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Freezing Behaviors of Frozen Foods Determined by ^{1}H NMR and DSC

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Abstract The freezing patterns of commercial frozen foods were characterized by using proton nuclear magnetic resonance (^{1}H NMR) relaxometry and differential scanning calorimetry (DSC). The liquid-like components like unfrozen water were investigated as a function of temperature (10 to –40°C) and then compared with the unfrozen water content measured by DSC. The formation of ice crystals and the reduction of water in the foods during freezing were readily observed as a loss of the NMR signal intensity. The proton NMR relaxation measurement showed that the decreasing pattern of the liquid-like components varied depending on the samples even though they exhibited the same onset temperature of ice formation at around 0°C. When compared with the unfrozen water content obtained by the DSC, the NMR and DSC results could be closely correlated at the temperature above –20°C. However, the distinct divergence in the values between 2 methods was observed with further decreasing temperatures probably due to the solid glass formation which was not detected by DSC.

Keywords: nuclear magnetic resonance (NMR), differential scanning calorimetry (DSC), unfrozen water, frozen food

Introduction

Freezing has been widely used as a mean to preserve foods at household and industrial levels. Reduction of available water coupled with the formation of ice crystals at subzero temperatures, provides an environment that favors retarded microbial growth and undesirable physical and chemical reactions. It is recognized that a variety of different physicochemical processes occur during the freezing process, including ice formation, freeze-concentration, and phase transitions (1,2). Besides, a number of detrimental physical and biochemical reactions by the growth of ice crystals and moisture migration take place during frozen storage and may be accentuated if proper processing and handling conditions are not maintained. Consequently, they lead to the deterioration in the quality attributes of frozen foods. It is thus imperative to monitor the quality of frozen foods during freezing and frozen storage.

As the freezing progresses, ice crystals grow and the solute concentration increases, eventually forming a maximally freeze-concentrated solution matrix. The unfrozen matrix exists as a kinetically metastable amorphous solid below the glass transition temperature, which is a major factor responsible for the physical changes of food components under frozen conditions (3,4). Therefore, the storage of frozen foods at or below the glass transition temperature, or formulation of food product to raise the glass transition temperature above storage temperature, enhances the storage stability of the frozen foods by maintaining the unfrozen phase in an amorphous solid matrix (glassy state) surrounding the ice. Thus, the stability of frozen foods could be well explained in terms of the mobility of the unfrozen phase. Consequently, the characterization of the freezing behaviors of foods by focusing on their unfrozen mobile phase would be helpful in minimizing the quality loss of frozen foods.

The phase behavior in a frozen system has been commonly studied using thermal analysis techniques like the differential scanning calorimetry (DSC) because of its ease of operation and widespread availability. However, the DSC measurement is sometimes problematic for some glass-forming materials, so that other techniques also have been reported to characterize a phase behavior. Especially, many researchers have demonstrated the usefulness of nuclear magnetic resonance (NMR) relaxation properties in relation to molecular mobility, availability of water, and transition temperatures (5-8). However, the most effective approach when studying the freezing behavior in a food system is in combination of various analysis techniques complimenting each other.

In this study, 4 typical commercial frozen foods (regular and low fat ice creams, frozen bread dough, frozen baked bread) were selected and their freezing behaviors were measured by ^{1}H NMR and DSC in terms of unfrozen phase (liquid-like component). Then, the NMR and DSC responses were correlated each other.

Materials and Methods

Sample preparations Food samples (regular and low fat ice creams, raw bread dough, and frozen baked bread) were purchased at a local store. Their water contents were measured by using an oven drying method (9). The contents (%) of moisture, carbohydrate, protein, and fat in each sample are presented in Table 1.

NMR measurements Based on the previous study (10), all NMR experiments were carried out with a Maran NMR

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Table 1. Compositions of food samples

Sample	%Water	%Carbo.	%Protein	%Fat
Regular ice cream	56.5	20.8	4.7	17.0
Low fat ice cream	59.9	28.3	5.0	5.0
Raw bread dough	42.0	45.5	7.3	4.5
Baked bread	36.7	49.6	7.9	5.0

spectrometer with a variable temperature (VT) controller (Resonance Instruments, Witney, UK) running at 15 MHz. Each sample (3 g) was placed into a glass NMR tube, labeled, and then inserted into the NMR probe. The samples were cooled down to −40°C and then warmed up stepwise to 10°C. For the free induction decay (FID) measurement, the samples were equilibrated at each temperature for 30 min to obtain the steady FID signal intensity. A single 90° radiofrequency pulse was then applied with the relaxation delay of 2 sec and the dwell time of 0.5 μsec. The number of acquisition points was 8,192 and the FID signals were averaged over 16 scans.

Since NMR signal intensity is functions of not only proton density but also temperature, further manipulation of data is required to correlate the signal intensity only with the number of proton. According to Curie's law (11), the signal intensity (M_0) is proportional to the number of proton (N) and inversely proportional to the temperature (T). In other words, the product of M_0 and T should be a function of N only as follows;

$M_0 \times T = A \times N$ (A is a proportional constant)

While the values of $M_0 \cdot T$ are hardly changed above freezing point, they are reduced during freezing due to the loss of liquid signals by ice formation.

By using a multiple exponential data analyzing software, WinFit (Resonance Instruments), the obtained FID signals were separated into 2 components (liquid and solid signal) and the liquid portion was extrapolated to time zero in order to obtain the liquid signal intensity (M_0) as shown in Fig. 1. The product of M_0 and T at any temperature below freezing point was then divided by that above 0°C in order to estimate the proportion of liquid-like components in samples.

DSC measurements DSC 2920 (TA Instruments, Inc., New Castle, DE, USA) was used to measure the proportion of unfrozen water. DSC was calibrated with indium (156.6°C, 28.591 J/g) and an empty pan was used as a reference. Samples were weighed (10 mg) and hermetically sealed in aluminum pans. Temperature was gradually decreased at 2°C/min, simulating slow freezing in a refrigerator, and then, standard DSC experiments were run from −40 to 10°C with a ramp speed of 5°C/min. The DSC results were further analyzed using Universal Analysis software (TA Instruments, Inc.). After the total enthalpy of ice melting was obtained from DSC endothermic peaks, a perpendicular drop analysis was conducted to extract the information of the enthalpy of ice fusion at particular temperatures, giving the proportion of unfrozen water.

Results and Discussion

Freezing behavior measured by ^{1}H NMR The proportions of liquid-like components in ice creams, dough, and bread were measured over temperatures (10 to −40°C) by using NMR. As can be seen in Fig. 2, the proportion of liquid-like components remained constant above 0°C and then decreased abruptly with a further decrease in the temperatures up to around −20°C, which implied the onset of freezing process, that is, the formation of ice crystals and the consequent reduction of liquid water. After the ice formation, the amount of liquid-like components gradually decreased through further concentration of the frozen matrix. However, the proportions of liquid-like components at subzero temperatures varied depending on the samples. Compared to raw bread dough and baked bread, the decrease in the amount of liquid-like components was more pronounced for ice creams. Approximately 90% of the liquid-like components in ice creams were frozen at −40°C whereas only 70% of them were frozen for dough and bread. In addition, the overall increase in the proportion of liquid-like components was observed in raw bread dough, compared to the baked bread. It might be due to higher water content in the raw bread dough, making more unfrozen phase. It is also known that fat may contribute to the signal intensity from liquid-like protons (12). Nonetheless, no noticeable difference in the proportion of liquid-like components was observed between ice cream samples. It was probably posited to be due to less contribution of fat

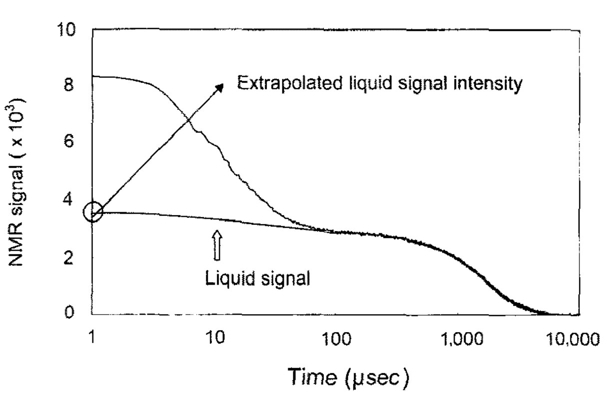

Fig. 1. A typical data fitting of FID curve for estimating liquid signal intensity.

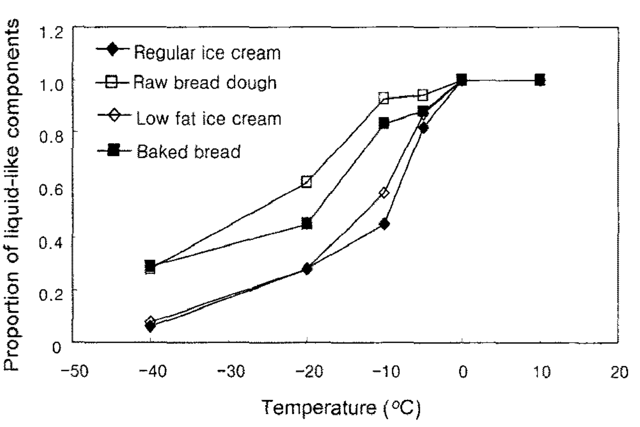

Fig. 2. Proportion of liquid-like components of food samples during freezing.

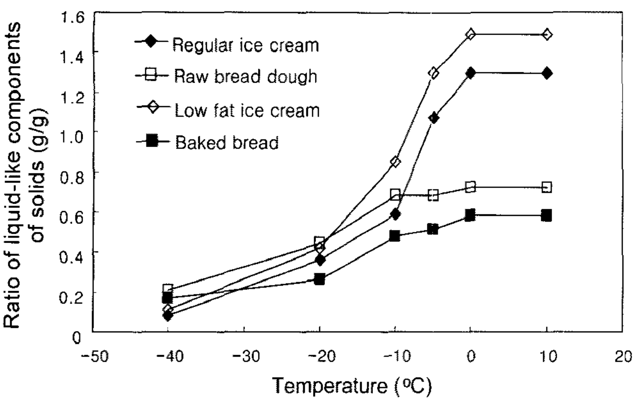

Fig. 3. Ratio of liquid-like components to solid of food samples during freezing.

to NMR signals in ice creams at low temperatures.

It is recognized that ^{1}H NMR measurement is limited to relatively mobile liquid-like protons. Thus, the signal intensity is proportional to the number of protons present in liquid phase. Therefore, the signals measured in this study appeared to have the relaxation time constants greater than hundreds of microseconds, which fell within the relaxation time range of liquids (13). Figure 2 suggests that the liquid components in the bread dough and baked bread were not readily frozen compared to those in the ice creams probably due to higher interaction between water and surrounding macromolecules.

Figure 3 presents the ratio of liquid-like components to solids for the ice creams, raw bread dough, and baked bread. Due to high amount of initial water content, the ice cream samples exhibited greater values at high temperatures. However, the decrease rate of the amount of liquid-like components per solids was distinctly different between the ice cream and bread samples. That is, more abrupt decreases with decreasing temperatures were observed in the ice creams than raw bread dough and baked bread. It suggests that the liquid-like components in the dough and bread samples were less temperature-dependent than those in ice creams. One of the possible explanations would be related to high solid contents of bread dough and baked bread, which could give more chances of liquid-like components to interact with the solids. Thus, their association with the solids could retard the mobility reduction, giving more NMR signals to the dough and bread samples as also shown in the Fig. 2.

Comparison of NMR an DSC results Figure 4 shows the comparison of the proportions of liquid-like components and unfrozen water obtained by NMR and DSC, respectively. Generally, there was a close correlation between the NMR and DSC results for ice creams in the range of temperatures above −20°C. However, they significantly diverged with further decreasing temperatures. A similar trend was observed in the bread dough samples except that the NMR and DSC data started to deviate at around −10°C. However, the proportion of liquid-like components (NMR) and unfrozen water (DSC) could be correlated well in the ice creams, relatively compared to the dough and bread samples.

The discrepancy between the amount of liquid-like components measured by NMR and unfrozen water measured by DSC was attributed to the solid glass formed during freezing, which was not considered in unfrozen water estimation by DSC. Non-equilibrium ice formation commonly occurs when commercial food products are frozen (14). The water to ice transition in a food matrix creates a freeze-concentrated phase with dissolved solutes. This process results in the formation of an amorphous freeze-concentrated liquid phase at a temperature far below

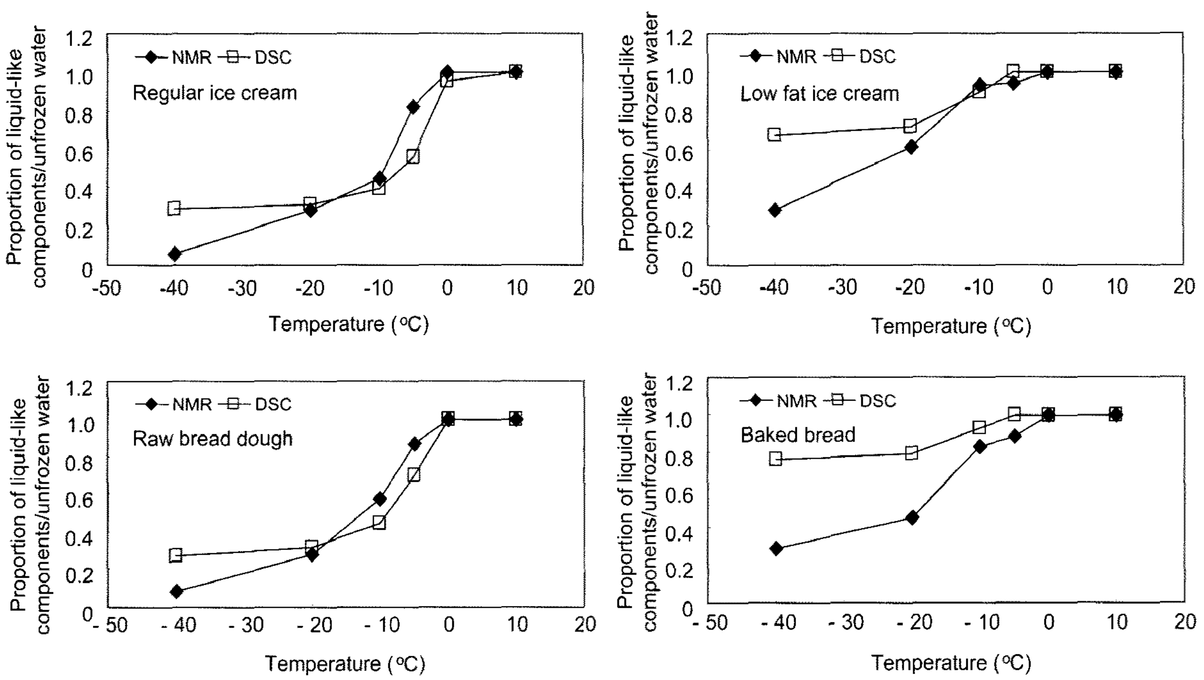

Fig. 4. Comparison of liquid-like components and unfrozen water.

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 (15,16). 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 (15). Undesirable quality losses in frozen foods such as enzymatic reactions and ice recrystallization may be minimized by maintaining the unfrozen phase in the glassy state. Hence, the knowledge of the unfrozen phase mobility in frozen foods would provide a great help in formulating, processing, and storing frozen foods to promote their shelf life. Therefore, the study on liquid-like components by NMR could be practically more important when the mobility of liquid phase is concerned in a food system.

Acknowledgments

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References

1. Goff HD, Sahagian ME. Glass transitions in aqueous carbohydrate solutions and their relevance to frozen food stability. Thermochim. Acta 280: 449-464 (1996)
2. Matveev YI, Ablett S. Calculation of the C'g and T'g intersection point in the state diagram of frozen solutions. Food Hydrocolloid 16: 419-422 (2002)
3. Wang YJ, Jane J. Correlation between glass-transition temperature and starch retrogradation in the presence of sugars and maltodextrins. Cereal Chem. 71: 527-531 (1994)
4. Goff HD. Low-temperature stability and the glassy state in frozen foods. Food Res. Int. 25: 317-325 (1992)
5. Auh J-H, Kim Y-R, Cornillon P, Yoon J, Yoo S-H, Park K-H. Cryoprotection of protein by highly concentrated branched oligosaccharides. Int. J. Food Sci. Tech. 38: 553-563 (2003)
6. Kim Y-R, Cornillon P. Effects of temperature and mixing time on molecular mobility in wheat dough. Lebensm.-Wiss. Technol. 34: 417-423 (2001)
7. Kou Y, Molitor PF, Schmidt SJ. Mobility and stability characterization of model food systems using NMR, DSC, and conidia germination techniques. J. Food Sci. 64: 950-959 (1999)
8. Lee S, Cornillon P, Kim Y-R. Spatial investigation of the non-frozen water distribution in frozen foods using NMR SPRITE. J. Food Sci. 67: 2251-2255 (2002)
9. AACC. Approved Methods of the AACC. 9th ed. Method 44-15A. American Association of Cereal Chemists, St. Paul, MN, USA (1995)
10. Kim Y-R, Yoo B-S, Cornillon P, Lim S-T. Effect of sugars and sugar alcohols on freezing behavior of corn starch gel as monitored by time domain H-1 NMR spectroscopy. Carbohyd. Polym. 55: 27-36 (2004)
11. Fukushima E, Roeder SBW. Experimental Pulse NMR. A Nuts and Bolts Approach. Addison-Wesely Publishing Company, Inc., Reading, MA, USA. pp. 125-126 (1981)
12. Kerr WL, Kauten RJ, McCarthy MJ, Reid DS. Monitoring the formation of ice during food freezing by magnetic resonance imaging. Lebensm.-Wiss. Technol. 31: 215-220 (1998)
13. Harz H-P, Weisser H, Liebenspacher F. Frozen food by nuclear magnetic resonance (NMR) spectroscopy. pp. 129-135. In: Technical Innovations in Freezing and Refrigeration of Fruits and Vegetables. Meeting of Commissions C2, D1, D2/3 of I.I.R. 9.-12. Juli. Davis, CA, USA (1989)
14. Roos Y. Phase Transition in Foods. Academic Press, San Diego, CA, USA. pp. 99-103 (1995)
15. Levine H, Slade L. Principles of cryostabilization technology from structure property relationships of carbohydrate water-systems-A review. Cryo-Lett. 9: 21-63 (1988)
16. Roos YH, Karel M, Kokini JL. Glass transitions in low-moisture and frozen foods: Effects on shelf life and quality. Food Technol. -Chicago 50: 95-108 (1996)