

Ice Recrystallization in Sucrose Solutions Stored in a Temperature Range of -21°C to -50°C

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The recrystallization of ice crystals in sucrose solution was investigated by cryo-SEM in a temperature range of -21°C to -50°C , including temperatures around T_g' . By using the technique of image analysis, the mean radius of the ice crystals was evaluated and recrystallization rates were calculated by a kinetic equation based on the Ostwald ripening principle. As the storage temperature decreased, a rapid decline in recrystallization rate was observed between -29°C and -35°C , which was consistent with the concept of glass transition of the freeze-concentrated matrix. Even at -50°C , at which the freeze-concentrated matrix was considered to be in glassy state, an increase in the mean crystal size was observed after 20 hr storage.

Keywords : recrystallization, ice crystal, cryo-SEM, glass transition, T_g' , T_m'

Introduction

The recrystallization of ice crystals is a cause of deterioration in many frozen desserts during storage and distribution. Generally, recrystallization is characterized by an increase in the mean size of ice crystals with storage time (Fennema, 1973 ; Hartel, 1998). In the case of ice cream, the growth of ice crystals often brings about a coarse, grainy and icy texture, resulting in unacceptable characteristics (Hartel, 1998 ; Hartel, 2001). Therefore, for proper design of storage and distribution process of frozen desserts, the recrystallization process must be well understood. Many studies of ice recrystallization have been conducted using frozen desserts and corresponding model systems as samples. Sutton *et al.*, (1996a) investigated the effects of storage temperature (-10 to -30°C) on the recrystallization rate of fructose solution. Hartel and co-workers performed quantitative analysis of recrystallization in ice creams to investigate the effects of storage temperature, temperature oscillations, sweeteners, and stabilizers on the recrystallization rate (Donhowe and Hartel, 1996 ; Hagiwara and Hartel, 1996 ; Miller-Livney and Hartel, 1997). The mechanism of inhibition of ice recrystallization by the addition of stabilizers has been also discussed (Hagiwara and Hartel, 1996 ; Miller-Livney and Hartel, 1997 ; Regand and Goff, 2003 ; Carrington *et al.*, 1996 ; Bollinger *et al.*, 2000 ; Goff *et al.*, 1999 ; Martin *et al.*, 1999 ; Sutton *et al.*, 1996b ; Sutton *et al.*, 1997 ; Sutton *et al.*, 1998), although the exact mechanisms have not yet been clarified (Hartel, 2001).

Despite the extent of the research, the range of storage temperatures examined in most of the previous studies was limited ; the lowest storage temperatures tested in

these studies were around -30°C . Since frozen storage is now available at much lower temperatures, it is strange that there are few reports dealing with recrystallization at lower storage temperatures. Experimental data on recrystallization at lower temperatures may be useful in the consideration of suitable conditions for long-term storage of frozen desserts. Furthermore, experiments at lower temperatures are interesting from the point of view of the the glass transition temperature of maximally freeze-concentrated solute matrix, T_g' . Levine and Slade (1988, 1991) have postulated that a frozen food is stable below its T_g' , because the movement of the reactants that cause deterioration is strongly restricted. There have been several reports confirming this hypothesis in the case of enzymatic reactions (Lim and Reid, 1991 ; Agustini *et al.*, 2001 ; Agustini *et al.*, 2003). Several studies have also suggested that the recrystallization rate is strongly reduced below the glass transition temperature (Hartel, 2001 ; Carrington *et al.*, 1996). However, there are few experimental studies of recrystallization in frozen desserts or model systems near or below T_g' , because the T_g' values of most components of frozen desserts, such as carbohydrates (sucrose, lactose, fructose etc.), are lower than -30°C (Slade and Levine, 1995 ; Roos, 1995).

The objective of this study is to provide experimental data on recrystallization rates in a model system of relevance to frozen desserts at lower storage temperatures, including near T_g' . Sucrose solution was chosen as a sample because sucrose is a typical sweetener used in various frozen desserts. We investigated the dependency of the isothermal recrystallization rate on storage temperature.

There is still debate on how to measure the T_g' value of sucrose, with different approaches producing different values. Until now, two different values have been cited

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frequently: -32°C (Slade and Levine, 1995), and -47°C (Roos, 1993; 1995). Both of these are values taken from DSC curves, the interpretation of which is still uncertain (Ablett *et al.*, 1992; Goff, 1995; Aubuchon *et al.*, 1998; Goff *et al.*, 2003). In this study we will discuss the temperature dependence of the recrystallization rate, taking both of these values into consideration.

Materials and Methods

Freezing and storage procedures A 30% sucrose solution was used. A volume of about 3 mL of the solution in a polypropylene tube (length 210 mm, inner diameter 5.6 mm, outer diameter 6.0 mm) was frozen at -60°C in ethanol brine. This temperature was chosen because it is sufficiently low compared to both the reported T_g' values of sucrose mentioned above. The temperature of the geometrical center of sample was monitored during freezing. After the temperature reached -60°C , the sample was rapidly transferred to stockers maintained by a thermostat at -21°C , -29°C , -35°C , and $-50 \pm 0.5^{\circ}\text{C}$, respectively. To reduce temperature fluctuation, the sample was kept in a styrene foam box located in the stockers. The storage times were set to 5, 10, 15 and 20 hr.

Observation of ice crystals by cryo-SEM An S-4000 microscope (Hitachi Ltd., Japan) was used. Figure 1 shows the outline of procedures for cryo-SEM observation in this study. After storage, the frozen samples were immersed in liquid nitrogen and the sample in the tube was transferred to a room maintained by a thermostat at -40°C . Pouring liquid N_2 on the sample, it was cut into 5-mm thick specimens using a knife. The cut samples were immersed in liquid N_2 , placed on a sample holder, and transported to the cryo-SEM room. The sample holder was set in a sample chamber of the SEM apparatus which had been precooled by liquid nitrogen. While monitoring the SEM image, the frost on the sample surface was sublimated by heating the sample to between -100 and around -120°C with a temperature controller in order to

make a cross section of the sample appear. Subsequently, the revealed cross section was sublimated until cavities created by sublimation of ice crystals could be observed clearly. The obtained SEM images were recorded as black-and-white photographs.

Image analysis The photographic images were scanned by an image scanner (GT-7000, Seiko Epson Corp., Japan) as bitmap images, which were finally converted to binary images. The projected area of each crystal was extracted by tracing the perimeter of the crystal on a CRT monitor. The size of each crystal was calculated as the radius of a circle with the equivalent projected area of the crystal. From the data set of each crystal size, the number-based mean crystal radius r was calculated. For these procedures, commercial image-analysis software WinROOF (Mitani Corp., Japan) was used.

To evaluate recrystallization rate, a theory based on the Ostwalds ripening principle (Lifshitz and Slyozov, 1961; Wagner, 1961) was used as preceding isothermal recrystallization studies (Sutton *et al.*, 1996a, 1996b, 1997, 1998; Hagiwara and Hartel, 1996; Miller-Livney and Hartel, 1997). According to the theory, the recrystallization process, after reaching the pseudo-steady state hypothesized in Ostwalds ripening (isothermal system), can be given by:

$$r^3 = r_0^3 + kt \quad (1)$$

where r is the number-based mean crystal radius, r_0 is the number-based mean crystal radius at time $t=0$ (time when the sample reaches a pseudo-steady state), and k is the recrystallization rate. Therefore, the recrystallization rate k can be evaluated as the slope of the cube of the mean radius vs. storage time.

All analyses were conducted for two or three different specimens under each set of conditions and the averaged values were obtained.

Results and Discussion

Determination of observation position The observed ice crystal size is dependent upon the position of observation due to variations in the cooling rate. Therefore, to investigate the effect of storage on ice crystal size it was necessary to fix the observation position. Prior to examination of recrystallization during storage, we determined an adequate position from which to evaluate ice crystal size immediately after freezing at -60°C . Figure 2 (a) illustrates schematically the positions we examined as candidates for the observation position. Figure 2 (b) shows typical SEM images of the candidate positions. From No. 1 (sample periphery) to No. 5, the ice crystal size tended to increase due to a reduction in cooling rate. From No. 6 to No. 8 (sample center), the ice crystals became smaller in spite of the lower cooling rate. This was probably caused by suppression of nucleation and growth of ice crystals due to increasing sucrose concentration at the sample center by freeze-concentration. We choose No. 5 as an observation position because the ice crystal particles were observed clearly and the size of ice crystals tended to be larger than those at other positions.

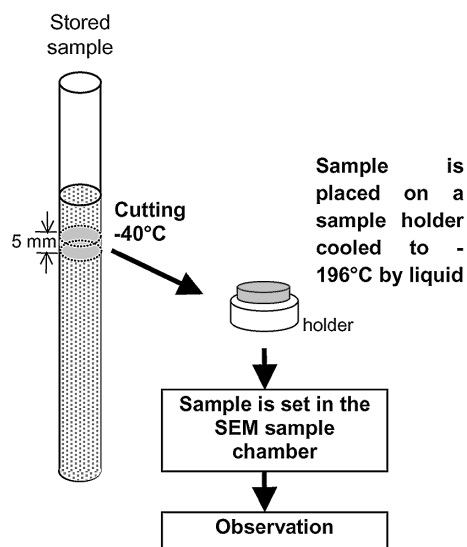


Fig. 1. Outline of procedures for cryo-SEM observation.

As stated above, the object of this study is to obtain experimental data on recrystallization rates at lower temperatures, including near T_g' . If we are to discuss the data from the point of view of T_g' , it is desirable that T_g at the

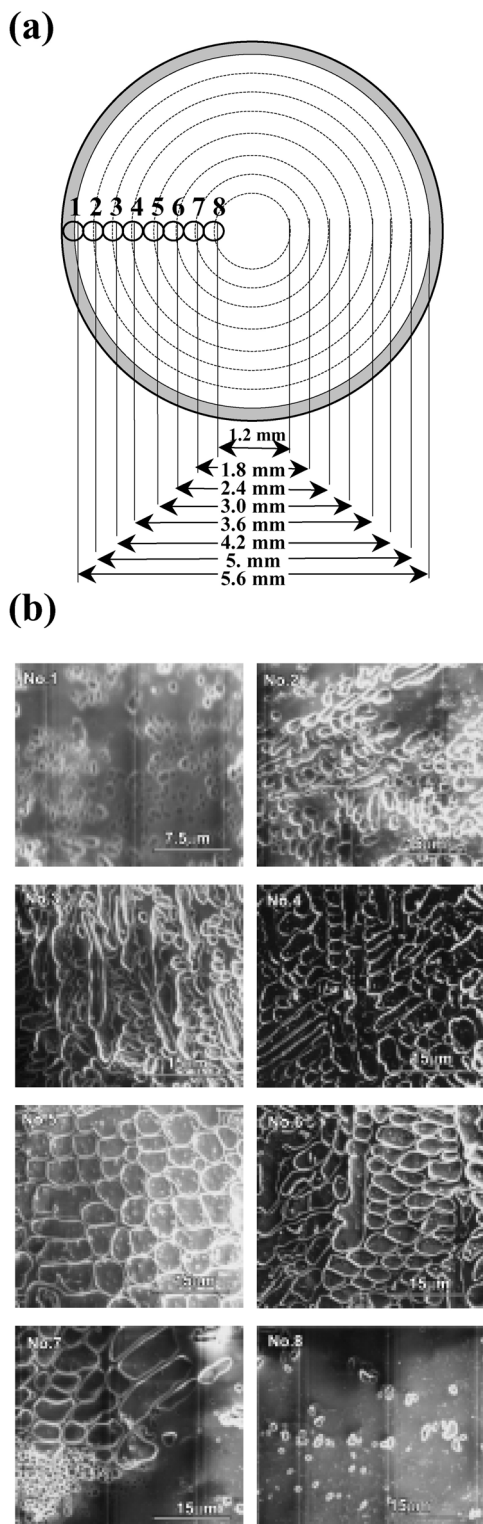


Fig. 2. Examination of observation positions for investigating the effect of storage on ice crystal size.

(a) Schematic illustration of candidates for observation position (Nos. 1-8). (b) Typical SEM images for each candidate.

observation position is near T_g' ; that is to say, vitrification without maximal concentration eventually occurs when the cooling rate is so rapid that ice crystals do not grow enough (Roos and Karel, 1991; Sahagian and Goff, 1994), which results in a value of T_g that is lower than T_g' . In the DSC experiment for measuring T_g' , an annealing treatment slightly above the expected T_g' is sometimes performed to ensure maximal freeze-concentration (Roos, 1993). We did not carry out such a treatment for the following two reasons. First, we attempted to investigate the isothermal recrystallization rate after initial freezing. Secondly, considering the practical conditions of frozen storage, such an annealing treatment may be unrealistic. In this study, we assumed that the T_g of No. 5 was not far from the T_g' of sucrose since the ice crystals grew large.

Recrystallization of ice crystals Figure 3 shows plots of r^3 vs. storage time at various storage temperatures. The plots can be reasonably approximated by a linear relation after 5 hours and the value of k could be evaluated from the slope of the plots according to Eq. (1), although there was an initial lag before r^3 could be fitted to a straight line. Apparent slopes before 5 hours seem to be smaller than those after 5 hours, indicating slower recrystallization. In this study, the samples were frozen at lower temperature (-60°C) than those typical during storage, and were stored in a box made of styrene foam. Due to the insulating effect of the styrene foam box, the sample temperature may have been kept lower than the storage temperature for significantly long periods, resulting in slower recrystallization.

Figure 4 shows a plot of recrystallization rate vs. storage temperature in the manner of Arrhenius plot. The plot did not show a single straight line, which indicates deviation from Arrhenius behavior. As the storage temperature decreased, a rapid decrease in recrystallization rate was observed between -29°C and -35°C . At -50°C , the value of the recrystallization rate ($1.29\mu\text{m}^3/\text{hr}$) was about 1.5% of that at -21°C ($82.4\mu\text{m}^3/\text{hr}$). In the following paragraphs, from the point of view of glass transition, we will discuss the temperature dependence of recrystal-

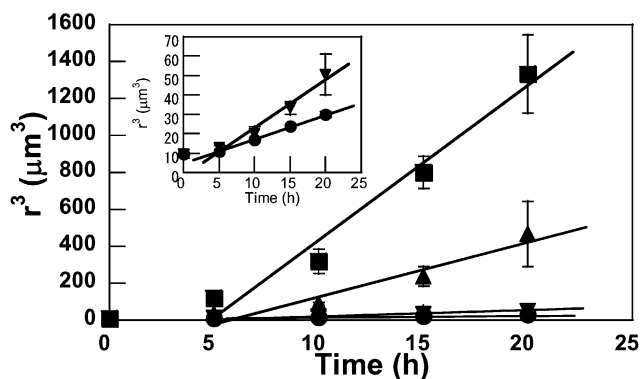


Fig. 3. Plots of r^3 vs. storage time at various temperatures.

■, -5°C ; ▲, -20°C ; ▼, -30°C ; ●, -50°C . The inset shows data plots at -35°C and -50°C on an expanded scale. The solid lines represent the results of fitting with Eq. (1).

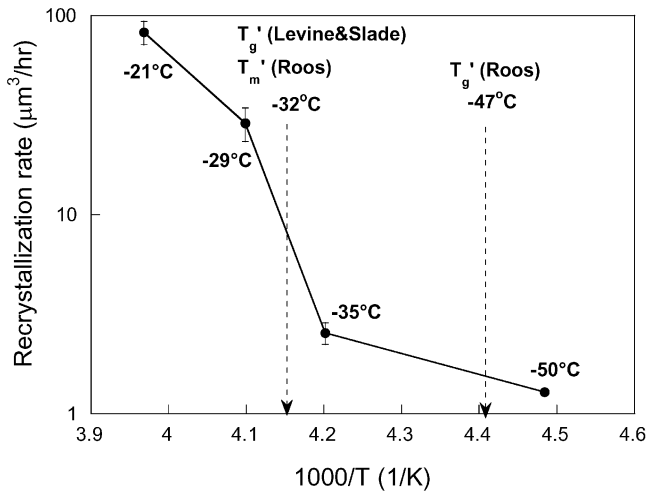


Fig. 4. Effect of storage temperature on recrystallization rate.

lization rates.

As stated before, two different T_g' values have been often used for sucrose, -32°C (Slade and Levine, 1995) and -47°C (Roos, 1993; 1995). Because there has been some debate over which is true T_g' , we must take both views into consideration when we discuss the temperature dependence of recrystallization rate. Levine and Slade (1986, 1988) have postulated that the rate of deterioration in frozen foods decreases dramatically below T_g' because the mobility of molecules in the glassy state is severely restricted. As shown in Fig. 4, the recrystallization rate decreased rapidly between -29°C and -35°C , which is in agreement with this hypothesis. On the other hand, according to Roos (1995), the reaction rate increases rapidly above T_m' ($>T_g'$, the onset melting temperature of ice in contact with a maximally freeze-concentrated solution) rather than T_g' . For a frozen sucrose solution, Roos (1993, 1995) reported a T_m' value of around -32°C , which is also consistent with the results shown in Fig. 4. From the discussion above, it may be concluded that the rapid decrease in recrystallization rate between -29 and -35°C , as shown in Fig. 5, can be explained using both of these views. It has been pointed out that the recrystallization rate of ice crystals is strongly reduced below the glass transition temperature (Hartel, 2001; Carrington *et al.*, 1996). However, little research on recrystallization near T_g' or T_m' has been conducted. The results shown in Fig. 4 may confirm experimentally that storage below T_g' or T_m' strongly suppresses recrystallization.

At -50°C , an unfrozen solute phase may be considered to be mostly in the glassy state. However, it should be noted that the mean crystal radius increased over 20 hr of storage. This suggests that over a realistic storage period, deterioration by recrystallization may be a problem even in the glassy state. In general, it is believed that food in a glassy state is very stable because its molecular motion is severely restricted. However, in the field of polymer science it is well-known that molecular movement, which leads to macroscopic structural relaxation over a practical period, is still present below the glass transition tem-

perature because of the non-equilibrium nature of glassy substances. Molecular movement in glassy polymers has been extensively investigated because changes in the internal structure of these materials directly affect their macroscopic properties, such as mechanical or transport properties or density (Matsuoka, 1992; Yoshida, 1995; Tiemblo *et al.*, 2001). Molecular mobility in glassy food and food component carbohydrates with low moisture content has also been studied recently (Hancock *et al.*, 1995; Urbani *et al.*, 1997; Noel *et al.*, 1999; Wungtanagorn and Schmidt, 2001; Kim *et al.*, 2003; Hashimoto *et al.*, 2004; Kawai *et al.*, 2005). As for frozen food systems, Pyne *et al.*, (2003) recently investigated molecular mobility in the freeze-concentrated phase of a trehalose solution below T_g' based on the concept of enthalpy relaxation. However, there is little research on molecular mobility in freeze-concentrated solutions in a glassy state. Molecular motion in a freeze-concentrated solute matrix may be sufficient to cause ice recrystallization over a realistic storage period even in the glassy state. Molecular movement in a glassy-state freeze-concentrated phase may be an important factor that should be taken into consideration in the further improvement of frozen food storage technology.

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