation on spray-dried skim milk protein but not on egg white since the latter produced an extremely cohesive gel with no water separation even at a high moisture level (85%). Spray-dried skim milk with and without sorbitol treatment was mixed with salt (40% on a dried milk weight basis) and the appropriate amount of water for 30 s in a 100-mL mini-Waring blender such that the water content was kept at 60% for all samples. Addition of 40% salt was found to be the minimum level necessary to facilitate gel formation. The reason for such a large amount of salt was believed to be the presence of lactose and sorbitol in high proportions (over 60% combined level). The resulting hydrated samples (12 g) were heated in a 40-mL centrifuge tube at 90 °C for 10 min for gelation and centrifuged for 20 min at 3000*g*. The amount of water retained after centrifugation was measured as thermal hydration ability (Chung and Lee, 1990).

Emulsifying Capacity (EC). For the determination of emulsifying capacity, 2 g of spray-dried egg white was mixed with 0.2 g of NaCl (10% on a dried egg white weight basis) and water in a 200-mL Waring blender jar for 30 s. A minimum of 10% salt was needed to produce a thick emulsion with a clear-cut break point, and water was added to adjust the final water content to 95%. Thirty grams of this slurry was transferred into a blender jar. While blending at a moderate speed (rheostat setting at 25), corn oil was added from a graduated cylinder at a constant rate of feed (15 mL/s). Blending was continued until the emulsion

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Table I. Effect of Sorbitol on Gel-Forming Ability of Freeze-Dried Refined Fish Mince[a]

sorbitol level,[b] %	compressive force ± SD, kg (% increase)	penetration force ± SD, g (% increase)
0.0	2.0 ± 0[a]	209 ± 4[a]
2.8	3.7 ± 0.2[b] (83.5%)	218 ± 3[b] (4.3%)
4.0	3.9 ± 0.1[c] (96.5%)	220 ■ 0[b] (5.3%)

[a] Means with different superscripts are significantly different ($p < 0.05$; $n = 3$). [b] Liquid sorbitol on a fish mince wet weight basis.

Table II. Effect of Sorbitol on Gel-Forming Ability and Denaturation Characteristics of Spray-Dried Egg White[a]

egg white	sorbitol added, %	penetration force,[b] g	ΔH, mcal/mg	T_d,[c] °C	extent of denaturation,[d] %
fresh	0	393 ± 6	0.30[a]	82.0	0
spray-dried	0	224 ± 4	0.16[c]	80.7	46.7
	1.4		0.16[c]	80.0	46.7
			(0.16)	(80.7)	(46.7)
	2.8		0.17[c]	80.3	43.3
			(0.13)	(80.7)	(56.7)
	4.3		0.25[b]	80.0	16.7
			(0.11)	(80.5)	(63.3)
	5.7	133 ± 4	0.26[b]	80.0	13.3
		(39 ± 2)	(0.11)	(80.0)	(63.3)

[a] Values in parentheses are for the samples in which sorbitol was added after spray-drying. Means with different letters are significantly different ($p < 0.05$). [b] Solid adjusted to 15% for all samples. [c] Measured on ovalbumin. [d] Based on the difference in ΔH values between fresh and spray-dried egg white.

collapsed. The amount of oil added to this break point was used as emulsifying capacity (milliliters of oil added per gram of solid).

Thermal Denaturation Behavior. A half gram of spray-dried egg white was mixed with the appropriate amount of water in a 10-mL test tube to adjust water content to that of fresh egg white (88.9%). Thermograms (enthalpy vs temperature) were obtained using a differential scanning calorimeter (Perkin-Elmer DSC-2). Approximately 15 mg of egg white solution (11.1% solid) was weighed in a DSC sample pan, sealed, and loaded into the instrument at 25 °C. After equilibration, the sample temperature was raised at a rate of 10 °C/min to 100 °C; sensitivity was set at 0.1 mcal/s mm.

The enthalpy for protein denaturation was calculated from the peak area as

$$\Delta H \text{ (mcal/mg)} = \frac{\text{area (mm}^2\text{)}}{\text{wt (mg)}} \times \text{time base (s/mm)} \times E \times \text{sensitivity (mcal/s mm)}$$

where E is the calibration coefficient from the thermogram of indium (reference metal).

Statistical Analysis. Analysis of variance was performed using the Statistical Analysis System (SAS, 1985). Differences in means were determined using Duncan's multiple-range test.

RESULTS AND DISCUSSION

As shown in Table I, the gel-forming ability of freeze-dried fish mince protein was improved with an increase in sorbitol concentration. This indicated that sorbitol protected the fish mince protein from losing its functional properties during freeze-drying as evidenced by 96.5% greater gel-forming ability at 4% of sorbitol as compared to that of the control. The thermoprotective effect of sorbitol in egg white was also evident from the finding that the addition of sorbitol prior to spray-drying resulted in a gel strength (133 g) 3.4 times greater than did addition after drying (39 g) (Table II). It should be, however, noted that addition of sorbitol reduced the gel strength of egg white from 224 to 133 g. Such a decrease in the gel strength of sorbitol-treated egg white was due to a high level of substitution for protein (18% of egg white after drying). It is believed that a high substitution of sorbitol would cause increased competition between sorbitol and protein for water binding, which leads to a limited availability of water for protein hydration necessary for optimum gelation. A similar observation was made in a surimi system (Yoon and Lee, 1990). The addition of sorbitol to

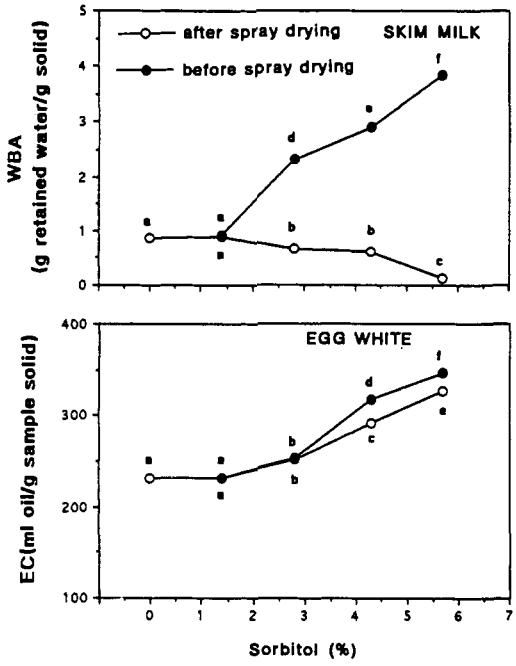

Figure 1. Effect of sorbitol on water-binding ability (WBA) of spray-dried skim milk protein and emulsifying capacity (EC) of spray-dried egg white. Data points with different letters are significantly different ($p < 0.05$). Sorbitol was added before and after spray-drying.

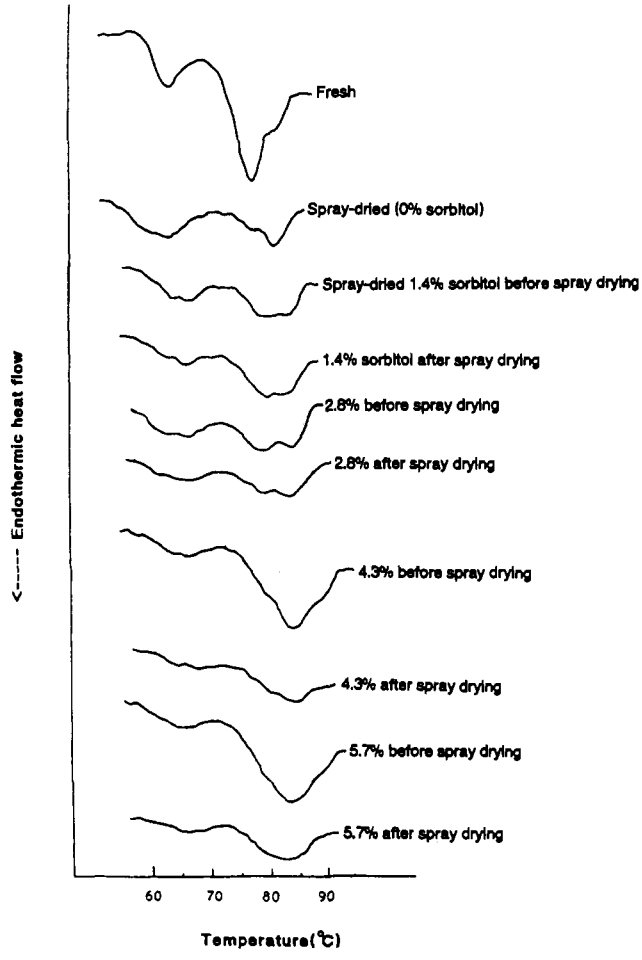

Figure 2. DSC thermograms of spray-dried egg white proteins treated at varying levels of sorbitol.

skim milk before spray-drying at 2.8% or more markedly increased the thermal hydration ability, while after 24 h of drying, it decreased the hydration ability as observed in egg white (Figure 1). The increase in thermal hydration ability appeared to be a linear function of the level of sorbitol added.

An increase in thermal hydration ability by addition of sorbitol prior to dehydration may be attributed to protected protein functionality and a possible formation of a protein–sorbitol complex. The protection of protein functionality would be accompanied by increased hydrophobic interaction, which was reported to stabilize the three-dimensional structure of proteins (Fersht, 1977). Back et al. (1979) showed that the mechanism by which sugars and polyols stabilize proteins against heat denaturation is through their effect on the structure of water molecules: hydrophobic interactions between pairs of hydrophobic groups in protein are stronger in sucrose solutions than in pure water. On the other hand, Lee and Timasheff (1981) and Arakawa and Timasheff (1982) suggested that the cohesive force of sugars responsible for the increase in the surface tension of water is an important factor governing the preferential interaction of proteins with solvent components in aqueous sugar systems and hence the stabilization of protein. The formation of a protein–sorbitol complex is suggested on the basis of the studies of Carpenter et al. (1990) and Crowe et al. (1990), who indicated the involvement of a direct interaction between sugar and protein in protein stabilization. Such formation could have increased hydrophilicity, which in turn could enhance the water binding.

Figure 1 also shows that sorbitol-treated egg white at 4.3% (3% solid) and 5.7% (4% solid) had a significantly greater ($p < 0.05$) emulsifying capacity than that treated after drying, suggesting that addition prior to spray-drying helped the protein retain greater functionality.

Figure 2 shows DSC thermograms of spray-dried egg white required proteins treated at varying levels of sorbitol. Consistently throughout the samples tested, sorbitol-treated egg white required significantly higher enthalpy (ΔH) for denaturation than those treated after drying (Table II). This indicated that the sorbitol-treated egg white protein underwent denaturation to a lesser extent, while changes in the denaturation temperature (T_d) of spray-dried egg white were insignificant. Thus, sorbitol treatment affected ΔH but not T_d. However, the study of Donovan et al. (1975) showed the addition of sucrose at a high concentration (10%) shifted T_d by approximately 2 °C. These two results differed because the spray-dried samples tested in our study already had undergone some degree of denaturation during drying, while the other had not.

CONCLUSION

The sorbitol treatment during a commercial dehydration process helped protein retain greater functional properties. The stabilization of protein functionality after the sorbitol treatment may be attributed to increased hydrophobic interaction, which helps stabilize the three-dimensional structure of the protein, and increased hydrophilicity from the formation of a protein–sorbitol complex.

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LITERATURE CITED

Arakawa, T.; Timasheff, S. N. Stabilization of protein structure by sugars. *Biochemistry* **1982**, *21*, 6536–6544.

Back, J. F.; Oakenfull, D.; Smith, M. B. Increased thermal stability of proteins in the presence of sugars and polyols. *Biochemistry* **1979**, *18*, 5191–5197.

Baldwin, R. E.; Cotterill, O. J.; Thompson, M. M.; Myers, M. High temperature storage of spray-dried egg white. 1. Whipping time and quality of angel cake. *Poult. Sci.* **1967**, *46*, 1421–1430.

Carpenter, J. F.; Crowe, J. H.; Arakawa, T. Comparison of solute-induced protein stabilization in aqueous solution and in the frozen and dried states. *J. Dairy Sci.* **1990**, *73*, 3627–3636.

Chung, K. H.; Lee, C. M. Relationships between physicochemical properties of nonfish protein and textural properties of protein-incorporated surimi gel. *J. Food Sci.* **1990**, *55*, 972–975.

Crowe, J. H.; Carpenter, J. F.; Crow, L. M.; Anchordoguy, T. J. Are freezing and dehydration similar stress vectors? A comparison of modes of interaction of stabilizing solutes with biomolecules. *Cryobiology* **1990**, *27*, 219–231.

Donovan, J. W.; Mapes, C. J.; Davis, J. G.; Garibaldi, J. A. A differential scanning calorimetric study of the stability of egg white to heat denaturation. *J. Sci. Food Agric.* **1975**, *26*, 73–83.

Fersht, A. R. *Enzyme Structure and Mechanism*; Freeman: Reading, England, 1977.

Galyean, R. D.; Cotterill, O. J. Chromatography and electrophoresis of native and spray-dried egg white. *J. Food Sci.* **1979**, *44*, 1345–1349.

Gerlsma, S. Y.; Stuur, E. R. The effect of polyhydric and monohydric alcohols on the heat-induced reversible denaturation of lysozyme and ribonuclease. *J. Pept. Protein Res.* **1972**, *4*, 377–383.

Gerlsma, S. Y.; Stuur, E. R. The effects of combining two different alcohols on the heat-induced reversible denaturation of ribonuclease. *J. Pept. Protein Res.* **1974**, *6*, 65–74.

Lee, C. M. A pilot study of surimi making properties of red hake, Urophycis chuss. In *International Symposium on Engineered Seafoods Including Surimi*; Martin, R., Collete, R., Eds.; National Fisheries Institute: Washington, DC, 1986; pp 225–243.

Lee, C. M.; Chung, K. H. Analysis of surimi gel properties by compression and penetration tests. *J. Texture Stud.* **1989**, *20*, 363–377.

Lee, J. C.; Timasheff, S. N. The stabilization of proteins by sucrose. *J. Biol. Chem.* **1981**, *256*, 7193–7201.

Matsumoto, J. J. Chemical deterioration of muscle proteins during frozen storage. In *Chemical Deterioration of Proteins*; Whitaker, J. R., Fujimaki, M., Eds.; ACS Symposium Series 123; American Chemical Society: Washington, DC, 1980; pp 205–224.

Matsumoto, I.; Ooizumi, T.; Arai, K. I. Protective effect of sugar on freezed-denaturation of carp myofibrillar protein. *Bull. Jpn. Soc. Sci. Fish.* **1985**, *51*, 833–839.

Niki, H.; Igarashi, S. Some factors in the production of active fish protein powder. *Bull. Jpn. Soc. Sci. Fish.* **1982**, *48*, 1133–1137.

Niki, H.; Deya, E.; Kato, T. The process of producing active fish protein powder. *Bull. Jpn. Soc. Sci. Fish.* **1982**, *48*, 999–1004.

Ooizumi, T.; Hashimoto, K.; Ogur, J.; Arai, K. I. Quantitative aspect for productive effect of sugar and sugar alcohol against denaturation of fish myofibrils. *Bull. Jpn. Soc. Sci. Fish.* **1981**, *47*, 901–908.

SAS. *SAS User's Guide*; SAS Institute Inc.: Cary, NC, 1985.

Tamoto, K.; Tanaka, S.; Takeda, F.; Fukumi, T.; Nishiya, K. Studies on freezing of surimi and its application. IV. On the effect of sugar upon the keeping quality of frozen Alaska pollock meat. *Bull. Hokkaido Reg. Fish. Res. Lab.* **1961**, *23*, 50–60.

Yoon, K. S.; Lee, C. M. Cryoprotectant effects in surimi and surimi/mince-based extruded products. *J. Food Sci.* **1990**, *55*, 1210–1216.

Zabik, M. E.; Brown, S. L. Comparison of frozen, foam-spray-dried, freeze-dried, and spray-dried eggs. *Food Technol.* **1969**, *23*, 262–266.

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