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The use of thermal analysis in the development of a better understanding of frozen food stability

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Abstract: Reactions causing deleterious changes in texture, structure and chemical composition of frozen foods occur frequently and result in a loss of quality or shelf-life. Polysaccharides, e.g., guar, locust bean gum, are added to many frozen food formulations at low concentrations and are effective at stabilizing products against rapid ice crystal growth. During freezing, an unfrozen phase (UFP) containing a high concentration of dissolved solutes is formed as water is separated from solution in the form of ice. This UFP is capable of undergoing a glass transition at low temperatures. Thermal analysis techniques (DSC, TMA, DEA) have been used extensively in the determination of thermal and physical properties at subzero temperatures for a number of model systems and frozen foods and in the elucidation of mechanisms of polysaccharide action.

INTRODUCTION

In North America and in other parts of the developed world, the frozen food industry has progressed from the freezing of a few raw commodities and meats to the processing of whole meals, bakery products, and desserts. The consumer is increasingly being presented with a wide selection of frozen foods that bring diversity and convenience to meal selection. This development has mostly occurred in the last 30 years, and is in part related to the widespread use of home freezers and microwave ovens. Freezing is based on the physical principles of separation of pure water from a solution by ice crystal formation. Many frozen foods contain an unfrozen phase (UFP) with a very low freezing point due to the process of freeze concentration of the solutes as water is removed in the form of ice (1,2). Detrimental reactions that are diffusion-controlled can occur within this UFP even at very low temperatures (3). These include those associated with the ice phase, related to size and growth of ice crystals and sublimation ("freezer burn"), and those related to the non-ice phase, including enzymatic and chemical reactions. Most raw vegetables can be stored only a short time even at -20 °C because of changes in texture, colour, flavour, and nutritional quality due to enzyme activity, and therefore must be blanched for adequate protection. Blanching, however, may also cause detrimental effects in many foods. Freezing and thawing also affect many food components. As a consequence of freeze-concentration, the properties of the unfrozen phase, including pH, titratable acidity, ionic strength, viscosity, freezing point, surface tension and oxidation-reduction potential, are altered. These can become detrimental to food components, e.g., protein denaturation leading to curdling and drip in thawed materials. Freezing of emulsions may cause coalescence of fat, e.g., frozen milk. The potential for these reactions cause frozen foods to possess a finite shelf life, despite the low temperatures and tremendous reduction in unfrozen water content. Frozen foods are different than cryopreserved biological materials in that storage and distribution, if not the freezing itself, is almost always carried out at temperatures where a significant amount of unfrozen water exists.

Extensive microstructural changes may occur inside food tissues during ice crystallization. The location of ice crystals depends mainly on the freezing rate. Slow freezing (<1 °C min^{-1}) causes ice crystals to grow in extracellular locations (3). This results in large crystals, maximum dislocation of water, a shrunken appearance of cells in the frozen state, drip loss, tissue shrinkage during thawing and a reduction in overall food quality. On the other hand, rapid freezing promotes uniform crystallization throughout the product (2) and produces smaller ice crystals, which leads to a superior product. Meat survives freezing better than does most plant tissue because of the flexible nature of muscle fibres, as compared to the semi-rigid nature of plant cells.

Ice crystals are relatively unstable, and during frozen storage, they undergo changes in number, size, and shape, known collectively as recrystallization, particularly as a result of temperature fluctuations, which are unavoidable throughout storage, distribution and retailing (3). This is probably the most important reaction leading to quality losses in frozen foods. Polysaccharides such as guar, locust bean gum, carrageenan, sodium alginate, or carboxymethyl cellulose, are used in many types of frozen foods to protect the product from the development of coarse texture as a result of ice recrystallization/ growth during heat shock (3). An example is ice cream (4). As temperatures increase during frozen storage, the proportion of frozen water decreases, and smaller crystals melt, since they have a lower melting temperature (2). Conversely, as temperatures decrease, water has greater tendency not to renucleate but rather to be deposited on the surface of larger crystals, so the net result is that the total number of crystals diminish and the mean crystal size increases (1,2). This leads to greater tissue disruption and poor food quality. Recrystallization can be minimized by maintaining a low and constant storage temperature.

GLASS TRANSITION AND THE PHASE DIAGRAM

Cryostabilization of frozen foods focuses on the reactivity of the UFP and its relationship to the shelf life of the frozen product (5). The basis of cryostabilization is a recognition and manipulation of the glassy state that can be formed in the UFP surrounding the ice crystals (6). A common amorphous or non-crystalline metastable state of solids is termed the "glassy" or vitreous state. A glass can also be characterized as a liquid with extremely high viscosity (10^{12} - 10^{14} Pa s). The glass/rubber transition is a kinetic phenomenon that is dependant on type and concentration of solute and temperature. In the "state diagram" (Fig. 1), a glass transition line as a function of temperature and concentration is shown. Above and to the left of the glass transition line, solutions or complex systems such as foods are in the rubbery or liquid state, in which they are unstable and reactive, so that ice crystal growth can occur in time frames significant to food storage. Below and to the right of the glass transition curve, the system transforms to the glassy (vitreous) state as a result of extremely high viscosity and exists as an unreactive, amorphous solid. The hypothesis has recently been stated that this transition greatly influences resulting frozen food stability, as the water in the concentrated serum phase becomes kinetically "immobilized", thus tremendously decreasing the rates of molecular diffusion, and therefore does not support or participate in reactions (5-7). Thus, successful storage of frozen products can be accomplished by storage at temperatures below the T_g or by formulating the frozen food to raise its T_g to temperatures above typical storage temperatures (7).

In Fig. 1, the equilibrium freezing line is shown in relation to the eutectic line and the glass transition line. Solute crystallization (especially sugars) at the eutectic point is unlikely, due to the very low temperatures, extremely high viscosities, and resulting low diffusion rates and limited solute mobility (1,2). Thus the solute concentration at temperatures less than the eutectic point may exceed the saturation point, resulting in a supersaturated state. If foods are subjected to an "equilibrium" freezing process in which they become maximally freeze-concentrated and temperature is sufficiently lowered, a metastable glass will form. This glass forms at a characteristic glass transition temperature lower than the eutectic temperature, designated the T_g', and the amount of unfrozen water present in the glass, designated W_g', will be minimized but still significant (6). If foods are subjected to more rapid freezing, a range of different, non-equilibrium glasses may form at $T_g < T_g'$ (dependent on the freezing rate), and more glass will form as a result of the unfrozen solution not being maximally freeze-concentrated, such that $W_g > W_g'$ (8). During warming, the UFW trapped in a glass may recrystallize after it devitrifies, because of its non-equilibrium state.

Subzero glass transition temperatures of maximally freeze-concentrated solutions of various solutes, T_g', were first reported by Levine and Slade (5-7,9). A considerable number of publications on this topic have occurred since, including those of Roos and Karel (eg., 10-12), Simatos and Blond (eg., 8,13), Ablett and Izzard (eg., 14,15), and a fairly recent comprehensive treatise (16). This recent body of literature has brought to light controversy over the interpretation of low temperature behaviour of aqueous solutions. Part of the difficulty lies in the fact that two thermal transitions are evident upon warming of frozen carbohydrate solutions. This was first reported 25 years ago by Luyet and Rasmussen (eg., 17,18) who identified the transitions as T_{am} (ante-melting) and T_{im} (incipient melting). It has recently been hypothesized that these transitions are the result of incomplete freeze-concentration, and extensive annealing needs to be used to maximally freeze-concentrate such samples (9,11). However, despite extensive annealing to promote maximal ice formation, two transitions always appear. In recent publications (19,20), we have preferred to call the transitions $Ttrs_1$ (onset temperature of transition 1, the lower temperature transition) and $Ttrs_2$ (onset temperature of transition 2, the higher temperature transition) as the research community collectively comes to some agreement as to the nature of these results. There seems to be three interpretations of this behaviour: a) $Ttrs_1$ is a T_g and $Ttrs_2$ is the T_g' (devitrification and recrystallization in the same experiment); b) $Ttrs_1$ is the T_g' only after extensive annealing and $Ttrs_2$ represents the onset of ice melting; c) $Ttrs_1$ is T_g' only after extensive annealing but $Ttrs_2$ contains both first- and second-order components, likely resulting from a complex relaxation of the glass and frequency dependence of the measurement (9,19).

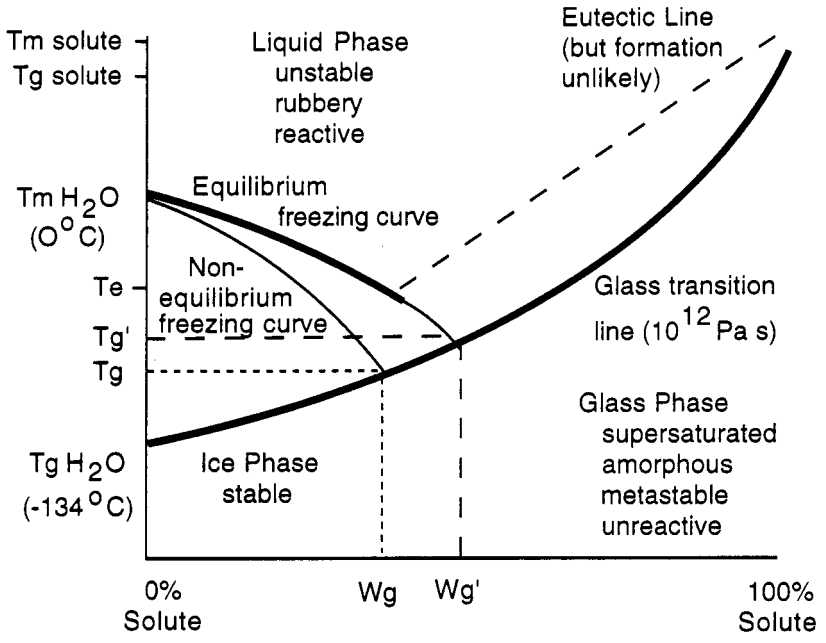

Fig. 1. A schematic temperature-concentration state diagram for an aqueous carbohydrate solution, showing the glass transition line, defined by viscosity, the equilibrium freezing line, a "non-equilibrium' freezing path, the theoretical eutectic line, and a description of the reactivity of the various 'states'. The glass transition, melting and eutectic temperatures of the solute and water are shown. Point T_g' represents the glass transition temperature of the maximally freeze-concentrated solution and W_g' represents the amount of unfrozen water (100-% solute) which becomes trapped in the glass. Points T_g and W_g represent an example of a temperature concentration relationship in a glass formed as a result of less than maximal ice formation.

RESEARCH OBJECTIVES

The objectives of our research have been to measure equilibrium and non-equilibrium glass formation in frozen systems using thermal analysis techniques, to determine the influence of polysaccharides on ice crystallization and glass formation in model systems and in frozen foods, eg., ice cream, at fluctuating subzero temperatures, and to gain further insight into stability of frozen foods and ways in which frozen stability may be enhanced through alternate ingredients or processing methods. A Thermal Analyst 2000 combined with a 2910 DSC with "Modulating" hardware and software, 2940 TMA with parallel plate rheometry, and 2970 DEA (TA Instruments, New Castle, DE, USA) have been used to measure the thermal events in the temperature range from -100 to 0 °C occurring in model aqueous solutions of sucrose or fructose of varying concentration, in the absence and presence of polysaccharide or protein stabilizers (guar, xanthan, gelatin), as a function of freezing rate. An EMscope SP2000A Sputter-Cryo Cryogenic Preparation System attached to an Hitachi S-570 scanning electron microscope has been used for low temperature scanning electron microscopy (cryo-SEM). Various freezing rates and storage times and temperatures in both model systems and ice cream have been studied by cryo-SEM and image analysis.

APPLICATION OF DIFFERENTIAL SCANNING CALORIMETRY

Differential scanning calorimetry (DSC) was used to examine low temperature behaviour of aqueous carbohydrate (sucrose + polysaccharide) solutions as a function of freezing rate, and to attempt to establish the T_g' (19). After rapid freezing, devitrification exotherms are readily apparent. Therefore, to determine T_g' values, one must utilize conditions that promote maximal ice formation, and hence annealing protocols are necessary. The glassy state begins as a highly disordered state containing excess enthalpy and free volume but relaxes toward thermodynamic and volumetric equilibrium in a process known as physical ageing. Annealing or holding at various sub T_g temperatures at constant time (60 min) should result in a

range of enthalpy compensation overshoots representing the excess enthalpy related to the degree of structural relaxation, leading to a maximum at the onset of T_g. We utilized this concept in the determination of appropriate annealing protocols to approach maximal freeze-concentration (19). Slowly frozen systems exhibited considerably less excess enthalpy than rapidly frozen systems, as expected. Slowly frozen systems annealed between -50 and -35 °C resulted in increased T_{trs1} onset values (-49 to -40 for 40% sucrose solution). However, annealing above -35 °C led to a large reduction in T_{trs1}. Thus -35 °C was optimal for annealing (19).

The data from extensive freezing rate and annealing studies are shown in Table 1. Solutions were slowly frozen at 2 °C min^{-1} to -100 °C, in the annealed case were armed at 5 °C min^{-1} to -35 °C, held 60 min to facilitate further crystallization, recooled to -80 °C and then scanned at 5 °C min^{-1}. Under annealing conditions, the two transitions directly follow each other but are both clearly resolvable. Annealing increases the T_{trs1}, but has no significant effect on the T_{trs2} of the 20% and 40% solutions. Annealing also greatly reduces the devitrification exotherm. The 80% solution approximates the C_g' (% solute in the glass at maximal freeze concentration) and has a ΔC_p in agreement with that observed at the T_{trs1}. Thus the value of -40 °C represents our best estimate of the T_g' (19) and correlates closely with recently published data (14). The second transition appears to be largely first-order. However, evidence of a small overshoot after T_{trs2} following annealing at -35 °C may suggest some second-order component to this transition in addition to ice melting. The T_{trs2} value may be the most technologically-significant transition (4,9), however, because, as we will see later, a tremendous increase in mobility occurs above this transition.The action of polysaccharides in these frozen solutions was also studied by DSC (20,21).

By DSC measurements (Table 2), they have had little if any effect on the transition temperatures, possibly delaying ice formation and thus resulting in slightly lower transition values and hindering devitrification of trapped undercooled unfrozen water (T_{trs1} was lowered from -40 to -42 °C by the addition of stabilizer in annealed samples). This would suggest that stabilizers are influencing the kinetics of ice formation within the time scale of the experiment. The DSC data regarding the stabilizer action are inconclusive, however, and we have had to use other thermal techniques to explore their action further.

TABLE 1. Transition temperatures (T_{trs}/°C) for 20%, 40%, 60% and 80% sucrose solutions, slowly frozen (2 °C min^{-1}) or slowly frozen and annealed (-35 °C for 60 min) and warmed at 5 °C min^{-1}, as determined by DSC.

Sample	Slow freezing		Slow freezing/annealing	
	T_{trs1}	T_{trs2}	T_{trs1}	T_{trs2}
20%	-46.7	-33.7	-40.5	-32.6
40%	-48.0	-33.8	-41.2	-32.8
60%	-97.3	-42.9	-45.6	-34.5
80%	-39.2	-	-	-

TABLE 2. Transition temperatures (T_{trs}/°C), as determined by DSC with a warming rate of 5 °C min^{-1}, and calculated compliance (Pa^{-1} x 10^{-5}) at -26 °C as measured by TMA under a constant stress of 140 Pa, for 20% sucrose solutions alone and in the presence of 0.5% stabilizers, rapidly (liquid nitrogen) or slowly (2 °C min^{-1}) frozen and annealed (-35 °C for 60 min). Mean values followed by the same letter within each column are not significantly different ($p>0.05$); n=4.

Sample	Rapid freezing			Slow freezing/annealing		
	T_{trs1}	T_{trs2}	Compliance	T_{trs1}	T_{trs2}	Compliance
Sucrose	-54.6 ab	-32.2 b	27.7 a	-40.5 a	-31.4 a	5.38 a
+ xanthan	-58.1 a	-34.7 a	3.45 c	-42.5 a	-32.2 a	3.17 b
+ gelatin	-53.9 b	-32.7 b	17.9 b	-42.8 a	-32.6 a	4.14 ab
+ guar	-54.2 b	-32.5 b	15.9 b	-42.5 a	-31.4 a	3.98 ab

APPLICATION OF THERMOMECHANICAL ANALYSIS

The manifestation of frozen food instability is very often mechanical (rheological) rather than thermodynamic and therefore thermomechanical analysis (TMA) techniques become readily applicable. TMA was therefore also used in the determination of transition temperatures and rates of mechanical collapse as a function of both freezing rate and stabilizers (20-23). After rapid freezing, sucrose solution demonstrate complete collapse after the first transition. Most stabilizers appeared to result in similar results, and only xanthan appeared to offer any mechanical stability to these quench frozen samples (20). However, an expansion of the frozen system above the first transition became readily apparent with the slow frozen annealed sample of sucrose and sucrose plus stabilizer (20). A gradual increase in volume expansion representing a volume recovery process, once adequate thermal energy was acquired, occurred from -52 °C to -30 °C, similar to the enthalpy recovery process observed in the DSC, at which point flow began.

Due to large differences in sample size, geometry and resulting thermal history, TMA T_{trs1} values were lower than those obtained by DSC. T_{trs2} values, however, were similar. After proper annealing, stabilizers had no effect on the transition temperatures. However, the influence of stabilizers on structural flow and collapse above $T_{trs}2$ was substantial, shown in Table 2 as compliance (strain/stress @ constant stress, inverse of modulus) at -26 °C (20). After rapid freezing, all stabilizers had a significant influence, however, the xanthan gum produced an extremely rigid mechanical system, very resistant to collapse. Slow freezing appeared to substantially reduce compliance values, consistent, with an overall reduction in free volume, and all stabilizers behaved similarly, further increasing the rigidity of the systems. The further influence of mechanical action of polysaccharides above T_{trs2} has come from a study utilizing TMA to determine the stress relaxation behaviour (23). Samples after either rapid or slow freezing were warmed to various temperature between -55 °C and -15 °C and equilibrated for 3 min. An instantaneous strain of 3.8% was then applied, the samples were allowed to relax and the resulting stress decay curve was monitored for 20 min. The addition of xanthan, gelatin, or guar to slowly frozen sucrose solutions substantially increased the relaxation time at -20 °C. A more stable system of lower mobility and free volume facilitated by slow freezing may have encouraged polymer entanglement and interactions leading to short-term elasticity.

APPLICATION OF DIELECTRIC ANALYSIS

Dielectric analysis (DEA) utilizes a periodic electrical field (sinusoidal voltage) to determine the capacitive and conductive nature of materials (ability to store and transfer electrical charge) and thus molecular relaxations by measuring the resulting current. Permittivity or dielectric constant, e', is related to capacitance and is a measure of the degree of alignment of dipoles to the electric field. The loss factor, e", is proportional to conductance and is related to the energy required to align dipoles or move ions. Temperature and frequency sweeps have been conducted on solutions of sucrose with the addition of the polysaccharide stabilizers and gelatin as before. Samples were slowly frozen on the ceramic plate in the machine, and simultaneously warmed at a rate of 1 °C per min and scanned at a frequency alternating between 0.1 and 1 Hz in the multiplexing mode. By taking the first derivative, the temperature and frequency dependence of each transition becomes more obvious (Fig. 2). The T_{trsl} is very frequency dependent upon slow cooling. The lowest peak maximum temperature at 0.1 Hz was -53 °C, and this was also shifted up by annealing. The T_{trs2} appeared at -28 °C and was not frequency dependent. This provides further evidence that the lower transition contains a significant second-order (glass) relaxation due to its frequency dependence while the warmer temperature transition contains a significant first-order transition with little second-order component. The effect of stabilizers was also apparent from DEA. Xanthan and gelatin had a large effect on the de"/dt (loss factor) maxima at 0.1 Hz. As these samples were slowly frozen but not annealed, this effect may have been due to the formation of less ice, hence more dilute glass, upon freezing, which then relaxes at lower temperature. Stabilizers, especially xanthan, also broadened the frequency dependence and thus the lower temperature at 0.1 Hz may have reflected a more complex relaxation of the same quantity of ice.

APPLICATION OF MODULATED DSC

Modulated DSC is a technique in which the temperature during the temperature scan is modulated by a controllable period and amplitude. Hence instantaneous fast heating rates for good sensitivity and slow underlying heating rates for good resolution are obtainable. Separation of the resultant experimental heat flow during this cyclic treatment provides not only the total heat flow signal available from conventional DSC, but also separates the total heat flow into its reversing (in phase) and non-reversing (out of phase) components. Typical glass transitions appear as reversing phenomena while cold crystallization as non-

reversing. As was evident from the earlier discussion, it is obvious that during warming of frozen solutions, we have a glass transition, possibly a devitrification exotherm, an endothermic peak representing an enthalpic relaxation, and the melting endotherm. Two transitions representing baseline shifts (changes in heat capacity) always appear despite extensive annealing. MDSC may be the technique to separate and understand these phenomena.

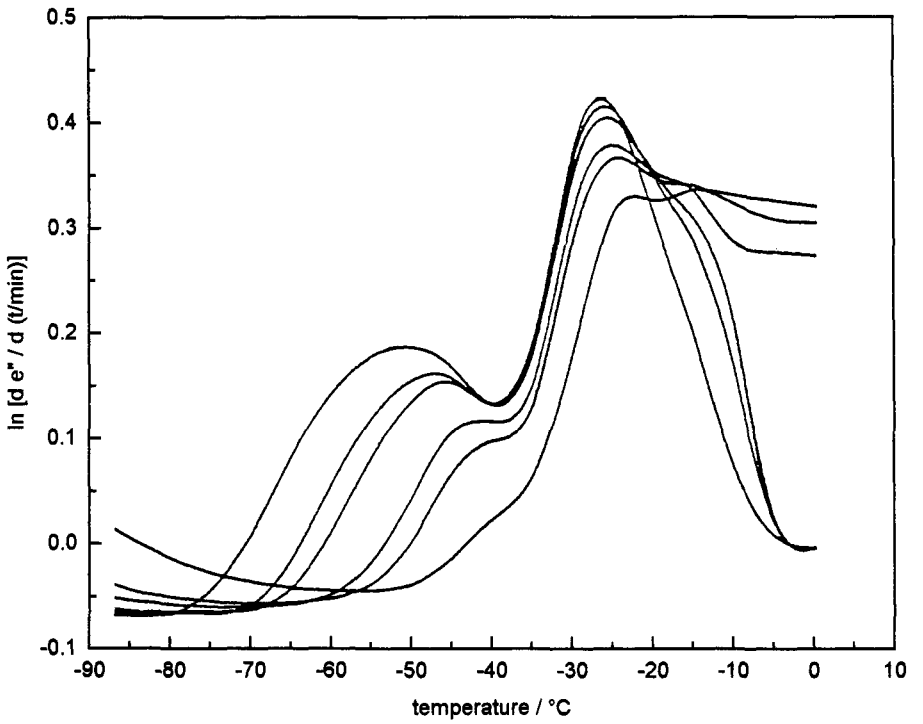

Fig. 2. First derivative of the loss factor (e") as a function of temperature (frequency from 0.1 to 50 Hz) as determined by dielectric analysis for a 20% aqueous sucrose solution.

Our results are quite preliminary at this time, but several observations indicate general trends. In slowly frozen but not annealed samples of 20% sucrose solutions, the total heat flow curve demonstrates previous results, a T_{trs1}, a devitrification exotherm, and a more distinct T_{trs2}. The non-reversing heat flow indicates that the devitrification shows up as a large exotherm, followed by an endotherm at the T_{trs2}, possibly associated with the onset of ice melting. The glass had to have relaxed at temperatures lower than T_{trs2} in order to have shown this devitrification. The reversing heat flow contains the T_{trs1} but also some portion of T_{trs2}. This may represent only the change in heat capacity associated with melting, or it may indicate a second-order component in the T_{trs2}. With proper annealing and a range of periods and amplitudes, this will become more evident. So, we are excited about the potential of this technique and its value in helping to understand these transitions.

APPLICATION OF CRYO-SCANNING ELECTRON MICROSCOPY

We have also used cryo-SEM to examine stability of stabilized solutions as a function of freezing rate and storage temperatures above and below the glass transition temperatures of model carbohydrate solutions and ice cream (24,25). Fig. 3 shows micrographs of ice crystals in quench frozen (Freon 22) 30% fructose (top left) and fructose plus 1% carboxymethyl cellulose (CMC; top right) after storage at -75 °C for two weeks and the same solutions at -25 °C for two weeks. Note the order of magnitude difference in magnification. Interestingly, ice crystal sizes in the sample quench frozen to -80 °C after storage at -25 °C for two weeks were similar in size to those obtained by freezing solutions slowly to -25 °C. Thus it was apparent that recrystallization following devitrification of the glass resulted in similar microstructures to slow freezing. Rapid freezing is desirable in food freezing to maximize nucleation, but it appears that rapid freezing to temperatures at or slightly above the T_g' are critical to ensure maximal freezeconcentration and reduce possibilities of extensive recrystallization.

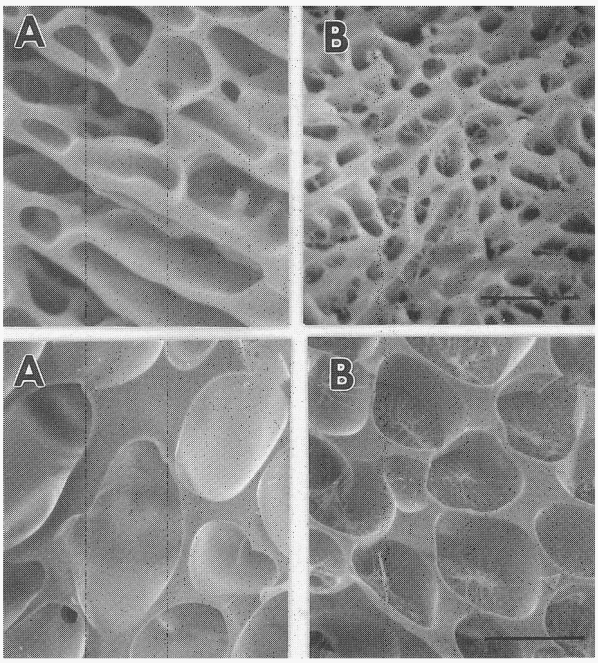

Fig. 3. Cryo-SEM micrographs of 30% fructose, 1% carboxymethyl cellulose (CMC) solutions after rapid freezing in Freon 22.
Top row: A) 30% fructose, 2 weeks storage at -75 °C, bar = 3 μm, B) 30% fructose + 1% CMC, 2 weeks storage at -75 °C, bar = 3 μm.
Bottom row: A) 30% fructose, 2 weeks storage at -25 °C, bar = 30 μm, B) 30% fructose + 1% CMC, 2 weeks storage at -25 °C, bar = 30 μm.
Note the order of magnitude difference in magnification between top and bottom row.

CONCLUSIONS

Two applications we will mention in closing involved the use of dextran as a polymer to increase the T_{trs2} and to observe its effect on sensory perception of iciness intensity in ice cream (26,27), and osmotic concentration of fruits as a pretreatment prior to freezing. Dextran is peculiar in that it has high molecular weight but contributes little to solution viscosity. Thus it can be incorporated into solution in relatively high concentration compared to other stabilizing gums. In ice cream formulations, the T_{trs2} was increased by 2 to 2.5 °C, and the stability of the ice cream was greatly enhanced as a result. Cryostabilization can come about from modifications of the physical characteristics of the frozen food, but this does not necessarily imply it is limited to formulated products. In the osmotic concentration of fruits as a pretreatment prior to freezing, modifications of cell turgor and both intracellular and extracellular solutes can greatly impact on the stability of the product to freezing, frozen storage and thawing.
The present understanding of structure/stability in frozen foods is rapidly increasing. We have tentatively concluded that the T_{trs1} approaches the true T_g', but only after measures have been taken to maximize ice formation. The T_{trs2} has both first- and second-order components, and represents the onset of ice melting together with the final relaxation of amorphous component. It may be the most technologically significant transition for frozen food stability, even if it is not the T_g'. However, further work is needed before these issues are resolved, and we are presently involved in extensive MDSC research which we hope will help to answer some of these fundamental questions surrounding the two transitions.
Freezing rate has emerged as an important variable to control in the freezing of foods. While rapid freezing may promote extensive nucleation, rapid freezing to temperatures below the T_g results in measurable devitrification and reduced mechanical resistance to structural flow. Storage and distribution of frozen foods is almost always conducted at temperatures greater than the T_{trs2}, unlike cryopreservation of biological materials. To compliment thermal analysis techniques, we have also used cryo-SEM and results indicated that recrystallization following devitrification of the glass resulted in similar microstructures to slow freezing. Rapid freezing is desirable to maximize nucleation, but it appears that rapid freezing to temperatures above the T_g critical to ensure maximal freeze-concentration and reduces possibilities of extensive recrystallization. It has also been evident from cryo-SEM that the high molecular weight stabilizers do offer some protection against ice crystal growth.
The role of polysaccharides is both important and intriguing, and it is certainly well known amongst industry practitioners that they play a crucial role. Stabilizer action likely does not involve modifications of thermodynamic behaviour but may involve a modification of the diffusion properties of components within the unfrozen phase above the T_{trs2}. Hyperentanglements resulting from freeze-concentration to greater than critical concentration values must play an important role in their action.

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