ELSEVIER

Effect of sugars and sugar alcohols on freezing behavior of corn starch gel as monitored by time domain ^{1}H NMR spectroscopy

Y.-R. Kim[a], B.-S. Yoo[b], P. Cornillon[c], S.-T. Lim[d],*

[a]Whistler Center for Carbohydrate Research, Department of Food Science, Purdue University, West Lafayette, IN 47907, USA
[b]Department of Food Technology, Dongguk University, Seoul 100-715, South Korea
[c]Danone Vitapole, Technovaleur-Responsable du groupe, Modélisation/Simulation, RD 128, F-91767 Palaiseau Cedex, France
[d]Graduate School of Life Sciences and Biotechnology, Korea University, 1,5-Ka, Anam-dong, Sungbuk-ku, Seoul 136-701, South Korea

Abstract

The freezing behavior of corn starch gels (40% starch solids) with 11 different sugars and sugar alcohols (10 or 30% based on starch weight) was investigated using time domain ^{1}H NMR spectroscopy. As estimated from the liquid signal intensity curve, the ice melting temperature linearly decreased and the relative amount of solid glass formed increased with the molar concentration of the added sugars. The observed ice melting temperatures were low (approximately $-10\,°C$ for non-sugar gel), because of the non-equilibrium state produced by rapid cooling (20 °C/min) and the large sample size. Sugar addition (up to 30%) depressed the ice melting temperature of the gel system by more than 10 °C. Sugar addition and rapid-cooling promoted solid glass formation in the freeze-concentrated solution with a consequent reduction in liquid mobility. The unfrozen liquid levels resolved from the relative liquid signal intensity data were not colligatively governed, showing a dependence on sugar structure. Among the sugars tested, the unfrozen liquid level produced by a sugar corresponded in the order: hexose > pentose, isomaltose > sucrose > trehalose > maltose. The sugar alcohols exhibited higher unfrozen liquid levels than did the corresponding sugars.
© 2003 Elsevier Ltd. All rights reserved.

Keywords: Starch gels; Ice melting temperature; NMR spectroscopy; Sugars; Glass transition

1. Introduction

Non-equilibrium ice formation commonly occurrs when commercial food products are frozen (Roos, 1995). The water to ice transition in a food matrix creates a freeze-concentrated phase with dissolved solutes, from which the substantial osmotic effect depresses the freezing temperature of the residual water. This process results in the formation of an amorphous freeze-concentrated liquid phase at a temperature far below that of ice formation. Further decrease in temperature increases the viscosity of the unfrozen amorphous phase, and the kinetic restriction due to the limited water mobility may transform the liquid phase into a solid glass (Levine & Slade, 1988; Roos, Karel, & Kokini, 1996).

The glass transition is often considered to be a major thermal characteristic responsible for physical and chemical changes occurring in the freeze-concentrated phase. Storing a food in a frozen state at a temperature below its glass transition temperature minimizes deleterious changes. The relationships between cryostability and glass transition temperature are well discussed in terms of the mobility of the solutes and water by Levine and Slade (1988).

In addition to the storage temperature, freezing rate is another important variable related to the cryostability (Reid, 1990). Rapid freezing promotes uniform crystallization throughout the product and produce smaller ice crystals, which thus minimizes stress-caused structural deterioration (Goff, 1992). Therefore, evaluating the transition temperatures of the amorphous glass phase and molecular mobility will help to control the stability of frozen food products.

Nuclear magnetic resonance (NMR) is one of the most powerful techniques for determining molecular mobility and dynamic molecular interactions. Many researchers have demonstrated the usefulness of the NMR relaxation

* Corresponding author. Tel.: +82-2-3290-3435; fax: +82-2-927-5201.
E-mail address: limst@korea.ac.kr (S.-T. Lim).

properties in relation to molecular mobility, availability of water, and transition temperatures for food carbohydrates (Ablette, Darke, Izzard, & Lillford, 1993; Auh, Kim, Cornillon, Yoon, Yoo, & Park, 2003; Chinachoti, Kim-Shin, Mari, & Lo, 1991; Chinachoti, White, Lo, & Stengle, 1991; Hanafusa, 1985; Kalichevsky, Jaroszkiewicz, & Blanshard, 1993; Kim & Cornillon, 2001; Kou, Molitor, & Schmidt, 1999; Lelievre & Mitchell, 1975; Le Meste, 1995; Lim, Setser, Paukstelis, & Sobczynska, 1992; Rasanen, Blanshard, Mitchell, Derbyshire, & Autio, 1998). Le Meste (1995) and Ablett et al. (1993) have reported that the mobility of water in glassy materials measured by T_2 relaxation time was relatively high, not sensitively affected by the physical state of surrounding medium.

Carbohydrate is one of the major constituents in foods, and plays significant roles in forming food matrices. Phase transitions in carbohydrate substantially influence the physical properties and stability of food matrices (Roos, 1995). Low molecular weight sugars or sugar alcohols are often added to food items to give plasticizing or cryostabilizing effects (Kalichevsky & Blanshard, 1993; Kalichevsky et al., 1993; Levine & Slade, 1988; Slade & Levine, 1995; Wang & Jane, 1994). Most of the researches to investigate the effect of sugars on freezing behavior have been carried out on model solutions. Little work has so far been carried out on a starch gel at high solute concentrations.

In this study, the effect of various sugars and sugar alcohols with different chemical structures on the freezing behavior of a corn starch gel was examined using ^{1}H NMR, in terms of phase transition temperature, the availability of liquid components, and the formation of solid glass.

2. Materials and methods

2.1. Materials

Normal type corn starch (about 25% amylose) was provided by Samyang Genex Company (Seoul, Korea). Eleven sugars and sugar alcohols were used for this study: monosaccharides (ribose, xylose, glucose, and fructose), disaccharides (sucrose, maltose, isomaltose and trehalose), and sugar alcohols (xylitol, maltitol and sorbitol). All the sugars and sugar alcohols were reagent-grade.

2.2. Sample preparation

Distilled water was added to a weighed corn starch to make 40% (dry solids) starch–water mixture. Sugars or sugar alcohols were added to the dispersion at a solid weight ratio of 1:10 or 3:10 based on starch (dry weight). The mixture was stirred for 5 min until sugars (or sugar alcohols) were dissolved, and then heated in a boiling water-bath while stirring continuously until homogeneous dough was formed. A portion (2.4 g) of the dough was transferred into a glass NMR sample cup (15 mm O.D. × 25 mm H.), and

then autoclaved (at 121 °C for 20 min) in a pressure-resistant vial. Immediately after the autoclaving, the sample cup was slid into a NMR tube (18 mm O.D.), and NMR measurements were performed. In order to prevent moisture loss during the measurements, the sample cup was covered with plastic wrap and the NMR tube was sealed with a rubber stopper.

2.3. NMR measurements

NMR relaxometry was performed using a Maran bench-top NMR spectrometer (Resonance Instruments Ltd, Witney, UK) running at 15 MHz with a variable temperature (VT) control system. A computer-based NMR software, provided by the manufacturer, was used for pulse sequencing and data acquisition. Compressed dry air was used as a cooling or heating medium, which passed through a cooling unit (FTS systems, Stone Ridge, NY) and then a VT control system, and was purged into the bottom of the NMR tube in a magnet module. First, samples were cooled down to −40 °C, and the Free Induction Decay (FID) and the spin–spin relaxation (T_2) curves were obtained as temperature increased stepwise to 3 °C. At each temperature, samples were equilibrated for 30 min to 1 h. All measurements were conducted continuously during the experiments using a built-in Visual Basic programming tool. All the measurements were duplicated. For the FID, a single 90° pulse was used, and the number of acquisition points and dwell time were 8192 and 0.5 μs, respectively. The Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence (90°-[τ-180°-τ]$_n$-) was used for the spin–spin relaxation time measurement. An interpulse time (τ) of 100 μs was used, and 4096 echoes were collected for each T_2 decay curve. For both experiments, the relaxation delay was 1 s, and the number of scans for signal averaging was 64.

2.4. Data analyses

The FID curves were analyzed to estimate the amplitude of liquid signal in the starch gel sample. Since solid protons decay much faster (within ~70 μs) than liquid protons, signals left after the solid decay should be from the liquid protons. As shown in Fig. 1, the total liquid signal intensity was estimated by extrapolating the liquid part of the FID curve at time zero, using an exponential decay function:

$$M = M_0 \exp\left(-\frac{t}{T_2^*}\right) \tag{1}$$

where t is time, T_2^* is the relaxation time for FID, and M_0 is maximum signal intensity (i.e. M at $t = 0$). The obtained liquid signal intensities were further used for estimating the proportion of unfrozen water. NMR signal intensity is a function not only of the number of protons
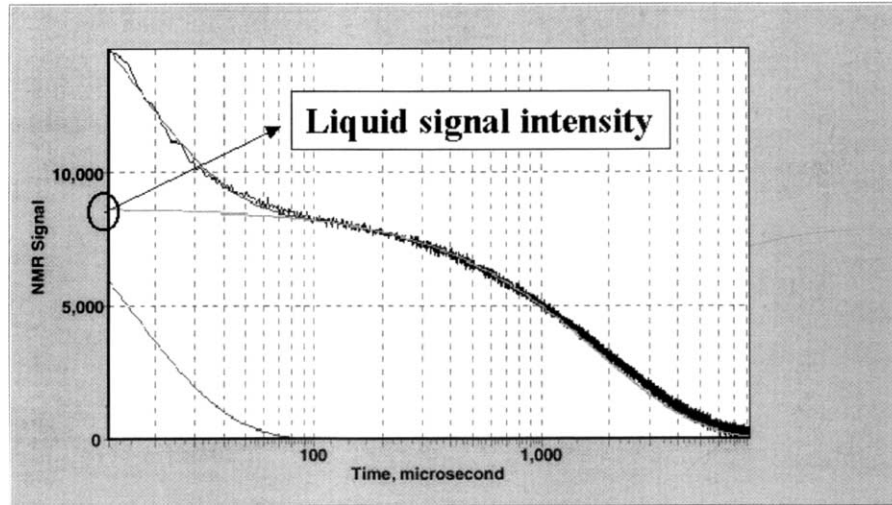
Fig. 1. Estimation of liquid signal intensity from FID curve.

but also of temperature, according to Curie's law:

$$M_0 = \frac{B_0 N \gamma^2 h^2 I(I+1)}{3kT} \qquad (2)$$

where M_0 is magnitude of magnetization (signal intensity), N is number of protons, γ is the gyromagnetic ratio (for proton, $\gamma(^1H) = 2.6752 \times 10^8 \, T^{-1} \, s^{-1}$), h is Planck constant $(6.626 \times 10^{-34} \, J \, s)$, I is spin quantum number (for proton, $I = 1/2$), k is Boltzmann constant $(1.38 \times 10^{-23} \, J \, K^{-1})$, T is temperature (K), and B_0 is magnetic field strength (Tesla). The signal intensity (M_0) is proportional to the number of spins (N) and inversely proportional to temperature (T) (Fukushima & Roeder, 1981). Since, for given experimental conditions, all the parameters are constant except N and T, the above equation can be rewritten as the following equation:

$$M_0 T = AN \qquad (3)$$

where A is a constant. Now, the new parameter, $M_0 T$, is temperature-corrected and a function of the number of protons (N) only. At above $0\,°C$ (1 and $3\,°C$ in this study), the $M_0 T$ values for a liquid signal were almost constant, which indicated the maximum amount of liquid protons in the sample. As temperature decreased below freezing point, the $M_0 T$ value decreased due to the loss of liquid signal caused by ice formation. The ratio of the $M_0 T$ at any temperature below freezing point to that above $0\,°C$ was defined as the Relative Liquid Signal Intensity (RLS), which represented the proportion of the liquid protons left unfrozen below freezing point.

The T_2 relaxation curves were analyzed to calculate the spin–spin relaxation times (T_2). Eq. (1) was also used for T_2 curve fitting, and the calculated time constant was T_2 instead of T_2^*.

3. Results and discussion

3.1. Ice melting temperature

Figs. 2–4 show the RLS of the frozen starch gels containing different types and amounts of the sugars or sugar alcohols, as the gel temperature increased. The proportion of liquid (RLS in figures) for starch gels containing sugars changed continuously even at the freezing temperature range (boxes in figures). The intensity gradually increased as temperature increased. The sugar-free starch gel, however, showed almost constant intensity in the freezing temperature range (up to about $-10\,°C$). When sugars were present, the onset for rapid increase of the liquid signal occurred at lower temperatures. The onset of the liquid signal increase was considered as an indication of ice melting (T'_m). The lowered onset by sugar addition evidenced that the sugar depressed the ice melting temperature. The sugar-induced changes in liquid signal intensity while the gel was frozen and the depression of ice melting temperaure were more substantial when more sugar was added (30 vs. 10%). In the RLS and ice melting temperature, there were some differences among the sugars and sugar alcohols tested.

Not all the protons composing the NMR liquid signal (Fig. 1) were necessarily from water, even though, in no doubt, water protons were major contributors to the total liquid signal, considering the composition of the starch gel samples used in this study. Therefore, the lowered liquid signal intensity with sugar addition did not necessarily mean that the amount of unfrozen water was also lowered. It could be rather related to the change in the mobility of sugar protons, possibly due to glass transition.

Similar trends were observed in the spin–spin relaxation times (T_2), as measured by using CPMG pulse sequence (Figs. 5 and 6). Due to the relatively long interpulse time
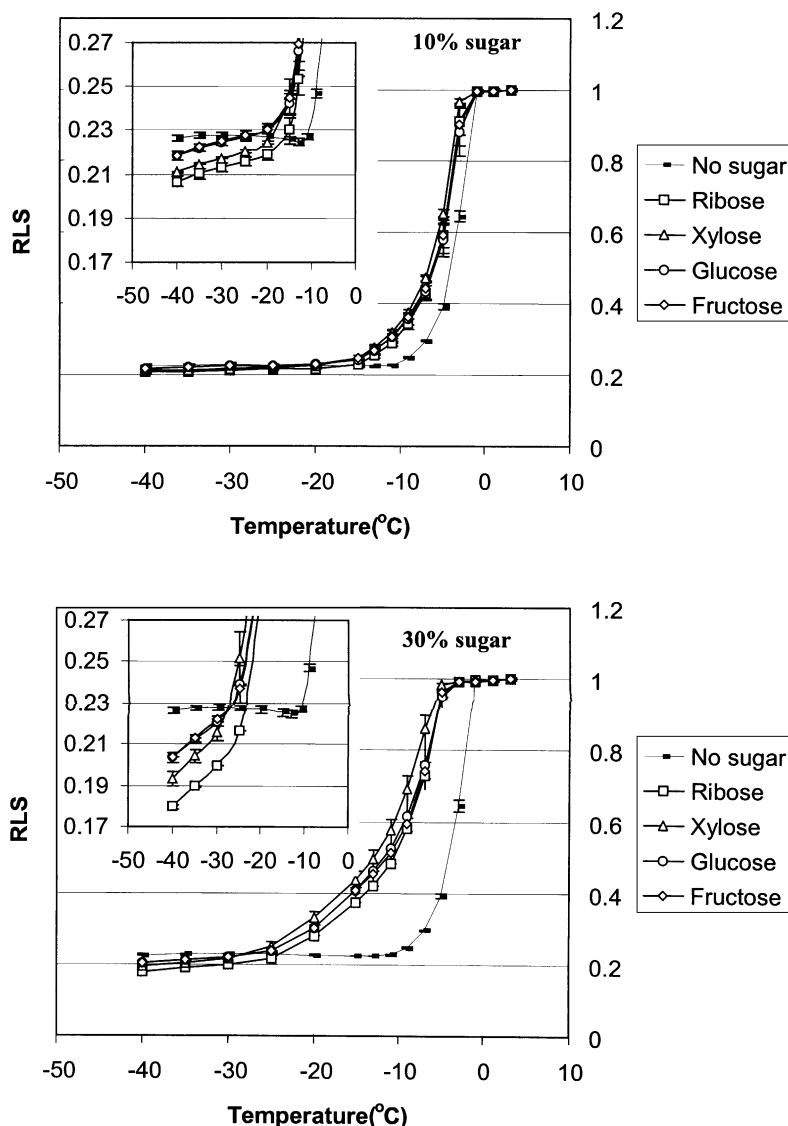
Fig. 2. Effect of sugars (monosaccharides) on liquid signal intensity of frozen starch gel.

($2\tau = 200 \, \mu s$) in CPMG pulse sequence compared to solid proton relaxation time ($<$ca. 20 μs), all the T_2 relaxation times measured in this experiment were considered to be exclusively for liquid protons. When ice melted, the T_2 increased rapidly as observed in Figs. 5 and 6. The onset temperatures for the T_2 increase agreed well with those for the liquid signal intensity (RLS) increase, measured from FID (Figs. 2–4).

The ice melting temperatures (T'_m) measured from the liquid signal intensity data were plotted against the molar concentration of the sugars and sugar alcohols added (Fig. 7). It was found that there was a linear and negative relation between the ice melting temperature and the molar concentration of sugars. Also, it appeared that disaccharides were slightly more effective in depressing the ice melting temperature than the monosaccharides, and hexoses (glucose and fructose) exhibited

lower melting temperatures than pentoses (xylose and ribose). In our previous work, using a differential scanning calorimeter (DSC) with the same starch–sugar samples, it was also observed that ice melting temperature decreased as proportional to molar concentration of sugar, and the difference among the sugars were minor (Baek, Yoo, & Lim, 2003).

Because the sample size for the analysis (2.4 g) was significantly larger compared to that used for the DSC measurement, non-uniformity in local temperature and ice formation inside the sample was expected. The considerably lower temperature for ice melting measured by NMR in comparison with that from DSC measurements (ca. -10 to $-2 \, ^{\circ}C$ for the non-sugar sample) might be caused by these differences in the samples. However, in the freezing process commercially practiced for processed foods, a moderately rapid cooling in bulk sizes is normally applied. Therefore,
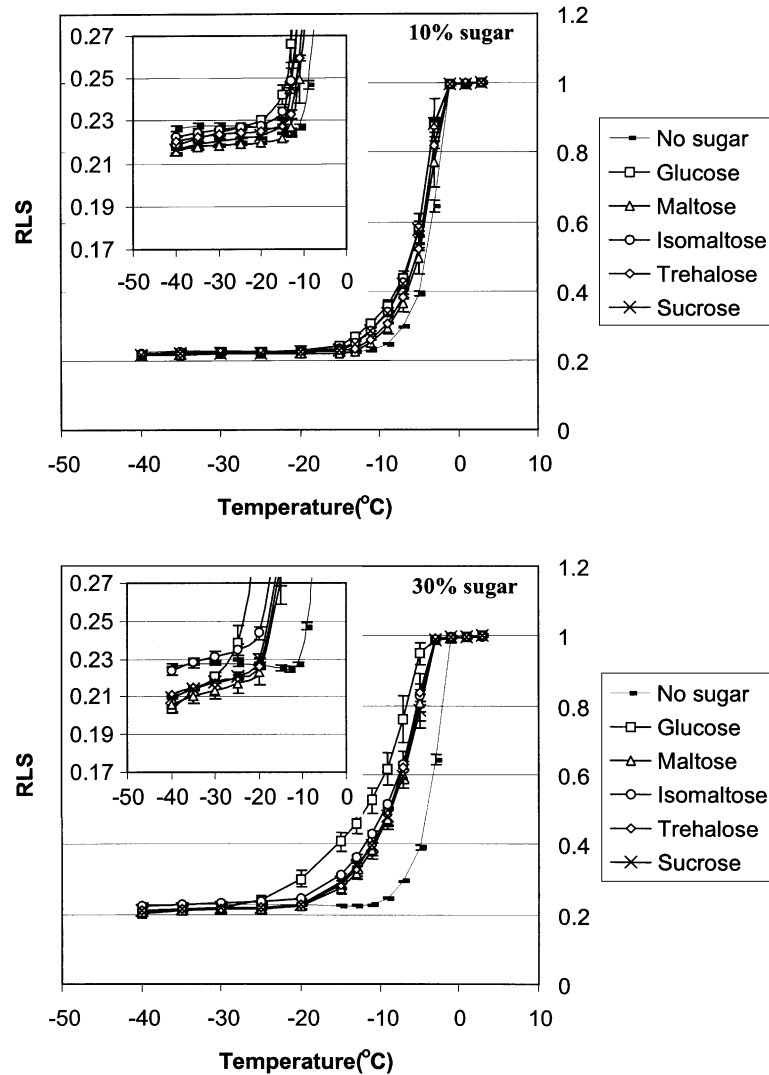
Fig. 3. Effect of sugars (disaccharides) on liquid signal intensity of frozen starch gel.

the experimental results from the present NMR measurements could be more applicable to the common freezing practice for foods.

3.2. Glass transition of frozen starch gel

The amount of unfrozen water in carbohydrate solutions, estimated by the enthalpy of endothermic ice melting peak in DSC thermograms, consistently showed an increasing trend as solute concentration increased (Izzard, Ablett, & Lillford, 1991; Kawai, Sakurai, Inoue, Chujo, & Kobayashi, 1992; Roos, 1995). The liquid signal intensity of the starch gels, measured by ^{1}H NMR in this experiment (Figs. 2–4), however, appeared to show an opposite trend. The starch gels containing sugars showed the higher liquid signal intensity than the sugar-free sample above ice melting temperatures (T'_m) (approximately $> -15\,°C$ for 10% sugar, and $-25\,°C$ for 30% sugar). However, below T'_m, the liquid signal intensity was higher for the sugar-free sample

(boxes in Figs. 2–4), and it further decreased as sugar concentration increased to 30%. Hanafusa (1985) and Auh et al. (2003) reported the similar trend for protein–sugar solutions, a decrease in the amount of unfrozen liquid with sugar addition measured by ^{1}H NMR. One possible explanation for these ^{1}H NMR results could be the further decrease in liquid signal due to the solid glass formation.

In order to examine the glass transition effect on NMR liquid signal intensity, a starch gel containing xylose (10%) was frozen at a lower cooling rate (about 0.4 °C/min) and compared with the rapidly frozen gel. The liquid signal intensity (RLS) and T_2 results are given in Fig. 8. Since the slower freezing promoted more ice formation than the rapid freezing (Roos, 1995), the liquid signal intensity and T_2 were expected to decrease by decreasing the freezing rate. However, the experimental data showed an opposite trend. The rapid cooling resulted in the lower liquid signal intensity and T_2 as presented in Fig. 8. This indicated that the ice formation would not be a single factor to affect
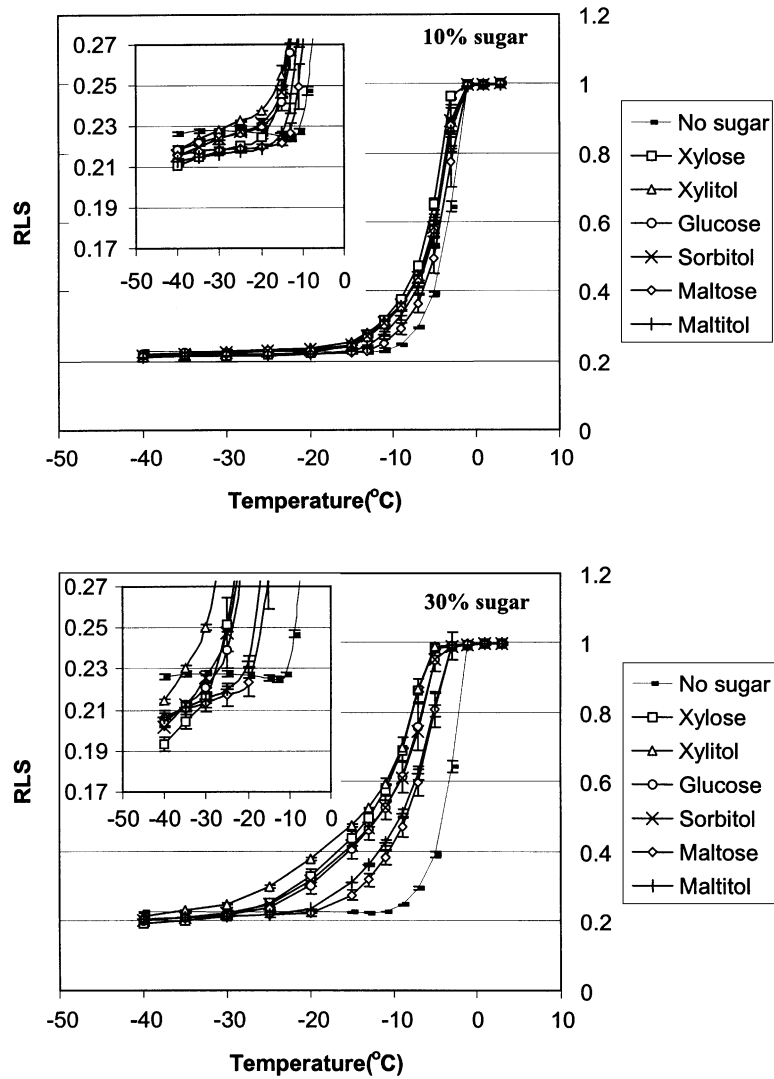
Fig. 4. Effect of sugar alcohols on liquid signal intensity of frozen starch gel.
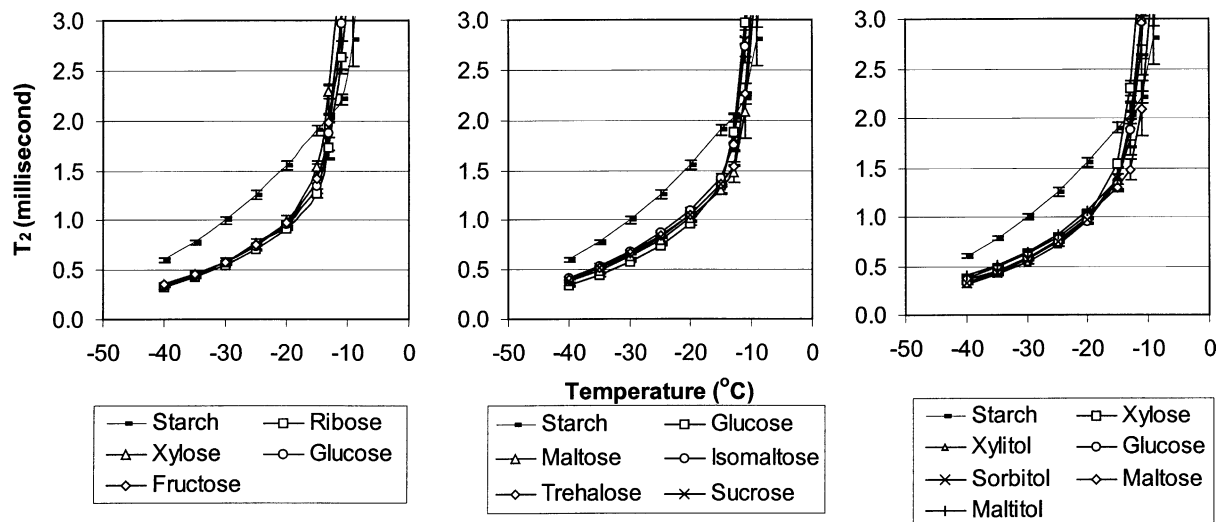

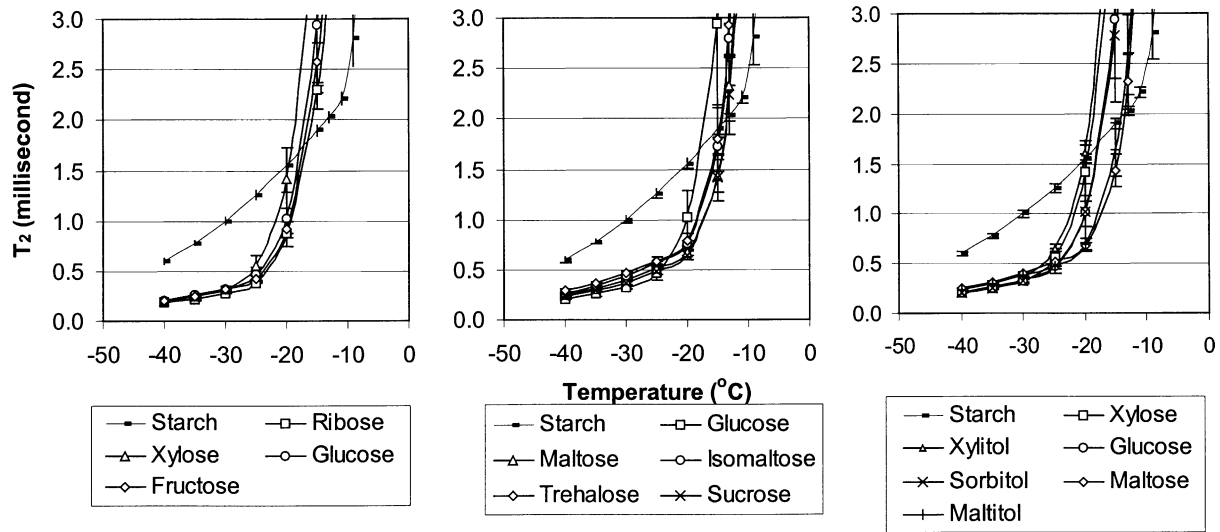Fig. 5. Effect of sugars (or sugar alcohols) on T_2 relaxation time of starch gel (10% sugar) during freezing.

Fig. 6. Effect of sugars (or sugar alcohols) on T_2 relaxation time of starch gel (30% sugar) during freezing.

the NMR liquid signal intensity. There should be a glass transition for each sample below the ice melting temperature, although the transition temperature was not obvious in Figs. 2–4. It was hypothesized that the changes induced by the glass transition of the freeze-concentrated phase was the major reason for the lowered liquid signal intensity by sugar addition.

It has been reported that the mobility of water in a material was not sensitively affected by glass transition, showing a relatively high mobility even in glassy materials (Ablett et al., 1993; Le Meste, 1995; Roos et al., 1996). In fact, as shown in Figs. 2–4, about 20% of the total liquid signal was remained in all frozen samples even at $-40\,^{\circ}\mathrm{C}$. Those signals might be from the unfrozen and still mobile water protons in the glassy state. The T_2 relaxation times for the samples at $-40\,^{\circ}\mathrm{C}$ was 200–300 μs, as presented in Figs. 5 and 6. These T_2 relaxation times also indicated that there still existed liquid-like protons at $-40\,^{\circ}\mathrm{C}$, even though their mobility was considerably restricted.

Therefore, a more reasonable explanation for the liquid signal intensity depression by the sugar addition would be a loss of the liquid-like protons of sugars caused by the solid glass formation. Starch gel without sugar did not show significant difference in the liquid signal intensity at $-40\,^{\circ}\mathrm{C}$ between the slow and rapid cooling (data not shown). The dependency of NMR liquid signal intensity on the cooling rate indicated that the dissolved sugar protons in the system were involved in NMR liquid signal, and their mobility was altered between liquid-like and solid-like on passing through glass transition. Accordingly, dramatic viscosity increases below T_g (five orders of magnitude for the 20 °C temperature interval) have been reported with the low molecular weight carbohydrates, which were thus classified as 'fragile' materials (Noel, Ring, & Whittam, 1990; Simatos, Blond, & Perez, 1995).

The glass transition temperature of a freeze-concentrated phase (T_g') in gelatinized starch or high molecular weight carbohydrates has been reported to be very close to
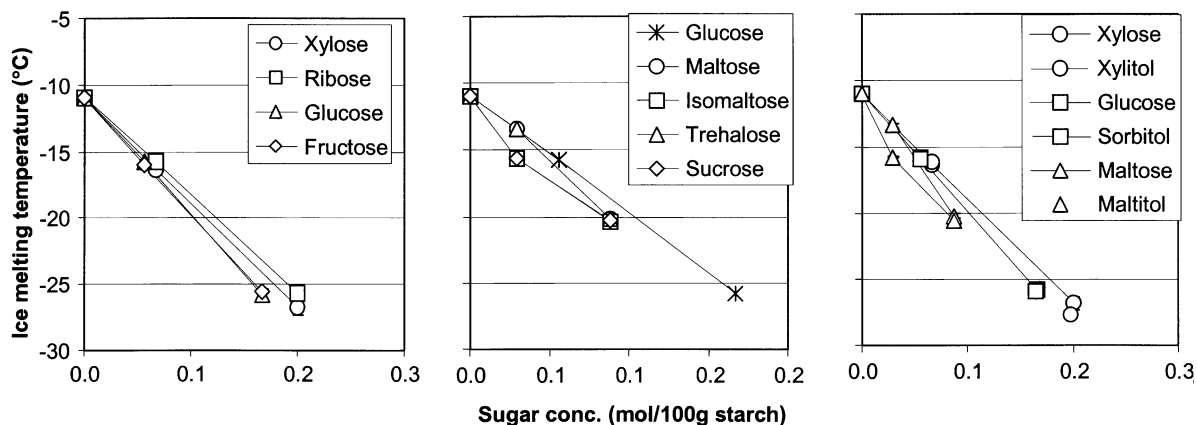

Fig. 7. Effect of sugars (or sugar alcohols) on ice melting temperature of starch gel as measured from onset of RLS increase.
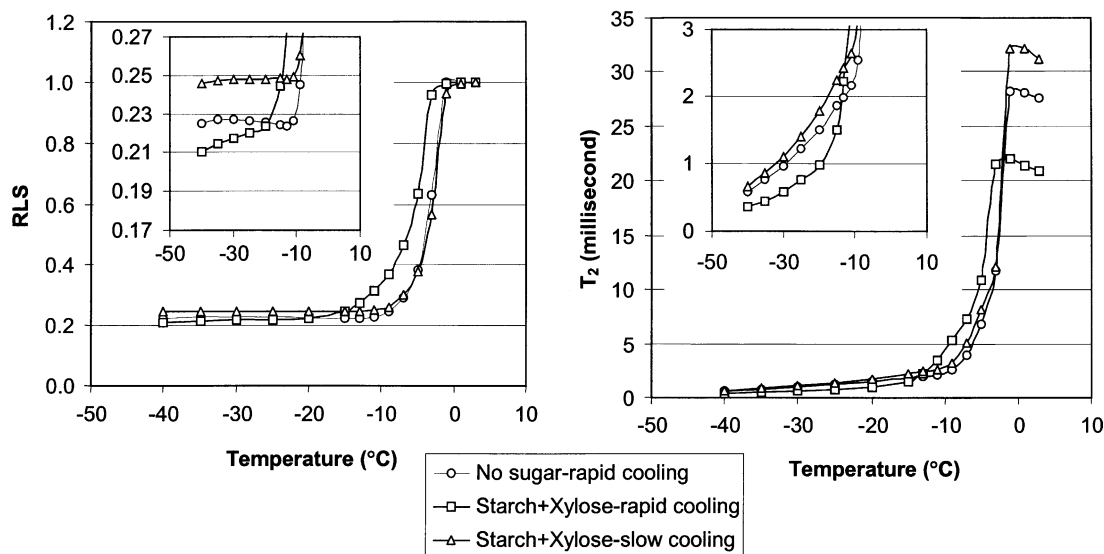
Fig. 8. Effect of freezing rate on relative liquid signal intensity and T_2 relaxation time of starch gel.

the ice melting (Roos, 1995; Roos & Karel, 1991). In a separate experiment with DSC, the sugar addition up to 30% as in this experiment depressed the T'_g as low as $-15\,°C$, whereas the counterpart of no sugar exhibited the T'_g of $-7.0\,°C$. The depression of T'_g was proportional to the molar concentration of sugar regardless of sugar structure (Baek et al., 2003). Therefore, the smooth and early increase of the liquid signal intensity for the sugar-added samples (Figs. 2–4) could be attributed to the glass transition temperature lowered by the sugar presence. Also, the large sample size used for the NMR measurement could generate local heterogeneity in ice formation and glass transition, which might consequently cause broadening of the glass transition.

If the liquid signal intensity change in the frozen state was due to the glass transition of sugars, difference in the liquid signal intensity (ΔRLS) between $-40\,°C$ and ice

melting temperature (Figs. 2–4) could be related to the amount of solid glass formed at $-40\,°C$. It should be noted that the ΔRLS value does not indicate the actual amount or proportion of solid glass in each sample, because the weight proportions of protons are different among water, sugars, and other soluble compounds, and it is difficult to evaluate which and how much molecules are involved in the total liquid signal. However, it could be qualitatively stated that the larger ΔRLS (between ice melting temperature and any temperature below that) indicates the more glass transition occurred below ice melting temperature. As shown in Fig. 9, the relative solid glass amount increased with molar concentration of sugar. It was also revealed that the sugar alcohols tested provided higher levels of solid glass than the counterpart sugars. This behavior could be related to the amount of supercooled freeze-concentrate in a starch polymer network.
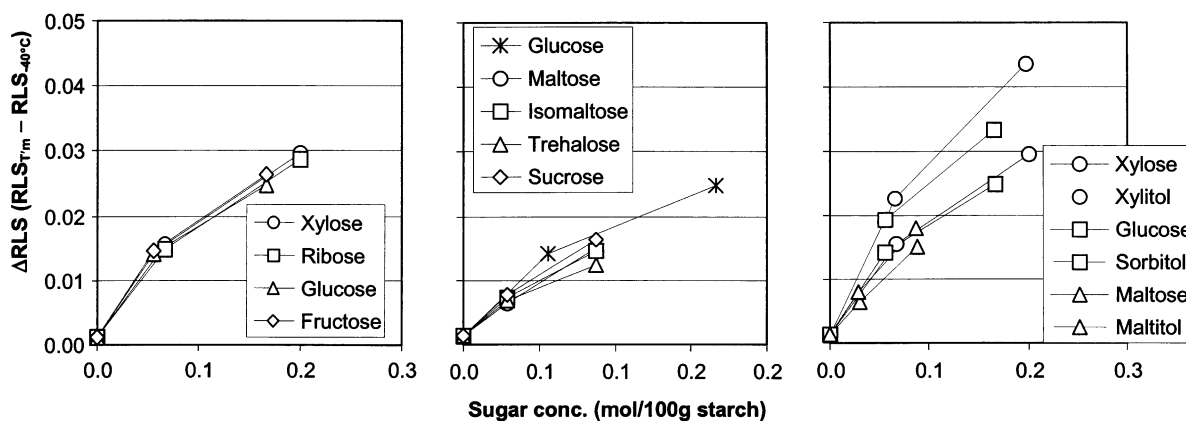

Fig. 9. Effect of sugars (or sugar alcohols) on relative amount of solid glass formed in starch gel at -40 °C, measured from the difference between liquid signal intensities at -40 °C ($RLS_{-40\,°C}$) and those at ice melting temperatures ($RLS_{T'm}$).

3.3. Unfrozen liquid levels in frozen starch gel

The changes in the RLS above ice melting temperatures could be mainly due to ice melting. Therefore, the relative liquid signal intensities at ice melting temperatures ($RLS_{T'm}$) could be related to the maximum liquid signal reduction mainly due to ice formation, even though the liquid signal intensity at ice melting temperature contained not only unfrozen water proton signal but also liquid-like sugar proton signal. By examining $RLS_{T'm}$, it was possible to compare the effects of different sugars (or sugar alcohols) on the relative unfrozen liquid level as given in Fig. 10, assuming that, with the same solute concentration, sample size, and cooling rate, the local heterogeneity could be similar for all the samples. The data revealed that the relative unfrozen liquid level depended on sugar structure. Frank (1985) has stated that the amount of unfreezable water does not show a simple dependence on molecular weight of solute. In the present experiment, hexoses provided more unfrozen liquid level in the starch gels than pentoses tested, with ribose showing the least unfrozen liquid level (Fig. 10). In comparison between sugars and the corresponding sugar alcohols, sugar alcohols provided the higher level of unfrozen liquid in the starch gel than the counterpart sugars did. The additional hydroxyl group at the anomeric carbon allowed more water molecules to interact, and the open structure for sugar alcohols provided more structural flexibility than the ring structure of sugars. Among the tested sugar alcohols, maltitol contained a glucosyl ring linked to sorbitol whereas xylitol and sorbitol were fully open. This might explain why maltitol showed no significant difference in the unfrozen liquid level compared with the maltose, whereas other two sugar alcohols did. Among the disaccharides tested, isomaltose showed the highest liquid level, and maltose showed the least. The alpha-1,6 anhydrous glycosidic linkage in isomaltose provides more angular freedom, whereas maltose has more rigidity due to the intermolecular hydrogen bond (C-2$'$ and C-3) between the adjacent glucose residues. The occupation of hydroxyl groups in the hydrogen bond may result in the reduced chance to interact with water. Thus, the availability of hydroxyl groups as well as molecular flexibility, which were provided more to isomaltose than to maltose, could be the main attributes to the higher level of unfrozen water in isomaltose-containing system.

3.4. Stability of starch–sugar gels upon freezing

The amount and size of ice crystals formed during freezing have been known to affect the structure and texture of freeze-thawed food matrix. Slow freezing causes lager ice crystal formation, which results in structural damage in food matrix (Goff, 1992). As shown in Fig. 8, after the ice was melted ($>0\ °C$), T_2 relaxation time of the slowly frozen starch–xylose gel ended up a much higher value (~ 30 ms) than that of the rapidly frozen gel (~ 20 ms). Because of the larger ice crystals formed by slow freezing, more water was supposed to be released from the polymer matrix during melting, resulting in the higher water mobility under NMR spectroscopy.

Solid glass formation in frozen foods has been reported to be advantageous in frozen storage by retarding water crystallization (Goff, 1992; Noel et al., 1990). As presented in Fig. 9, the amount of solid glass formation was proportional to the sugar concentration. As more solid glass was formed, the mobility (T_2) of unfrozen liquid protons was accordingly lowered (Figs. 5,6 and 8). However, the relative amount of unfrozen liquid was not simply determined colligatively. Also, transition temperatures and the amount of glass and ice formed were largely influenced by the kinetics. In other words, in commercial freezing of food materials, the stability also depends on both cooling rate and sample size, which affects the freezing kinetics.
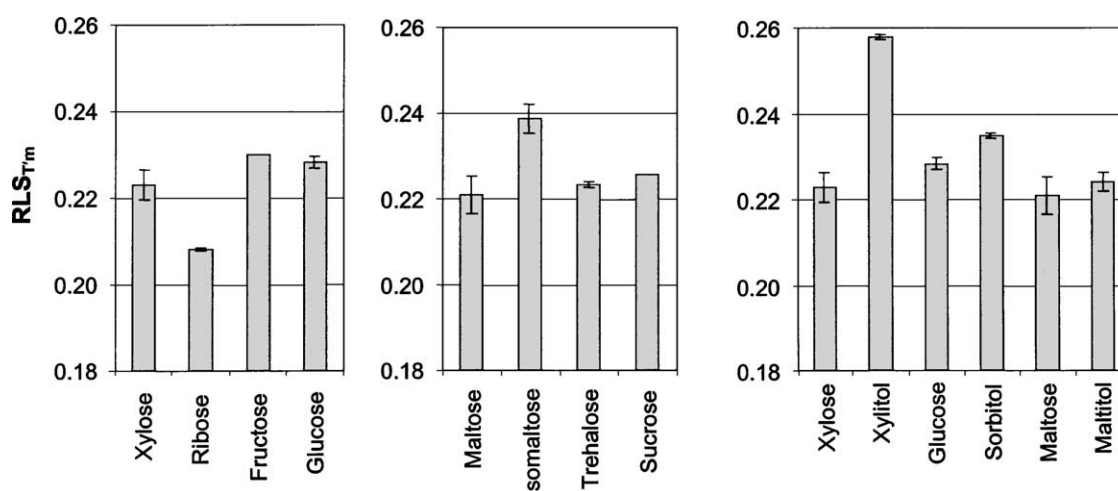

Fig. 10. Effect of sugars (or sugar alcohols) on relative liquid signal intensities of starch gel (30% sugar) at ice melting temperatures ($RLS_{T'm}$).

4. Conclusions

NMR technique well resolved the freezing behavior of the starch gels containing sugars or sugar alcohols, in terms of ice melting temperature, amount of ice and solid glass formed, and amount and mobility of liquid components. There was additional decrease of NMR liquid signal due to the solid glass formation of the sugars in freeze-concentrated phase. The solid glass formation also caused a decrease in liquid mobility. The ice melting and solid glass formation depended on molar concentration of sugar solutes. The NMR analysis with rapid freezing to mimic the commercial freezing for food products revealed that the effectiveness of the low molecular weight sugars or sugar alcohols as cryoprotectants by depressing the ice melting temperature and by increasing the solid glass content. The unfrozen liquid level depended on sugar structures. The availability of the hydroxyl groups and structural flexibility in sugar molecules appeared to be one of the attributes in determining the unfrozen liquid level.

Acknowledgements

References

Ablett, S., Darke, A. H., Izzard, M. I., & Lillford, P. (1993). Studies of the glass transition in malto-oligomers. In M. V. Blanshard, & P. J. Lillford (Eds.), *The Glassy State in Foods*. Loughborough: Notthingam University Press.

Auh, J. H., Kim, Y. R., Cornillon,, Yoon, P. J., Yoo, S. H., & Park, K. H. (2003). Cryoprotection of protein by highly concentrated branched oligosaccharides. *International Journal of Food Science and Technology*, 38(5), 553–563.

Baek, M. H., Yoo, B., & Lim, S.-T. (2003). Effect of sugars and sugar alcohols on thermal transition of corn starch gel during cold storage. *Food Hydrocolloids*, [in press].

Chinachoti, P., Kim-Shin, M., Mari, F., & Lo, L. (1991). Gelatinization of wheat starch in the presence of sucrose and sodium chloride: Correlation between gelatinization temperature and water mobility as determined by oxygen-17 nuclear magnetic resonance. *Cereal Chemistry*, 68(3), 245–248.

Chinachoti, P., White, V. A., Lo, L., & Stengle, T. R. (1991). Application of high-resolution carbon-13, oxygen-17, and sodium-23 nuclear magnetic resonance to study the influences of water, sucrose, and sodium chloride on starch gelatinization. *Cereal Chemistry*, 68(3), 238–244.

Frank, F. (1985). Complex aqueous systems at subzero temperatures. In D. Simatos, & L. Multon (Eds.), *Properties of water in foods*. Dordrecht: Martinus Nijhoff.

Fukushima, E., & Roeder, S. B. W. (1981). *Experimental pulse NMR*. Massachusetts: Eddison-Wesely Publishing Company, Inc.

Goff, H. D. (1992). Low-temperature stability and the glassy state in frozen foods. *Food Research International*, 25(4), 317–325.

Hanafusa, N. (1985). The interaction of hydration water and protein with cryoprotectant. *Refrigeration Science and Technology*, 1(1), 59–64.

Izzard, M. J., Ablett, S., & Lillford, P. J. (1991). Calorimetric study of the glass transition occurring in sucrose solutions. In E. Dickinson (Ed.), *Food polymers, gels, and colloids*. Cambridge: The Royal Society of Chemistry.

Kalichevsky, M. T., & Blanshard, J. M. V. (1993). The effect of fructose and water on the glass transition of amylopectin. *Carbohydrate Polymers*, 20(2), 107–113.

Kalichevsky, M. T., Jaroszkiewicz, E. M., & Blanshard, J. M. V. (1993). A study of the glass transition of amylopectin-sugar mixture. *Polymer*, 34(2), 346–358.

Kawai, H., Sakurai, M., Inoue, Y., Chujo, R., & Kobayashi, S. (1992). Hydration of oligosaccharides: anomalous hydration ability of trehalose. *Cryobiology*, 29(5), 599–606.

Kim, Y., & Cornillon, P. (2001). The effect of temperature and mixing on molecular mobility in wheat dough. *Lebensmittel Wissenschaft und Technologie*, 34(7), 417–423.

Kou, Y., Molitor, P. F., & Schmidt, S. J. (1999). Mobility and stability characterization of model food systems using NMR, DSC, and conidia germination techniques. *Journal of Food Science*, 64(6), 950–959.

Lelievre, J., & Mitchell, J. (1975). A pulsed NMR study of some aspects of starch gelatinization. *Starch/Staerke*, 27, 113–117.

Le Meste, M. (1995). Mobility of small molecules in low and intermediate moisture foods. In G. V. Barbosa-Canovas, & J. Welti-Chanes (Eds.), *Food preservation by moisture control*. Lancaster: Technomics.

Levine, H., & Slade, L. (1988). Principles of cryostabilization technology from structure property relationships of carbohydrate/water systems—a review. *Cryo Letters*, 9(1), 21–63.

Lim, H., Setser, C. S., Paukstelis, J. V., & Sobczynska, D. (1992). ^{17}O nuclear magnetic resonance studies on wheat starch-sucrose-water interactions with increasing temperature. *Cereal Chemistry*, 69(4), 382–386.

Noel, T. R., Ring, S. G., & Whittam, M. A. (1990). Glass transitions in low-moisture foods. *Trends in Food Science and Technology*, 1(3), 62–67.

Rasanen, J., Blanshard, J. M. V., Mitchell, J. R., Derbyshire, W., & Autio, K. (1998). Properties of frozen wheat doughs at subzero temperatures. *Journal of Cereal Science*, 28(1), 1–14.

Reid, D. S. (1990). Optimizing the quality of frozen foods. *Food Technology*, 44(7), 78–82.

Roos, Y. (1995). *Phase transitions in foods*. San Diego: Academic Press.

Roos, Y., & Karel, M. (1991). Water and molecular weight effects on glass transitions in amorphous carbohydrates and carbohydrate solutions. *Journal of Food Science*, 56(6), 1676–1681.

Roos, Y. H., Karel, M., & Kokini, J. L. (1996). Glass transitions in low moisture and frozen foods: Effects on shelf life and quality. *Food Technology*, 50(11), 95–108.

Simatos, D., Blond, G., & Perez, J. (1995). Basic physical aspects of glass transition. In G. V. Barbosa-Canovas, & J. Welti-Chanes (Eds.), *Food preservation by moisture control*. Lancaster: Technomics.

Slade, L., & Levine, H. (1995). Polymer science approach to water relationships in foods. In G. V. Barbosa-Canovas, & J. Welti-Chanes (Eds.), *Food preservation by moisture control*. Lancaster: Technomics.

Wang, Y.-J., & Jane, J. (1994). Correlation between glass transition temperature and starch retrogradation in the presence of sugars and maltodextrins. *Cereal Chemistry*, 71(6), 527–531.