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Glass transition properties of frozen and freeze-dried surimi products: Effects of sugar and moisture on the glass transition temperature

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Abstract

Frozen surimi mixtures containing four types of sugar (sorbitol, glucose, sucrose, and trehalose) and freeze-dried surimi–trehalose mixtures were made from live carp, and their glass transition properties were investigated by differential scanning calorimetry. For comparison, a commercial frozen cod surimi was also investigated. The frozen carp surimi samples showed two freeze-concentrated glass transitions, at −42 to −65 °C and at −21 to −41 °C, depending on type and the sugar content of samples. These glass transition temperatures were denoted as T'_{g1} and T'_{g2}, respectively. With increasing sugar content, the T'_{g1} and T'_{g2} of the samples increased and decreased, respectively. At each sugar level, the T'_{g1} and T'_{g2} of carp surimi–trehalose mixtures were higher than those of the other mixtures. From the sugar content effects on T'_{g1} and T'_{g2}, the T'_{g1} and T'_{g2} of the carp surimi without sugar were estimated to be −55 to −65 °C and −15 °C, respectively. The commercial cod surimi showed $T'_{g1} = -65$ °C and $T'_{g2} = -22$ °C. These were reasonable values in comparison with those of carp surimi–sugar mixtures. From the result, it was suggested that the difference of fish stock did not greatly affect the T'_{g1} and T'_{g2} of surimi–sugar mixtures. The moisture content effect on the glass transition temperature of freeze-dried carp surimi–trehalose mixtures was investigated, and a state diagram of the surimi-moisture pseudo-binary system was developed. From the viewpoint of "glass transition" concept, it is expected that these results are useful in the production and storage of frozen and freeze-dried surimi products.

Keywords: Surimi; Myofibrillar proteins; Glass transition; Trehalose; DSC

1. Introduction

Surimi, minced and water-washed fish muscle tissue, has been used as a primary material for gelling foods enriched in myofibrillar proteins, such as kamaboko and shellfish analog products. Surimi is a labile product chemically, physically, and microbiologically, and thus frozen surimi is produced for practical use. To allow shipping and preservation at ambient temperature, the production of freeze-dried surimi has been attempted (Reynolds, Park, & Choi, 2002). The myofibrillar proteins in surimi, however, undergo unfolding and aggregation during processing and storage, and then their functional properties (such as gel-forming ability and water holding capacity) are significantly diminished. Extensive efforts have been devoted to improve the stabilization of frozen or freeze-dried surimi products by adding stabilizers (Auh et al., 1999; Carvajal, MacDonald, & Lanier, 1999; Lim & Reid, 1991; MacDonald, Lanier, & Giesbrecht, 1996; Park, Lanier, & Green, 1988; Reynolds et al., 2002; Rodríguez Herrera, Pastoriza, & Sampedro, 2000; Saeki, 1996; Somjit, Ruttanapornwareesakul, Hara, & Nozaki, 2005; Sultanbawa & Li-Chan, 1998; Sych, Lacroix, Adambounou, & Castaigne, 1990; Yoon & Lee, 1990; Zhou, Benjakul, Pan, Gong, & Liu, 2006).

The stabilizing effects associated with stabilizers have been widely explained by "preferential interaction" and

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"glass transition." The "preferential interaction" involves preferential interaction of protein with water rather than stabilizers, which are preferentially excluded from the protein's hydration shell; thus, unfolding of the protein is prevented and its native conformation is stabilized (Crowe, Carpenter, Crowe, & Anchordoguy, 1990; Kita, Arakawa, Lin, & Timasheff, 1994). This mechanism, however, is proposed empirically from a protein stabilization mechanism in the liquid state and cannot completely explain protein stabilization in the frozen state. For example, Nema and Avis (1993) reported that there was no apparent correlation between the preferential interaction of stabilizers and their protective effects on protein during freeze-thawing. On the other hand, the "glass transition" involves embedding of the frozen protein in a glassy matrix formed by freeze-concentrated stabilizers leading to a highly viscous state, and thus molecular rearrangement of the protein and the rates of chemical and physical degradation are inhibited by immobilization (Franks, 1990; Le Meste, Champion, Roudaut, Blond, & Simatos, 2002; Levine & Slade, 1988; Roos, 1995). Furthermore, the "glass transition" is extended to the stabilizing mechanism of freeze-dried protein; the glassy matrix formed by freeze-concentration maintains its state even at an elevated temperature due to the decrease in the number of water molecules that play the role of plasticizers. In the "glass transition" mechanism, glass transition temperature is one of the most significant parameters. The glass transition temperatures of a maximally freeze-concentrated system and a freeze-dried one are usually denoted as T'_g and T_g, respectively. At each glass transition temperature, amorphous materials show a transition between a solid-like glassy state and liquid-like rubbery state; for the preservation of frozen or freeze-dried foods, the storage temperature should be at a temperature below the glass transition temperature. In addition, the glass transition temperature means the temperature at which the viscosity of amorphous materials becomes a constant viscosity ($\sim 10^{12}$ Pas); it is thought that the higher the glass transition temperature, the lower the molecular mobility is at a reference temperature.

There are some studies on the effect of glass transition on the cryostabilization of frozen surimi. For example, Lim and Reid (1991) investigated the effects of glass-forming solutes (sucrose, maltodextrin, and carboxymethylcellulose) on the storage stability of frozen myofibrillar proteins. They demonstrated that the salt-solubility of actomyosin in the myofibrillar proteins showed no significant change at storage temperatures above and below the T'_g of their glass-forming solute aqueous solutions. Similarly, Carvajal et al. (1999) investigated the effects of glass-forming solutes (sucrose, a sucrose–sorbitol mixture, and various molecular weights of maltodextrin) on the storage stability of frozen surimi products. They demonstrated that loss of the Ca^{2+}-ATPase activity of actomyosin extracted from the surimi products showed no significant change at storage temperatures above and below the T'_g of their glass-forming solute aqueous solutions. The interpretations of these studies, however, remain outstanding problems as follow.

The first point to be considered is that freeze-concentrated aqueous systems exhibit two glass transitions. For example, in differential scanning calorimetry (DSC) studies, first a small endothermic shift and subsequently a large one are observed in the DSC heat-scanning thermograms. In this paper, these glass transition temperatures are denoted as T'_{g1} and T'_{g2}, respectively. The origin of these glass transitions is not completely understood. In the case of aqueous sugar solutions, it is suggested that T'_{g1} corresponds to a maximally freeze-concentrated glass transition and T'_{g2} is an ante-melting temperature where the viscous flow initiates in the amorphous region occurring a few Kelvin below the beginning of ice-melting (MacKenzie, 1977; Shalaev & Kanev, 1994) or the temperature of the beginning of ice-melting (Ablett, Izzard, & Lillford, 1992; Roos, 1995; Shalaev & Franks, 1995). Additionally, there is an interpretation that T'_{g1} and T'_{g2} correspond to glass transitions of two phases of different solute concentrations, with the former due to the transition in a freeze-concentrated region containing a large amount of unfrozen water and the latter due to the transition in a maximally freeze-concentrated region (Levine & Slade, 1988; Pyne, Surana, & Suryanarayanan, 2003). In the case of protein aqueous solutions, it has been suggested that T'_{g1} corresponds to a glass transition of a part of unfrozen water (Inoue & Ishikawa, 2000; Kawai, Suzuki, & Oguni, 2006; Sartor, Hallbrucker, & Mayer, 1995) or a glass transition of the side chain of the hydrated protein (Green, Fan, & Angell, 1994), and T'_{g2} corresponds to a glass transition of the primary chain of the hydrated protein (Green et al., 1994; Kawai et al., 2006). At temperatures above T'_{g2}, structural collapse, crystallization of solute, and growth of ice occur significantly (Chang & Randall, 1992; Levine & Slade, 1988; Shalaev & Franks, 1995; Wang, 2000). In the temperature range between T'_{g1} and T'_{g2}, a part of the unfrozen water crystallizes (Pyne et al., 2003; Roos, 1995). At temperatures below T'_{g1}, the amorphous matrix shows only slow dynamic properties such as enthalpy relaxation on a time scale of hours to days (Inoue & Suzuki, 2006). From these observations, it has been noted that both the T'_{g1} and T'_{g2} of frozen surimi products should be understood as the T'_g.

The second point that requires clarification is that the glass transition temperature of multicomponent systems such as surimi–stabilizer mixtures depends strongly on the components. For example, Chang and Randall (1992) investigated the effects of water-soluble proteins (bovine serum albumin, lysozyme, ribonuclease, and elastase) on the T'_{g2} of various aqueous solutions (lactose, sorbitol, and glycerol). They demonstrated that the T'_{g2} of the aqueous mixtures increased up to the T'_{g2} of the protein aqueous solutions with an increase in the protein content. Similar results were reported for T'_{g2} of sucrose–dextran aqueous mixtures (Blond & Simatos, 1998) for T'_{g1} and T'_{g2} of sucrose–gelatin and sucrose–peptone aqueous mixtures

(Shalaev, Varaksin, Rjabicheva, & Aborneva, 1996), and for T'_{g1} and T'_{g2} of sucrose–glycine aqueous mixtures (Shalaev & Kanev, 1994; Suzuki & Franks, 1993). Some aqueous mixtures, however, show a drastic increase in T'_{g1} and T'_{g2}. For example, the T'_{g1} and T'_{g2} of sugar and/or polyol aqueous solutions increase remarkably with the addition of borate; the T'_{g1} and T'_{g2} of the aqueous mixtures are higher than those of the sugar and/or polyol and borate aqueous solutions (Izutsu, Ocheda, Aoyagi, & Kojima, 2004; Izutsu, Rimando, Aoyagi, & Kojima, 2003). It is suggested that borate accomplishes this by forming a reversible cross-linked network between hydroxyl compounds. There is a similar report on the T'_{g2} of tripolyphosphate–sugar aqueous mixtures (Kawai & Suzuki, 2006). On the other hand, the T_g of freeze-dried amorphous foods depends primarily on the moisture content, and thus the effect of moisture content on the T_g is a subject of not only practical but also fundamental interest (Franks, 1990; Le Meste et al., 2002; Levine & Slade, 1988; Roos, 1995). Glass transition temperatures of frozen and freeze-dried surimi–cryostabilizer mixtures, however, have never been investigated in detail.

Low-molecular-weight sugars, including glucose (Somjit et al., 2005), sorbitol (Yoon & Lee, 1990), sucrose (Carvajal et al., 1999; Somjit et al., 2005), and trehalose (Zhou et al., 2006) are among the most effective cryostabilizers of not only isolated proteins (Crowe et al., 1990) but also surimi. Furthermore, disaccharides are effective lyostabilizers of isolated proteins (Crowe et al., 1990). Among the disaccharides, it is thought that trehalose is well suited as a lyostabilizer of freeze-dried surimi because of its relatively low chemical reactivity (non-enzymatic browning reaction), calorimetric value, and sweetness. In this study, thus, glass transition properties of frozen and freeze-dried surimi–sugar mixtures were investigated by DSC. First, surimi–sugar mixtures were made from live carp and four types of sugar (sorbitol, glucose, sucrose, and trehalose), and then effects of the sugars on the T'_{g1} and T'_{g2} were investigated. For comparison, a commercial frozen cod surimi was also investigated. Second, freeze-dried carp surimi–trehalose mixtures were prepared as typical freeze-dried surimi products, and the effect of moisture content on the T_g was investigated.

2. Materials and methods

2.1. Preparation of frozen surimi–sugar mixture

Analytical grade sorbitol (Wako Pure Chem. Ind. Ltd., Japan), sucrose (Kokusan Chem. Works Ind. Ltd., Japan), and glucose and trehalose (Sigma Chem. Co., USA) were obtained. A commercial frozen cod surimi from Alaska pollack (FA Grade) and live carp (*Cyprinus carpio*) were purchased from Nichiro Co. Ltd., Japan and a retail market, respectively.

Carp surimi was prepared according to a commonly used procedure, as illustrated in Fig. 1. Every procedure was carried out at 0–4 °C to minimize degradation of the fish muscle. The moisture content of the surimi was determined gravimetrically by drying at 105 °C. For preparing carp surimi-sugar mixtures, 10% (w/w) aqueous sugar solutions (sorbitol, glucose, sucrose, and trehalose) were kneaded manually into the surimi. The sugar content of the mixtures ranged from 16.9% to 87.0% (dry matter basis). DSC measurement was carried out within 24 h after preparation.

Killing of carp (*cyprinus carpio*)
↓
Collecting of dorsal meat
↓
Mincing for 30 sec by using a homogenizer
↓ (Ace AM-3 Homogenizer, Nihonseiki Kaisha Ltd., Tokyo, Japan)
Washing with 0.1 % (w/w) NaCl solution
↓ (removing water soluble protein)
Centrifuging at 11,000 g for 15 min
↓ (collecting carp mince)
Straining
↓ (concentrating carp mince)
Addition of 10 % (w/w) sugar aqueous solution
(preparation of carp surimi-sugar mixture)

Fig. 1. Procedure of carp surimi preparation.

The commercial frozen cod surimi was used without further treatment. The moisture content of the surimi was determined gravimetrically to be 74.5% (w/w) by drying at 105 °C. It is known that commercial frozen surimi generally contains 4% (w/w) sorbitol, 4% (w/w) sucrose, and 0.2% (w/w) polyphosphate salt as cryostabilizers (Carvajal et al., 1999; MacDonald & Lanier, 1991; Ohshima, Suzuki & Koizumi, 1993); the sugar content corresponds to about 31.4% (dry matter basis). DSC measurement was carried out after the frozen surimi was thawed atmospherically.

2.2. Preparation of freeze-dried surimi–trehalose mixture

A carp surimi–trehalose mixture containing 80.0% trehalose (dry matter basis) was used to prepare typical freeze-dried surimi samples. The mixture was freeze-dried at 3.0×10^{-2} Torr at −40 °C for 6–300 h. The freeze-dried mixtures were hermetically sealed in a vial and preserved at −50 °C until they were used for DSC measurement. The moisture content of each freeze-dried sample was determined gravimetrically by drying at 105 °C.

2.3. DSC measurement

Glass transition properties of frozen and freeze-dried surimi–sugar mixtures were investigated by using a DSC (DSC-50: Shimadzu Co. Ltd., Japan). Temperature and heat flow were calibrated with indium and distilled water, and α-alumina powder was used as a reference. The sample (25–45 mg) was put into an aluminum pan and sealed hermetically. DSC measurement was performed as

follows: the sample was cooled at 6 °C/min from ambient temperature to −100 °C and reheated at 3 °C/min up to maximally 200 °C. The measurement was carried out in triplicate. The glass transition temperature was determined from the mid-point of an endothermic shift of baseline using Ta-60 software interfaced with the DSC.

3. Results

3.1. Freeze-concentrated glass transition properties of surimi–sugar mixtures

Typical DSC thermograms for carp surimi–trehalose mixtures of varying trehalose content (0–75.4% in dry matter) are shown in Fig. 2. The mixtures containing 0% and 16.9% trehalose showed no apparent thermal event except ice-melting. The mixtures containing 34.2%, 54.2%, and 75.4% trehalose, on the other hand, showed two characteristic thermal events: a small endothermic shift at a low temperature and an endothermic shoulder immediately before ice-melting. These endothermic events were judged to be freeze-concentrated glass transitions, and the T'_{g1} and T'_{g2} of each mixture were determined. The magnitude of the endothermic events reflecting T'_{g1} and T'_{g2} increased with increasing trehalose content. Additionally, the T'_{g1} and T'_{g2} increased and decreased, respectively. The mixtures containing other types of sugar showed similar results to those in Fig. 2, and the T'_{g1} and T'_{g2} of each mixture were determined.

For comparison, freeze-concentrated glass transition properties of a commercial frozen cod surimi were also investigated. A typical DSC thermogram for the cod surimi is shown in Fig. 3. The DSC thermogram showed an apparent endothermic shift and a gradual endothermic shoulder; these results were judged to be the freeze-concentrated glass transitions corresponding to T'_{g1} and T'_{g2}, respectively. The T'_{g1} and T'_{g2} of the commercial cod surimi were determined to be −65 and −22 °C, respectively.

The T'_{g1} and T'_{g2} of these surimi samples are plotted against sugar content in Fig. 4. It was found that the T'_{g1} and T'_{g2} depended strongly on the types and content of sugar. With an increase in sugar content, the T'_{g1} and T'_{g2} of carp surimi–sugar mixtures increased and decreased, respectively. At each sugar level, the T'_{g1} and T'_{g2} were, from highest to lowest, trehalose, sucrose, glucose, and sorbitol. At sugar contents of less than 30%, apparent glass

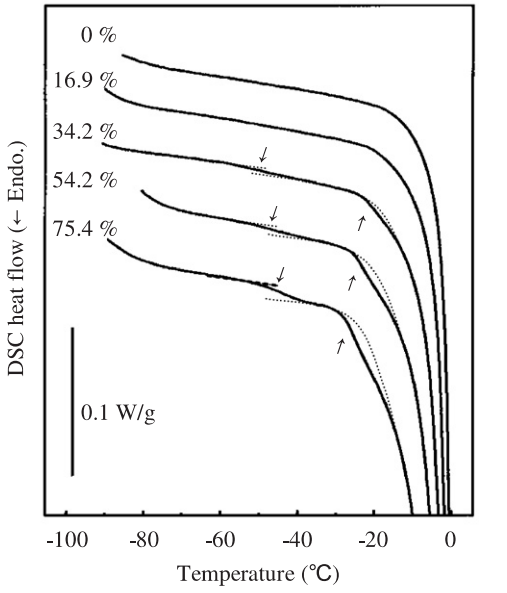

Fig. 2. DSC thermograms for carp surimi–trehalose mixtures of varying trehalose content. Down-arrows, up-arrows, and dotted lines indicate T'_{g1}, T'_{g2}, and expected baseline, respectively.

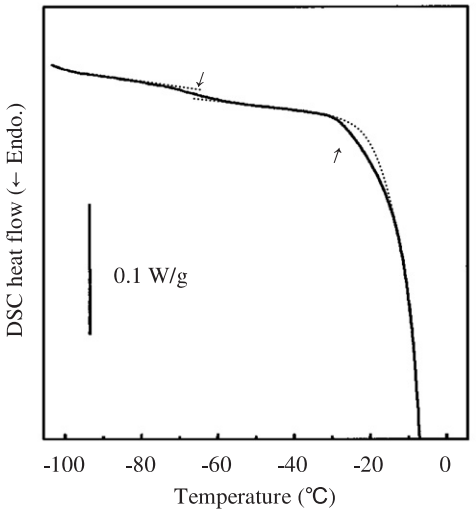

Fig. 3. DSC thermogram for a commercial frozen cod surimi. A down-arrow, an up-arrow, and a dotted line indicate T'_{g1}, T'_{g2}, and expected baseline, respectively.

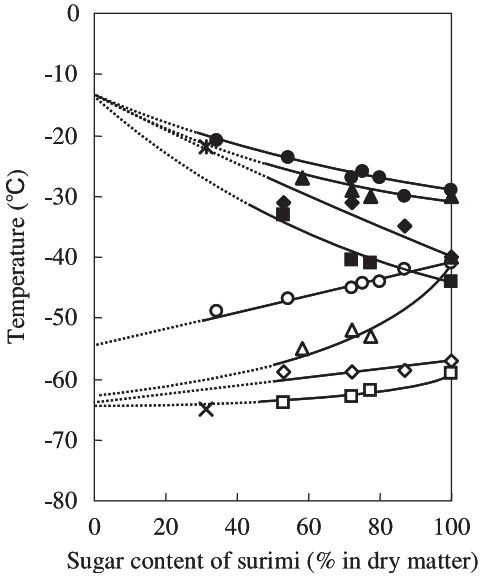

Fig. 4. Sugar content dependence of T'_{g1} and T'_{g2} of carp surimi–sugar mixtures: circle, trehalose; triangle, sucrose; diamond, glucose; square, sorbitol. Open and closed symbols indicate T'_{g1} and T'_{g2}, respectively. The sugar content of a commercial frozen cod surimi was assumed to be 32.2% (dry matter basis), and the T'_{g1} (cross) and T'_{g2} (asterisk) were plotted.

transitions could not be observed. From the sugar content effects on the T'_{g1} and T'_{g2} of the carp surimi–sugar mixtures, the T'_{g1} and T'_{g2} of the surimi without sugar was roughly evaluated to be −55 to −65 °C and −15 °C, respectively. The sugar content of the commercial cod surimi was assumed to be 31.4%, and the T'_{g1} and T'_{g2} of the cod surimi were plotted. The T'_{g1} and T'_{g2} of the cod surimi were in a reasonable temperature range in comparison with those of the carp surimi–sugar mixtures.

3.2. Glass transition properties of freeze-dried surimi–trehalose mixtures

DSC thermograms for freeze-dried carp surimi–trehalose mixtures of varying moisture content (2.5–47.9% w/w) are shown in Fig. 5. The samples containing 2.5–16.2% (w/w) moisture had a clear glass transition, and the T_g of each sample was determined. It was found that the T_g decreased significantly with an increase in the moisture content. This result is ascribed to the plasticizing effect of water. The sample containing 47.9% (w/w) moisture showed ice-nucleation during the pre-cooling process (data not shown), and thus T'_{g1} and T'_{g2} were observed before ice-melting, as shown in Fig. 5. These results are summarized as a state diagram of the surimi-moisture pseudo-binary system in Fig. 6. The T_g-curve in Fig. 6 was obtained by fitting the Gordon–Taylor equation (Eq. (1)) to the T_g data.

$$T_g = \frac{W_{(s)}T_{g(s)} + kW_{(w)}T_{g(w)}}{W_{(s)} + kW_{(w)}}, \quad (1)$$

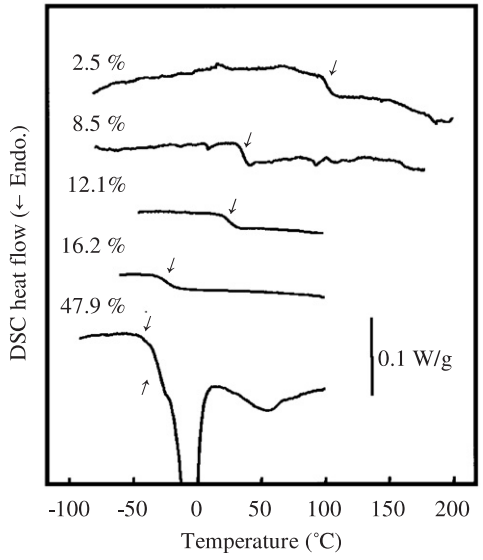

Fig. 5. DSC thermograms for freeze-dried carp surimi–trehalose mixtures (80% trehalose in dry matter) of varying residual moisture. Down-arrows shown in the DSC thermograms for samples containing 2.5–16.2% (w/w) moisture indicate T_g. Down-arrow and up-arrow shown in the DSC thermogram for a sample containing 47.9% (w/w) moisture indicate T'_{g1} and T'_{g2}, respectively.

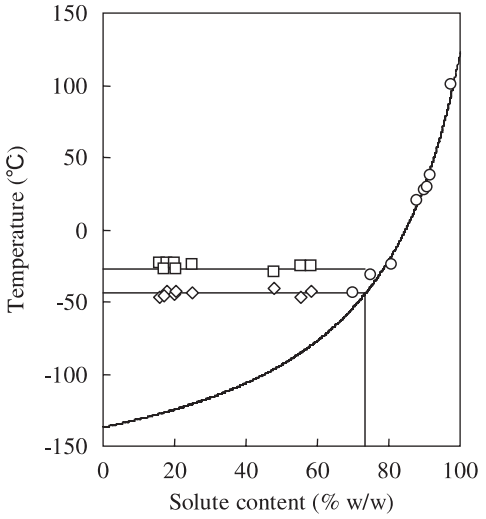

Fig. 6. State diagram of surimi-moisture pseudo-binary system. The surimi consists of carp surimi and 80% trehalose in dry matter. The symbols of circle, diamond, and square are T_g, T'_{g1}, and T'_{g2}, respectively. The T_g-curve was obtained by fitting the Gordon–Taylor equation to the T_g-data.

Table 1
The values of $T_{g(s)}$, k, and C'_g of a carp surimi-trehalose mixture and trehalose

Formulation	$T_{g(s)}$ (°C)	k	C'_g (% w/w)	
Surimi–trehalose	123	5.0	73.5	This study
Trehalose	107	6.5	81.6	Roos (1995)
	115	7.5	—	Crowe et al. (1996)

where $T_{g(s)}$ and $T_{g(w)}$ are T_g of the pure solute (anhydrous surimi–trehalose mixture) and pure water (the unit is Kelvin), $W_{(s)}$ and $W_{(w)}$ are the mass fraction of the solute and water, and k is a constant depending on the system, respectively. The value of k corresponds to resistance to a decrease in T_g induced by the plasticizing effect of water; the larger the value of k, the greater the moisture content dependence of T_g. The value of $T_{g(w)} = 136$ K (ca. −137 °C) was obtained from a previous publication (Angell et al., 1994), and $T_{g(s)} = 396$ K (ca. 123 °C) and $k = 5.0$ were obtained as fitting parameters. Maximally freeze-concentrated solute content (C'_g) was determined to be 73.5% (w/w) as the solute content at a crossover temperature between the T'_{g1} and T_g-curve. The value of C'_g corresponds to the amount of unfrozen water in the maximally freeze-concentrated glassy state; the less the value of C'_g, the more the amount of the unfrozen water. The parameters characterizing the state diagram, $T_{g(s)}$, k, and C'_g, are listed in Table 1, along with values for trehalose obtained in previous studies (Crowe, Reid, & Crowe, 1996; Roos, 1995).

4. Discussion

4.1. Glass transition of frozen surimi products

Our investigations of carp surimi–sugar mixtures indicated the presence of two freeze-concentrated glass transitions; this is similar to many other aqueous systems. The T'_{g1} and T'_{g2} of each mixture are shown in Fig. 4. It was found that the T'_{g1} and T'_{g2} of carp surimi–sugar mixtures depended strongly on the type and content of sugar. At each sugar content, the T'_{g1} and T'_{g2} were, from highest to lowest, trehalose, sucrose, glucose, and sorbitol. As mentioned above, it is thought that the higher the T'_{g1} and T'_{g2}, the lower the molecular mobility at a reference temperature. The finding that the T'_{g1} and T'_{g2} of the surimi–trehalose mixtures were higher than those of the other mixtures suggests that trehalose has a greater cryostabilizing effect on surimi than the other sugars do. Zhou et al. (2006) investigated the effects of trehalose on the storage stability of frozen surimi samples and stated that the stabilizing effect of trehalose was greater than those of other solutes (sucrose–sorbitol mixture and sodium lactate).

The mixtures containing sugar at concentrations of less than 30.0% (dry matter basis) showed no apparent glass transition. This means that the endothermic shift due to glass transition was outside the detectable limits. The magnitude of the endothermic shift depends mainly on heat capacity change at glass transition (ΔC_p) and thermal histories (e.g., rates of pre-cooling and re-heating and annealing). It is known that the ΔC_p reflects the freezing degree of conformational freedom induced by glass transition, and the ΔC_p of water, protein, and their mixture is much less than that of sugar (Angell et al., 1994; Green et al., 1994). Consequently, it is thought that the ΔC_p of surimi–sugar mixtures decreased with the decrease in sugar content.

Since the relaxation accompanying a glass transition is from non-equilibrium to equilibrium states, the magnitude of the endothermic shift due to a glass transition is affected by thermal histories. For example, the magnitude of the endothermic shift increases with an increase in the rates of pre-cooling and re-heating; Sartor et al. (1995) disclosed the T'_{g1} of hydrated globular proteins by using DSC measurement at the pre-cooling rate of up to ~2700 °C/min and at the re-heating rate of 30 °C/min. In addition, annealing, which means isothermal holding treatment, can distinguish glass transition from overlapped thermal events in the DSC thermogram; Brake and Fennema (1999) demonstrated that cod and mackerel showed $T'_{g2} = -7$ to -14 °C by using annealing treatment. In this study, only a customary DSC scanning (pre-cooling at 6 °C/min and re-heating at 3 °C/min) was employed. In order to detect experimentally the T'_{g1} or T'_{g2} of surimi–sugar mixtures containing sugar at concentrations less than 30% (dry matter basis), further study will be required.

From the sugar content effects on the T'_{g1} and T'_{g2} of carp surimi–sugar mixtures, the T'_{g1} and T'_{g2} of the surimi were roughly evaluated to be -55 to -65 °C and -15 °C, respectively. These values are in reasonable agreement with those of fish muscles: $T'_{g1} = -77$ to -87 °C for cod (Nesvadba, 1993), $T'_{g1} = -63$ to -73 °C for tuna (Agustini et al., 2001; Inoue & Ishikawa, 1997), and $T'_{g2} = -7$ to -14 °C for cod and mackerel (Brake & Fennema, 1999). In addition, there are some similar reports: $T'_{g1} = -73$ to -103 °C (Sartor & Johari, 1996) and $T'_{g2} = -12$ °C (Brake & Fennema, 1999) for beef muscle, and $T'_{g1} = -67$ to -87 °C and $T'_{g2} = -9$ to -15 °C for many types of protein (Chang & Randall, 1992). As mentioned above, the origin of T'_{g1} and T'_{g2} is not completely understood, and thus various interpretations are proposed. It is beyond the scope of this paper to discuss the origin of T'_{g1} and T'_{g2}, and thus it is noted that the carp surimi will show $T'_{g1} = -55$ to -65 °C and $T'_{g2} = -15$ °C at this stage.

The commercial cod surimi showed $T'_{g1} = -65$ °C and $T'_{g2} = -22$ °C; these are reasonable values in comparison with those of carp surimi–sugar mixtures, as seen in Fig. 4. From the result, it is suggested that the difference of fish stock does not greatly affect the T'_{g1} and T'_{g2} of surimi-sugar mixtures. The commercial frozen cod surimi will be in the rubbery state at a typical frozen storage temperature, -18 °C. As mentioned above, various degradations (crystallization of cryostabilizers and grows of ice) occur significantly at temperatures above T'_{g2} (Chang & Randall, 1992; Levine & Slade, 1988; Wang, 2000), and thus the myofibrillar proteins in surimi will be damaged during storage. For cryostabilization of the surimi product, it is thought that the storage temperature should be reduced at least to -22 °C due to the T'_{g2}. Furthermore, it is noted that a part of unfrozen water crystallizes in the temperature range between T'_{g1} and T'_{g2} (Pyne et al., 2003; Roos, 1995). Although storage at temperature below -65 °C due to T'_{g1} is impracticable from the viewpoint of economic problem, it is noted that the crystallization of unfrozen water may bring chemical and physical damages to myofibrillar proteins in surimi during long-term storage at temperature above the T_{g1}.

4.2. Glass transition of freeze-dried surimi products

The moisture content dependence of the T_g of the freeze-dried carp surimi–trehalose mixtures was summarized as a state diagram of the surimi-moisture pseudo-binary system (Fig. 6). A state diagram is useful as a practical guide in the production of freeze-dried foods. For example, an optimal pathway of freeze-drying is proposed as follows: the drying temperature should be maintained consistently higher than the T_g-curve by only a few degrees in order to prevent structural collapse, and the residual moisture should be reduced to the moisture content at which the T_g is above ambient temperature. The state diagram, thus, has been reported as for many freeze-dried foods: fruits (Moraga, Martínez-Navarrete, & Chiralt, 2006; Silva, Sobral, & Kieckbusch, 2006; Telis & Sobral, 2001), vegetable (Telis & Sobral, 2002), and marine products (Rahman, Kasapis,

Guizani, & Al-Amri, 2003; Sablani, Kasapis, Rahman, Al-Jabri, & Al-Habsi, 2004). To our knowledge, the present study is the first to present a possible state diagram of surimi-moisture pseudo-binary systems.

Three parameters characterizing the state diagram, $T_{g(s)}$, k, and C'_g, are listed in Table 1 with those of trehalose. The $T_{g(s)}$ of the surimi–trehalose mixture was slightly higher than that of trehalose; this is because surimi plays the role of anti-plasticizer to trehalose. The k and C'_g of the surimi–trehalose mixture were lower than those of trehalose; the surimi–trehalose mixture has greater resistance to a decrease in T_g induced by the plasticizing effect of water and a greater amount of unfrozen water in the maximally freeze-concentrated glassy state than trehalose does. These observations suggest that the glassy properties of trehalose were affected by intermolecular interaction between surimi and trehalose. The trehalose content dependence of T_g-curves of surimi–trehalose mixtures was not investigated in this study. A foreseeable extension of this research would involve understanding the glass transition properties of freeze-dried surimi as a state diagram of a surimi–trehalose–water pseudo-ternary system.

5. Conclusion

The glass transition properties of frozen and freeze-dried surimi products were investigated in this study. It was demonstrated that the frozen surimi samples showed two freeze-concentrated glass transitions at −42 to −65 °C and at −21 to −41 °C, depending on the types and content of sugar. The moisture content dependence of the T_g of freeze-dried surimi–trehalose mixtures was summarized as T_g-curve in the state diagram. From the viewpoint of "glass transition" concept, it is expected that these results are useful in the production and storage of frozen and freeze-dried surimi products.

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