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Glass transitions in aqueous carbohydrate solutions and their relevance to frozen food stability[1]

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Abstract

During the freezing of aqueous low molecular weight carbohydrate (sugar) solutions, the solute is freeze-concentrated into a supersaturated solution of high viscosity that is capable of undergoing a transition into the vitreous (glassy) state. If the freezing process follows the equilibrium liquidus line, maximal ice formation results in a glass transition temperature known as the T'_g. However, it is apparent from freezing rate studies that the concentration of solute in the glass may be lower than expected as a result of less than maximal ice formation, resulting in a $T_g < T'_g$. The addition of polysaccharides to sugar solutions has no significant effect on T'_g, but increases the mechanical properties of the solution at $T > T'_g$. Sugar and sugar/polysaccharide solutions can be used as model systems to determine the behaviour of several categories of frozen foods, such as fruits, frozen dairy desserts, or other types of sugar-added food systems. Storage of such frozen foods under conditions where the unfrozen, freeze-concentrated phase is in the vitreous state may greatly improve stability and shelf-life. An examination of the low-temperature thermal behaviour of carbohydrate solutions also provides considerable insight into the action of polysaccharides in improving the stability of frozen foods to ice recrystallization.

Keywords: Glass; Frozen food; Stability; Transition

1. Introduction

Over the last two decades, food freezing as a means of preservation has gained increasingly widespread attention. Reduction of available water as ice, coupled with sub-zero temperatures, provides an environment that favours reduced chemical and enzymatic reactions, microbial growth and structural collapse of tissues, hence leading to increased storage stability. Frozen prepared foods are also convenient for the

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[1] Dedicated to Professor Hiroshi Suga.

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consumer. However, fundamental factors that accompany ice formation are relatively complex. The freezing process, which includes under-cooling nucleation (homogeneous and heterogeneous), ice crystal propagation and maturation, is strongly influenced by thermodynamics (heat transfer properties), kinetics (mass transfer properties), and product (composition and size) variables. Modification of these variables can ultimately lead to large changes in ice distribution and subsequent quality of the product.

Current research interests focus more on kinetic rather than thermodynamic processes that occur during freezing of aqueous systems. The formation of a supersaturated state or glassy domain resulting from freeze-concentration has potential implications for improvement of sub-zero temperature stability. This cryostabilization approach to frozen food stability draws non-equilibrium concepts and ideas from glassy polymers. The mechanisms involved during formation of the glass state, kinetic and thermodynamic stability above and below the glass transition temperature (T_g), the concept of partial or dilute glass formation, and the process of devitrification all become relevant to food freezing.

Research from this laboratory has focussed on the determination of thermal and mechanical behaviour of aqueous solutions between −80 and 0°C as a function of freezing rate and the addition of polysaccharide stabilizers, and the relevancesac of this behaviour to frozen food systems, particularly frozen dairy products. The objectives have been to gain a better understanding of the formation of the glassy state as a function of both composition and processing variables, to elucidate the mechanisms of polysaccharide stabilizer functionality, to examine the relevance of the glass transition to frozen food stability, and to explore alternative mechanisms to improve the stability of frozen foods. This review will therefore focus on issues related to these research areas and objectives.

2. The glassy state in frozen systems

Food systems are very heterogeneous and many contain relatively high solute concentrations, which increase through the process of freeze-concentration during freezing. Hence, non-ideal, non-equilibrium behaviour becomes increasingly more dominant as the quantity of ice increases with a decrease in temperature. An approach for the study of concentrated non-equilibrium systems known as "Food Polymer Science" has recently evolved, mainly from the pioneering works of Levine and Slade [1] and Slade and Levine [2]. Using relationships developed from the field of synthetic polymer science, functional properties of completely amorphous or partially crystalline food systems during processing and storage can be predicted and explained based on mobility, as dictated by structure and composition of products. Time-and temperature-dependent functional properties and solute mobility are characterized by the glass transition temperature T_g and the effect of water on the diffusion-limited temperature range (rubbery, liquid states) above T_g during processing and storage [1–3]. The focal point of the approach is that the behaviour of food materials is governed by dynamics (mobility, diffusion, kinetics) rather than energetics (thermodynamics).

The foundation of the glass dynamic concept of food stability evolves around the physical properties of the glassy state and the change in translational mobility at T_g.

A glass is a non-equilibrium, metastable, amorphous solid of extremely high viscosity, i.e. 10^{10}–10^{14} Pas. The formation of a glassy supersaturated state in frozen food materials is achieved by the removal of large quantities of water by the process of freeze-concentration, since water is a readily crystallizable plasticizer [3,4]. During freezing, the glass-forming constituent of the system, e.g. a monosaccharide, disaccharide or polymeric material, must not precipitate and form a eutectic mixture, otherwise the system will be unable to supersaturate and vitrify. Fortunately, however, eutectic formation rarely occurs largely due to the high viscosities and low thermal energy [5–9]. Within the unfrozen, freeze-concentrated phase, translational mobility decreases to a point where the molecules are unable to achieve their equilibrium conformation and packing [10–12]. As a result, solidification (vitrification) occurs and the molecules within the unfrozen phase (UFP) are captured into a disordered state of excess volume and enthalpy, in contrast to the favourable (crystallized) state at the same temperature [11]. Hence, the glassy state is a non-equilibrium disordered solid (no lattice structure) that is unable to achieve any long-range cooperation relaxation behaviour and therefore achieves real "time structural stability" through high viscosity on a practical time scale [9]. It is this intrinsic slowness of molecular reorganization below T_g that the food technologist seeks to create within the concentrated phase surrounding constituents of food materials.

Consider the freezing of a 20% sucrose solution (large aqueous phase) having a theoretical T_g close to that of water (−135°C). The supplementary state diagram showing the solid/liquid coexistence boundaries and glass transition profile for a binary sucrose/water system is shown in Fig. 1. The initial T_g of this solution at room

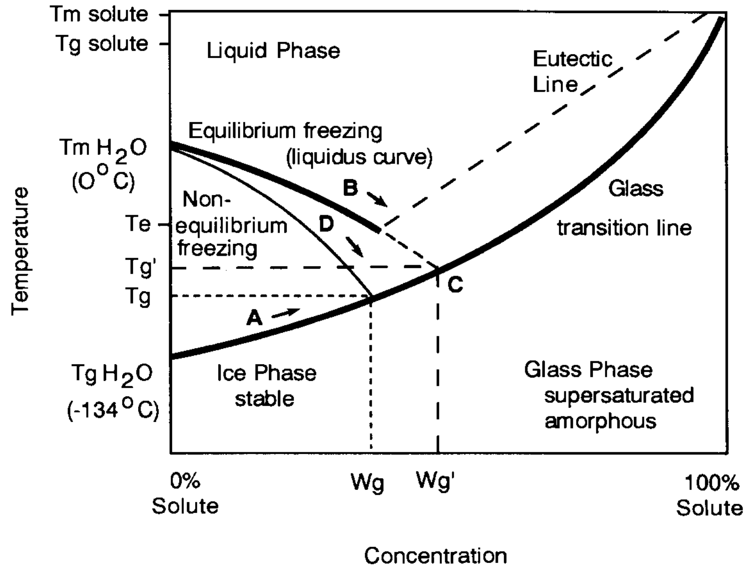

Fig. 1. Supplementary state diagram (not to scale) for an aqueous sucrose solution showing the glass transition line, liquidus curve, theoretical eutectic line and the various physical states defined within the boundaries. Details of specific points are provided in the text (from Ref.[8]).

temperature before phase separation is marked as A. Upon cooling of the system somewhat below its equilibrium freezing point (undercooling), nucleation and subsequent crystallization initiates the freeze-concentration process removing water (plasticizer) in its pure form. As ice crystallization proceeds, the continual increase in solute concentration (removal of plasticizer) further depresses the equilibrium freezing point of the UFP in a manner which follows the liquidus curve (shown as B in Fig. 1) while the T_g of the system moves up the glass transition line with a rapid increase in viscosity in a non-Arrhenius manner, particularly in late stages of the freezing process [13]. At all points on the liquidus line in Fig. 1, a ratio of ice to unfrozen water will exist, increasing with decreasing temperature, to satisfy equilibrium conditions based on the depressed freezing point. Assuming equilibrium freezing conditions are satisfied, which is unlikely during freezing of real food systems, the T_g continues to increase with increased ice formation and the system becomes supersaturated beyond the eutectic curve. It is important to note, however, that only the liquidus curve up to the eutectic (T_e) represents an equilibrium state, while beyond T_e, it depicts a state of thermal instability far from equilibrium [14]. As ice is separated from the liquid phase, T–T_g decreases causing a concurrent increase in viscosity.

When a critical, solute-dependent concentration is reached [15–17], the unfrozen liquid exhibits very resisted mobility and the physical state of the UFP changes from a viscoelastic "rubber" to a brittle amorphous solid. Using Fig. 1, the intersection of the non-equilibrium extension of the liquidus curve and kinetically determined non-equilibrium glass transition curve, denoted as C, represents the solute-specific, maximally freeze-concentrated T_g of the frozen system, denoted T_g', where ice formation ceases within the time-scale of the measurement [2, 18, 19]. The corresponding W_g' and C_g' define the maximum (perhaps best defined as practical) amount of plasticizing water unable to crystallize within the glass and sucrose concentration of the glass at T_g', respectively [2, 3, 18]. It is worth noting that this unfrozen water is not bound in an "energetic" sense, rather it is unable to freeze within practical time frames [3, 5, 7, 19–21].

At the T_g', the supersaturated solute takes on solid properties because of reduced molecular motion, which is responsible for the tremendous reduction in translational, not rotational, mobility [13, 15, 17, 19, 22, 23]. The significant reduction in free volume and the associated viscosity increase (10^{14} Pas) has been estimated to reduce the diffusion of a water molecule to 1 cm per 3×10^5 years[24] and hence, viscous flow of this supercooled liquid takes place at rates of μm per year. However, warming through the T_g' results in a tremendous increase in diffusion, not only from the effects of the amorphous to rubber transition but also from increased dilution as ice melting of small ice crystals occurs almost simultaneously ($T_g' = T_m'$). This is in contrast to polymers where, at $T > T_g'$, composition does not change, and hence is the source of some difficulty when assessing the effect of temperature above T_g'.

Despite the thermodynamic driving force to achieve the unfrozen water content corresponding to W_g', one must also consider the large kinetic factors which "overtake" the freezing process. At sub-zero temperatures, the formation of an amorphous state is time-dependent since the limiting factor of the process (water removal in the form of ice) becomes more difficult as concentration increases. The exponential effect of viscosity on mass transfer properties acts as the limiting factor for growth. In addition, under conditions where heat removal is rapid, a high level of undercooling at the interface will only add to a further decrease in propagation rate. The net result is that freezing becomes progressively slower as ice crystallization is hindered and consequently more time is required for lattice growth at each temperature. Therefore the kinetic restriction imposed on the system can lead to a situation in which non-equilibrium freezing, resulting in a partial dilute glass, can occur [13, 15].

The typical pathway a system may follow during non-equilibrium freezing is shown in Fig. 1 as the line leading to lower T_g (point D) than T_g' with a corresponding lower C_g due to excess undercooled water plasticized within the glass. The magnitude of deviation from the equilibrium curve may be regarded as a function of the degree of departure from equilibrium. Systems possessing this undesirable structure may undergo various relaxation–recrystallization mechanisms in order to maximally freeze-concentrate and minimize the unfrozen water content. As a result, during warming, systems formed under these conditions may lead to one or more low temperature transitions, followed by an exothermic devitrification peak due to crystallization of immobilized water, and finally the onset of ice melting, T_m [13, 15, 25, 26,].

3. Methods for determination of T_g

Since the glass transition manifests itself as a change in thermal and mechanical properties, thermal analysis techniques are most commonly used to study this phenomena [27]. In particular, measurement of the change in heat capacity (C_p) using differential scanning calorimetry (DSC) has become the established method, largely because of its ease of operation and widespread availability. However, a measurable heat change at T_g may be problematic for some glass-forming materials since ΔC_p can be small, which depends largely on the type of glass, i.e. "fragile" or "strong" as described by Angell et al. [28], and the degree of polymerization, as in the case of maltose oligomers, which showed a decrease in ΔC_p with increasing polymerization [29]. The relationship between ΔC_p and the accompanying T_g is obscure and still poorly understood [9, 22]. Recent advances in modulated (M)DSC, where sinusoidal, oscillating temperature profiles are overlaid onto linear heating gradients, may prove to be more useful in defining glass transitions than conventional DSC. Other techniques, namely dynamic mechanical analysis (DMA or DMTA) [13, 30–32] and thermomechanical analysis (TMA) [8, 16, 33–38], have been used to characterize phase behaviour in frozen systems. Because T_g is a time and frequency dependent phenomenon (occurs over a temperature range), dielectric analysis (DEA) as well as other spectroscopic methods, such as NMR [37, 39–41] and electron spin resonance (ESR) [42–44], are also useful since they allow for the measurement of relaxation times more applicable to time/frequency dependent materials. However, as with most investigations, the effective approach when studying glass transitions and glasses in food systems is the combination of various thermal analysis techniques (DSC, DEA, TMA, DMTA) each complimenting the other.

4. Interpretation of sub-zero events: the identification of T_g'

The practical importance of T_g' from a theoretical standpoint is well established and agreed upon by many in the field. However, there exists a wide variation in published T_g' values, mainly as a result of different interpretations of the same DSC-measured transitions of warming curves of many low molecular weight (MW) carbohydrates. A typical warming DSC curve for a slowly cooled (5°C min^{-1}) 40% sucrose solution is presented in Fig. 2 (dotted line). Based on the original description of T_g', consistent with the state diagram, glass relaxation and ice melting should coincide within the time-frame of the DSC experiment. However, due in part to the possibility of partial freeze-concentration, biphasic behaviour is always evident with a measurable devitrification peak between the transitions once sufficient translational mobility is regained. Ablett et al. [17], using optical microscopy, confirmed that the exotherm in vitrified sucrose solutions is the result of delayed crystallization. Sahagian and Goff [16] have also shown that the magnitude of this exotherm is related to the applied freezing rate (increases with increased rate) and the initial concentration, consistent with the effect of imposed kinetic constraints on the process of ice formation. For discussion purposes, these two transitions are labelled as $T_{Tr1}$and T_{Tr2} in Fig. 2 similar to the method adopted by Sahagian and Goff [16].

At present, there are three major interpretations regarding the origin of each transition and the assignment of T_g': (1) the T_g' is represented by the major transition at T_{Tr2} (Fig. 2) while the lower temperature T_g (partial glass) is usually ignored [1–

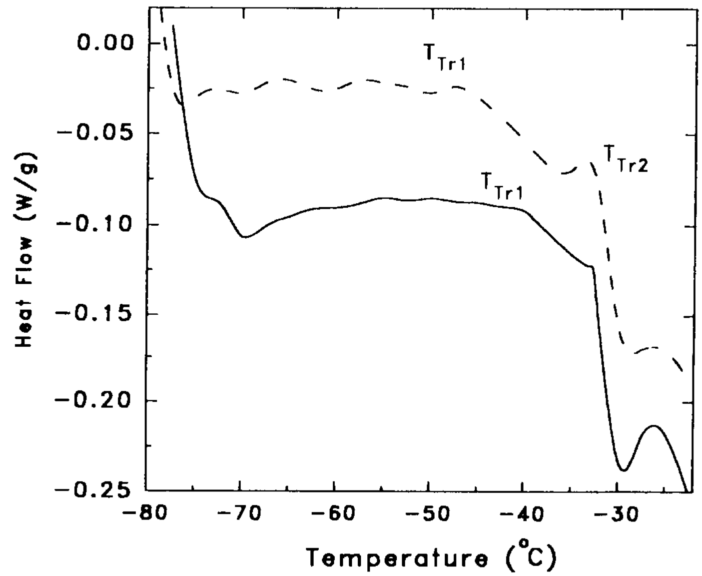

Fig. 2. Thermal behaviour of 40% sucrose solutions following slow freezing (- - -) (2°Cmin^{-1}) and slow freezing after annealing at −35°C for 60 min (——) showing the onset of transitions T_{Tr1} and T_{Tr2} (from Ref. [45]).

3, 5, 14, 18, 46, 47]; (2) the T_g' occurs at the lower transition (T_{Tr1}) while the warmer transition is not a glass transition but rather the onset of ice melting (first-order transition) initiated soon after the T_g' [13, 15–17, 30, 48, 49]; and (3) the T_g' is close to T_{Tr2} [47] or somewhere between the two transitions [31, 50, 51] and the complex behaviour is partially the result of a relaxation process manifested as an excess enthalpy overshoot close to the T_g'. Table 1 represents a more complete summary of the recently published T_g' and C_g' values for freeze-concentrated carbohydrate solutions of sucrose, glucose and fructose. In the case of sucrose, it is evident from the table that there are three different temperature (T_g') ranges: −32– −35°C; −37– −42°C; and −46°C below. Adding to the difficulty is the fact that some reported values are the midpoint of the transition [3, 18, 47], while others represent the onset [13, 15, 16]. Moreover, annealing temperature and times are not consistent and, in the case of values reported by Levine and Slade [3, 18], T_g' values are from non-annealed systems. In any case, it is evident that clarification of these thermal transitions in frozen systems is a priority since new technologies and processes that attempt to use this approach must rely on the position of T_g' under specific conditions.

Some of the debate on whether T_g' labelled by group (1) above is a glass transition stems from recently reported ΔC_p values at this transition [16, 17, 49]. These researchers have reported that ΔC_p is $\approx$ 3 times larger than expected, as determined from completely amorphous (no ice) supersaturated systems. Sahagian and Goff [16] have shown that the lower transition has a ΔC_p in close agreement with ΔC_p for a low-level plasticized sucrose glass of approximately 80%. However, an analysis of ΔC_p for the galactose–water system by Blond and Simatos [50] showed that changes in C_p are associated with the T_g of the freeze-concentrated phase and the second (higher) transition overlaps the melting endotherm. However, as pointed out by Slade and Levine [22], and discussed in detail by Angell et al. [28], "fragile" and "strong" glass-formers have substantially different relaxation properties and magnitudes in ΔC_p. In addition, Hatley and Mant [47] have shown that at the T'_g in dilute solutions, a significant contribution from a relaxation process, as well as the onset of ice melting, gives rise to a large distorted T'_g. The identification of an enthalpy relaxation at T'_g was previously reported by Blond and Simatos [50] and Simatos and Blond [51], and recently confirmed by isochronal annealing experiments performed by Sahagian and

Table 1
Published T_g' and C_g' values for freeze-concentrated sucrose, glucose and fructose solutions

Glass former	T_g' (°C)	Ref.	C_g' (%)	Ref.
Sucrose	−32	1,2,5,14,18,35,42,46,47	64	2,5,14,18
	−40	16,17,31	80	13,15,34,49
	−46	13,34	83	46,47,52
Fructose	−42	1,5,14,18,40	51	2,5,14,18
	−53	13,52	79	13
			83	52
Glucose	−43	1,5,14,18,35,40	71	2,5,14,18
	−52	13,52	80	13,52
			83	51

Goff [16]. Furthermore, an increase in plasticizer and mean MW increases free volume, which tends to result in higher ΔC_p at T_g [15]. Therefore, it appears there may be no simple relationship between ΔC_p and T'_g, making identification using ΔC_p rather difficult.

Several researchers have demonstrated that proper annealing protocols within a narrow temperature range between T_g and onset of melting T_m are necessary to allow for delayed crystallization [13, 15–17, 49]. It is worthy of note, however, that it is very difficult to form ice at concentrations above 70% solute without extensive annealing (long times) and/or temperature cycling and therefore it is virtually impossible to maximally freeze-concentrate within realistic time-frames [7, 44, 45, 49, 53]. Changes in T_{Tr1} with annealing temperature (T_a, 1 h) for freeze-concentrated 40% and 60% sucrose solutions are shown in Table 2. The increase in T_{Tr1} has been interpreted as the T_g of diluted glass approaching the T'_g of the system by removal of excess undercooled water through the process of devitrification. Consider an initial T_g of approximately −46°C [13, 15, 16]. Annealing at −55 and −50°C is within the amorphous state and hence little change in ice fraction is expected or observed. However, above the initial T_g and after annealing for 60 min between −45 and −35°C, the T_{Tr1} shows a continual increase, but never reaches the specific T_a. This result again demonstrates the effect of extreme viscosity on ice formation in highly concentrated solutions and consequently more time is required for the UFP to reach a concentration associated with T_a. Ablett et al. [17] demonstrated that annealing times of 16 h were required for T_g of the UFP to reach the annealing temperature, i.e. maximal ice formation at that temperature.

An important point to note from the annealing study of Sahagian and Goff [16] is that two transitions are still evident, although the devitrification peak is no longer detectable (Fig. 2, solid line). However, because the ΔT between the observed T_{Tr1}(onset) and T_a is relatively large ($\Delta T \approx 5$°C) and a substantial reduction in T_{Tr1} is seen at $T_a > -35$°C (ice melting, increased plasticizer), it is possible that this transition is not the "true" T'_g but is rather best defined as being very close to the experimental maximum freeze-concentration — a "practical" T'_g. Another possibility is that this

Table 2
T_{Tr1} onset of 40 and 60% (w/w) sucrose solutions slowly frozen (2°C min^{-1}) following isothermal annealing at various temperatures for 60 min

Annealing temperature/°C	T_{Tr1} onset /°C, 40% (w/w)	T_{Tr1} onset/°C, 60% (w/w)
−55	−54.2 (1.19)[a]	−54.6 (1.23)
−50	−49.4 (0.93)	−52.1 (1.38)
−45	−47.9 (0.85)	−50.3 (0.95)
−40	−46.1 (1.15)	−48.1 (0.91)
−37	−44.5 (0.88)	−47.4 (0.98)
−35	−40.1 (0.78)	−45.0 (0.99)
−32	−48.2 (0.96)	−46.2 (1.15)
−28	−49.1 (0.91)	−47.4 (0.95)
−20	−51.8 (1.19)	−52.9 (2.23)

[a] Standard deviation of 3 determinations.
Source: Ref [45].

small separation is simply the result of a finite warming rate and infinitely small heating rates may lead to one transition.

These data also suggest that T_{Tr2} has at least a significant first-order component, namely the onset of ice melting representing the "achievable" freezing point depression temperature of the UFP. From Fig. 2, the two transitions appear to be strongly related in origin. An increase in T_a with 60% solutions also results in fairly linear increases in T_{Tr2}, not an expected decrease as concentration increases [45]. This may suggest other factors: possibly a reduced free volume, facilitated by aging, plays a role in the position of T_{Tr2}, and the small change in T_{fp} with C_g is not significant enough to be measured at such high concentrations. Another curious result is the occurrence of an overshoot at T_{Tr2} at T_a up to $-35°C$ as shown in Fig. 2. This strongly suggests that an amorphous component or domain above $-35°C$ can undergo relaxation and therefore must exist. The complexity of this phenomenon is exemplified by the fact that the 60% sucrose system does not follow exactly the same trend (Table 2). This may represent an overall change in kinetics of the system. Therefore, it is clear that the present understanding is far from complete and time-temperature relationships, crucial to the kinetics of ice formation, are of prime importance when dealing with non-equilibrium materials. Much of the ambiguity that exists may be attributed to a poor recognition of the significance of time (hence, kinetics) during freezing.

Modulated DSC may provide further information regarding the true nature of these two transitions. 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. In slowly frozen but not annealed samples of 20% sucrose solutions (Fig. 3), the total heat flow curve demonstrates previous results: a T_{Tr1}, a devitrification exotherm, and a more distinct T_{Tr2}, leading directly into the melting endotherm. The non-reversing heat flow indicates thatthe devitrification shows up as a large exotherm, followed by an endotherm at the T_{Tr2}, possibly associated with the onset of ice melting. The glass had tohacerelaxed at temperatures lower than T_{Tr2} in order to have shown this devitrification. The reversing heat flow contains the T_{Tr1} but also some portion of T_{Tr2}. This may represent only the change in C_p associated with melting, or it may indicate a second-order component in the T_{Tr2}. With proper annealing and a range of periods, amplitudes and heat-heat or heat-cool warming profiles, this will become more evident.

5. Relevance of the glassy state to frozen food stability

5.1. *Mobility at temperatures below* T_g *or* T'_g

One of the major components of the "glass dynamics" approach to stability is that within the amorphous phase ($T < T_g$), the extremely high viscosity (10^{14} Pas) hinders the diffusion of water molecules to the ice interface, thereby preventing crystallization/recrystallization mechanisms within realistic time frames [3, 5, 7]. However,there is evidence from NMR and ESR that unfrozen water is relatively mobile, possibly

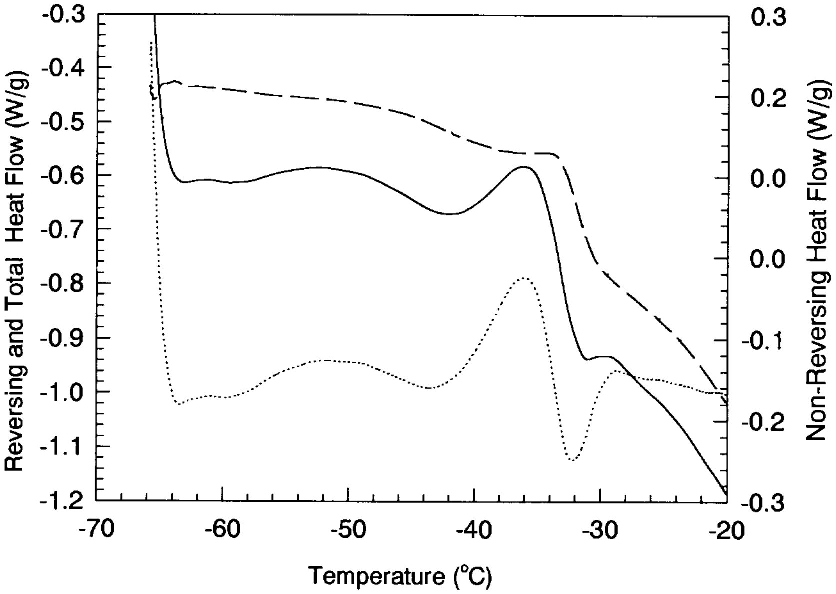

Fig. 3. Thermal behaviour of 20% sucrose solutions following slow freezing (2°C min^{-1}) as determined by Modulated DSC, showing the total heat flow (——), reversing heat flow (– – –), and non-reversing heat flow (. . . .) as a function of temperature.

resulting from sample inhomogeneity leading to components or domains within the sample of varying degrees of mobility. Water binding to sites has been found to be highly transient, occurring within the picosecond time-scale [54]. Moreover, there is NMR evidence to suggest that rotational and translational properties of water below T_g in amylopectin solutions continue to decline and show no temperature dependence at T_g, in contrast to the polymer itself [9, 55]. Within the glass, water molecules are substantially hindered and cannot crystallize, but indeed appear to be able to diffuse rapidly in comparison to the glass-forming molecules [9]. Also of particular scientific interest is the migration and removal of unfrozen water from amorphous materials during freeze drying at $T < T_g$, since it signifies that sufficient translational mobility is present to allow for secondary drying [56]. Therefore, a link may exist between the factors affecting the diffusion of residual water in solid solutions below T_g or T'_g and the nature and rates of measurable chemical reactions, in particular those which are not translational diffusion-limited [9,22]. Hence, storage below T'_g may not necessarily assure complete stability.

Slade and Levine [22] have recently suggested an explanation for this behaviour. They theorized that below the T_g of the blend but above the T_g of the diluent (water in foods), the free volume of the mixture is such that the critical volume for transport of the diluent is adequately met and hence it is able to diffuse. Additionally, as the temperature approaches the T_g of the diluent, free volume will continue to decrease towards the hydrodynamic volume of the diluent at which time diffusion becomes severely hindered. Based on this interpretation, frozen food stability at T_g' may depend on the resultant free volume of the UFP. It follows that the kinetics of relaxation processes below T_g', which substantially alters the total free volume of the glass, may modify diffusion and subsequent related effects (collapse, recrystallization and enzymatic activity).

With regard to frozen food stability, it must be appreciated that movement of water molecules may occur at appreciable rates and may be a function of initial stability and rate of relaxation, which in turn is dependent on freezing rate and temperature. Blond [31] has shown that 10% dextrin can substantially reduce the degree of physical aging and mobility of a freeze-concentrated sucrose glass. This may be a significant result based on the premise that mobility of the total system can affect the mobility of the constituents. However, the presence of the polymer may simply reduce the free volume enough to limit transport of the glass-forming solute but not the diluent. The question of how macromolecules affect structural rearrangement during aging is one that deserves further investigation since it may lead to better understanding of mobility below T_g.

Another study has recently been published [57] that focused on the effect of the glassy state on two diffusion-limited reactions, oxidation of ascorbic acid and an enzyme hydrolysis reaction, as well as the rate of protein insolubilization. The results demonstrated the potential difficulty in assigning a stable region in relation to T_g' since stability below T_g' appeared to be dependent on the glass former. For example, a maltodextrin glass system effectively controlled all reactions below T_g'. However, the same is not true for a CMC glassy system, where none of the reactions were inhibited at $T < T_g'$, while only partially true for the sucrose system, in which the protein insolubilizing reaction was effectively controlled close to T_g'. These results also suggest other factors may be important, e.g. free volume distribution related to the characteristics of the glass, i.e. degree of polymerization or branching. In an unrelated study, Carrington et al. [58] reported that the presence of CMC resulted in larger numbers of smaller ice crystals in rapidly frozen fructose solutions after storage at −75°C for two weeks, in comparison to fructose alone. Although a full explanation is not yet available, one possibility is that CMC stabilized the amorphous phase and reduced recrystallization. Stability below the T_g is not guaranteed and requires an experimental analysis to be confident that the chosen storage temperature is adequate.

5.2. *Mobility and ice recrystallization at temperatures above* T_g or T_g'

The effects of storage temperature on frozen food stability is of prime importance because of the large influence this temperature has on deleterious reaction rates that result in losses in nutrients and quality [51]. It has been proposed that frozen food stability, particularly the large effect of temperature on reaction rates of the UFP, may be modeled using Williams–Landel–Ferry (WLF) kinetics [18]. Unlike the Arrhenius equation, WLF relates the logarithm of time–temperature mechanical properties of amorphous materials to $T - T_g$ instead of 0 K. The WLF equation predicts a large decrease in viscosity (increased translational mobility) when the temperature is raised slightly above T_g [10]. However, Blond and Simatos [50] found that the rate of degradation in various frozen products did not follow WLF kinetics closely, especially the change in crystal growth in beef during storage. Although the WLF equation has successfully been applied to aqueous sucrose–fructose mixtures [4], it appears that a large number of other factors must be considered at temperatures above T_g'. Of these, the most important are the effects of ice melting. Specifically, increasing unfrozen water with temperature adds an additional reduction in viscosity, in addition to the WLF contribution, by diluting the systems. As well as increasing the ΔT, the T_g is plasticized to lower temperatures with increasing storage temperatures. Another important factor is the dilution of reactants, which could largely offset the effect of high viscosity. This dilution would be expected to reverse the large rate enhancement by freeze-concentration discussed earlier. Moreover, a possible change in the order of the reaction kinetics with concentration [5, 59], changing values of the WLF constants with moisture and composition [2, 3], and the fact that diffusivity at or above T_g' may or may not be directly related to increased free volume because of the size of the dilute molecule [51, 60], will only add to the complexity of evaluating the adequacy of the WLF equation. Recent TMA results on frozen sucrose solutions clearly demonstrate that translational mobility is greatly enhanced after the onset of melting [16] and show an increase in the rate of collapse above $-10°C$ [34], possibly greater than expected from WLF kinetics [2]. A rough estimate of the contribution of each variable to the overall decrease in viscosity has been presented by Simatos and Blond [51].

Ice crystals are relatively unstable and undergo changes in number, size and shape during frozen storage, known collectively as recrystallization. This process is a consequence of the surface energy between the ice and UFP [5]. Although the amount of ice remains relatively constant, over time this phenomenon can be extremely damaging to the texture of frozen products, e.g. ice cream [61]. At constant temperature, Ostwald ripening involves the growth of large crystals at the expense of smaller ones and is the result of the system moving towards reduced surface area. During temperature fluctuations, as temperature increases, small ice crystals tend to melt, while on cooling, this proportion of unfrozen water does not renucleate but rather is deposited on the surface of larger crystals [5]. The net result of both processes is that the number of crystals decreases whereas the average size and distribution will increase [5, 6, 8, 62, 63]. Recrystallization in frozen beef has been shown to increase with storage temperature leading to greater tissue disruption, protein denaturation and exudate production [63].

The frozen dairy products industry combats the problem of recrystallization by adding low concentrations of high MW stabilizers to the mix formulation [61]. In the presence of these compounds, recrystallizing processes are substantially reduced at normal storage temperatures where the UFP is relatively large. However, despite numerous studies, the mechanism involved is not well understood. Buyong and Fennema [64] studied the effect of gelatin on ice crystal growth in ice cream and concluded that the stabilizer had no effect on the amount, size and shape of ice crystals after freezing, or on the rate of recrystallization. The presence of locust beam gum (0.5%) in 35% corn syrup solutions was found to have a limited effect on ice crystal growth during temperature fluctuations [65]. However, stabilizers were found by cryo-scanning electron microscopy, in an ice cream study, to alter both initial crystal size and recrystallization rate after storage [66].

It has been difficult to demonstrate any real relationship between thermodynamic parameters or linear crystallization rates and the macroscopic effect of stabilizers [67–70]. Nor do stabilizers significantly affect thermodynamic transition after annealing in 20% sucrose solutions during warming in the DSC [32, 37] (Table 3). However, an interesting observation was that under rapid freezing conditions, the presence of xanthan gum appeared to hinder initial nucleation and/or ice crystal formation as demonstrated by the lower T_{Tr1} in Table 3. This is consistent with the claim that xanthan gum can exhibit a small inhibitory effect on nucleation [67]. In addition, xanthan systems showed significantly smaller ΔC_p values at T_{Tr2} [37]. The reason for this is not clear but may evolve from a fundamental change in the relaxation mechanism at this temperature.

What has been clearly demonstrated is that stabilizers strongly influence mechanical and hence the diffusion properties of the highly mobile UFP above T_{Tr2} in sucrose solutions, xanthan having the largest effect [36, 37]. Subsequent studies have also shown that stabilizers alter stress relaxation properties of frozen sucrose solutions, suggesting that rearrangement mechanisms for the dissipation of stress are modified [38]. It was hypothesized that once the critical concentration of the polymer was reached, extensive entanglement would occur, reducing free volume and greatly increasing the viscosity relative to a non-stabilized system [8, 36–38]. Blond [31] and Simatos et al. [32] have shown that the addition of a polymer, particularly guar gum, to partially frozen sucrose solutions greatly increased the elastic (storage) modulus in DMTA experiments. Entanglement phenomena contributed to these effects on rheological properties; however, these researchers also concluded that the ice fraction of the system plays a role in the rigidity of frozen systems. The same conclusion emerged from a recent study [71] on the rheological behaviour of ice cream as a function of temperature and stabilizer addition. Stabilizer addition was seen to decrease the storage and loss moduli and this may be attributable to its effect on ice crystal size

Table 3
Thermodynamic data for 20% (w/w) sucrose with and without 0.5% (w/w) xanthan gum, gelatin (275 bloom) and guar gum, rapidly cooled (liquid N_2) or slowly cooled (2°C min^{-1}) and annealed at −35°C for 60 min, as determined by DSC[a]

Sample	Rapidly frozen				Slowly frozen/annealed				
	T_{Tr1}[b]	$\Delta C_p T_{\mathrm{Tr1}}$[c]	T_{Tr2}[d]	$\Delta C_p T_{\mathrm{Tr2}}$[c]	T_{Tr1}[b]	$\Delta C_p T_{\mathrm{Tr1}}$[c]	T_{Tr2}[d]	$\Delta C_p T_{\mathrm{Tr2}}$[c]	ΔH_{m}[e]
Sucrose	−54.6	0.75	−32.2	3.4	−40.5	1.23	−31.4	3.81	220.2
Sucrose/xanthan	−58.1	0.48	−34.7	2.6	−42.5	1.31	−32.2	3.12	219.1
Sucrose/gelatin	−53.9	0.84	−32.7	3.6	−42.8	1.22	−32.6	3.75	206.2
Sucrose/guar	−54.2	0.85	−32.5	3.8	−42.5	1.24	−31.4	3.76	222.3

[a] Source: Ref. [37]. [b] Onset in °C. [c] Per gram of sucrose in Jg^{-1}K^{-1}. [d] Midpoint in °C. [e] Enthalpy of melt in Jg^{-1}.

distribution. These results warrant further investigation of the distribution of ice crystals since uniformity facilitates ordered packing and, on a theoretical level, greater rigidity. In summary, our present understanding of stabilizers is still limited, but based on the experimental evidence they appear to involve modification of kinetic parameters rather than changes to the thermodynamics of frozen foods.

6. Conclusions

Freezing involves the separation of water as ice in its pure form. This may appear to be a rather simple task largely controlled by a reduction in temperature. However, as our understanding of freezing has improved, it has become clear that the overall driving force of freezing and stability of frozen products is a complex balance of thermodynamic and kinetic factors. The potential of a glassy state for enhancement of frozen food stability relies on the individual steps in the freezing process that create maximum ice formation; otherwise, the glassy state may be fairly unstable. Therefore, it would be advantageous to develop freezing systems that take into account the relative importance of kinetics and thermodynamics as temperature is lowered such that "equilibrium" conditions can be approached.

We have tentatively concluded that the $T_{\mathrm{Tr}1}$ approaches the true T_g', but only after measures have been taken to maximize ice formation. The $T_{\mathrm{Tr}2}$ 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 MDSC research may help to answer some of these fundamental questions surrounding the two transitions. The role of polysaccharides is both important and intriguing, and it is certainly well known amongst industry practitioners that they play a crucial role. It is likely that stabilizer action does not involve modifications of thermodynamic behaviour but may involve a modification of the diffusion properties of components within the UFP above the $T_{\mathrm{Tr}2}$. Hyperentanglements resulting from freeze-concentration to greater than critical concentration values must play an important role in their action. Being aware of and respecting the complexities and interplay that exist among all variables in a freezing process will ensure that frozen foods will continue to enjoy widespread success.

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