Original article

Effect of trehalose on the glass transition and ice crystal growth in ice cream

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Summary The effects of trehalose and sucrose on the rate of ice crystal growth in ice cream during accelerated shelf-life were compared. Experimental and theoretical freezing curves were shown to be in good agreement. Glass transition temperatures (T_g) of maximally freeze concentrated trehalose and sucrose solutions (40% w/w) were found to be −39.5 °C and −47 °C respectively. For ice cream mixes, the T_g value increased from −46.4 °C for the 100% sucrose-based mix to −42.0 °C for the 100% trehalose sweetened ice cream. However, no differences in viscosity, nucleation rate or inhibition of ice crystal growth were observed with increasing trehalose concentration in ice cream.

Keywords Glass transition, ice cream, ice recrystallisation inhibition, trehalose.

Introduction

Ice crystal growth leading to iciness is one of the most problematic defects in ice cream (Marshall *et al*., 2003). Good quality ice cream has a smooth texture without any noticeable presence of detectable ice crystals when eaten. If present in sufficient numbers, ice crystals >40–55 μm can be perceived by the tongue as rough particles resulting in undesirable quality (coarse, grainy or icy texture) (Hartel, 1996; Russell *et al*., 1999; Marshall *et al*., 2003). The shelf-life of ice cream is strongly dependant on formulation, process and storage conditions. Composition, especially the sweetener system, has been shown to affect the freezing point, amount of water frozen at any given temperature and the glass transition temperature (T_g) of ice cream mixes (Bradley, 1984; Goff *et al*., 1993). It is generally accepted that the main factor influencing recrystallisation and ice crystal growth is the storage temperature. During temperature fluctuations (heat shock) melted water does not re-nucleate to form small ice crystals, instead, it re-freezes onto existing crystals making them bigger, thus over time large ice crystals grow at the expense of smaller ones (Flores & Goff, 1999a). Ice crystal growth rate was shown to increase as mean temperature increased from an almost negligible rate at −30 °C (Flores & Goff, 1999a, b) to a very rapid rate at −5 °C (Hagiwara & Hartel, 1996). Depending on the formulation, the T_g of ice cream can fall within the range −40 ± −10 °C. The temperature difference between T_g and the storage temperature is the main driving force in ice recrystallisation (Goff *et al*., 1993).

Trehalose is a naturally occurring non-reducing disaccharide composed of two glucose molecules bound together by anα, α1, 1 linkage. The T_g of pure trehalose is the highest of any disaccharide (115 °C) and is approximately 43 °C higher than that of sucrose (Nabors, 2001). It has been proposed that the higher T_g of the trehalose/water system may be responsible for its superior cryo- and lyo-protective properties *in vivo* (Green & Angell, 1989), although dextran, which has a significantly higher T_g than trehalose, is a much less effective *in vitro* cryoprotectant than trehalose (Crowe *et al*., 1994). A wide range of glass transition temperatures of amorphous trehalose has been reported (79 °C < T_g < 115 °C, Table 1). Similarly, a wide range of glass transition temperatures of maximally freeze concentrated trehalose solutions (T_g') has been reported (−46 to −22 °C) with some authors reporting two glass transitions (Table 1). The discrepancy may be because of many factors: purity of the sample, heating and cooling rate, annealing time and temperature, etc. Tadanori *et al*. (2002) demonstrated that trehalose delayed to a larger extent the growth of ice crystals compared to sucrose, especially at high concentrations (i.e. 41.7 wt%).

Recently, a novel enzymatic system has allowed for a considerable reduction in the production costs of trehalose, making it feasible for use in widespread applications (Nakada *et al*., 1995a,1995b; Cerdeira

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Table 1 Reported glass transition values for trehalose

	Trehalose (T_g) °C	Freeze concentrated trehalose (T_g') °C	Freeze concentrated trehalose (T_m') °C
(Slade & Levine, 1988)		−29.5	
(Green & Angell, 1989)	79		
(Roos, 1993)	107	−35	
(Saleki-Gerhardt & Zografi, 1994)	115		
(Her & Nail, 1994)		−31.8	
(Nicolajsen & Hvidt, 1994)		−30	
(Roos, 1995)		Onset −40 Midpoint −35 Endpoint −30	T_m'−30 C_g' 81.6
(Cardona *et al.*, 1997)	110		
(Miller *et al.*, 1997)	114.9	−22	
(Wang & Haymet, 1998)		−37.5	
(Pyne *et al.*, 2003)			
Cooling rate 0.1 °C min^{-1}		−41	−31
Quench cooled		−46	−34
Annealing −36 °C, 2 h		−38	−31
Annealing −38 °C, 8 h		−37	
(Singh & Roos, 2005b)	112	−42 ± 1	T_m' = -32 ± −1
Annealing −31 °C, 15 min			C_g' = 79%

et al., 2005; Vega *et al.*, 2007). Trehalose has been shown to have a clean taste profile with no aftertaste and a sweetness profile that is characterised by a rapid onset but unlike sucrose, the sweetness of trehalose increases in a non-linear fashion with increasing concentration. At a concentration of 22.2% it has been reported to be ~45% as sweet as a 10% sucrose solution (Portmann & Birch, 1995). Table 2 shows a comparison of various properties of sucrose and trehalose.

The objective of this study was to assess the potential application of trehalose in an ice cream formulation by comparing its effects on delaying ice recrystallisation to sucrose.

Materials and methods

Ice cream mix formulations contained 10% anhydrous milk fat (Gay Lea Foods, Guelph, Canada), 12% milk-solids-not-fat (from skim milk powder, Gay Lea Foods, Guelph, Canada), 15% sucrose (Redpath Sugar, Toronto, Canada) or trehalose (Hayashibara International, Okayama, Japan) or combinations thereof, 0.06% guar gum, 0.015% carrageenan and 0.075% mono- and di-glycerides (Danisco, Toronto, Canada), for a total solids of approximately 37% and a theoretical freezing point of −2.6 °C. The content of sucrose (15–0%) and trehalose (0–15%) was varied at 3% increments.

Dry ingredients were dispersed in warm water at 40 °C. At 50 °C the fat source was added. Ice cream mixes were batch pasteurised at 75 °C for 15 min, homogenised in two stages (21 MPa/3 MPa, APV Gaulin, Everett, MA, USA), cooled to 4 °C and aged overnight. Ice cream was frozen in a batch freezer (Taylor Freezer Model 104, Rockton, IL, USA) to a draw temperature of −5.0 °C ± 0.5 °C. The residence time in the freezer was held constant at 10 min. Overrun was measured and samples were drawn into cylindrical plastic containers (250 mL) and immediately placed into a hardening room at −35 °C for storage until analysis.

Mix viscosity measurements were performed at 4 °C after ageing at increasing shear rate from 0.01 to 10 s^{-1} with a TA AR2000 controlled stress rheometer (TA Instruments, New Castle, DE, USA) using an aluminium DIN concentric cylinder (14/15 mm diameter).

The theoretical ice cream mix freezing point and freezing curve were calculated based on the method of Leighton (1927) as subsequently modified (Bradley & Smith, 1983; Bradley, 1984; Marshall *et al.*, 2003).

Freezing points and glass transition temperatures were measured with a modulated temperature differential scanning calorimeter (DSC, Q1000 TA Instrument, New Castle, DE, USA). The instrument was calibrated using sapphire, gallium (mp 29.8 °C; ΔH = 80 J g^{-1}) and indium (mp = 156.6 °C; ΔH = 28.45 J g^{-1}). Nitrogen (150 mL min^{-1}) was used as a purge gas. Hermetically sealed alod-al pans (TA Instruments) were used; sample size was ~15 mg. About 40% sucrose (Fisher, Toronto, Canada) and trehalose (Hayashibara International, Okayama, Japan) solutions were prepared and tested immediately. Ice cream mix samples were also analysed. The temperature protocol applied during DSC was similar to that used by Goff *et al.* (2003): equilibrate at 25 °C, ramp 2 °C min^{-1} to −25 °C, ramp 2 °C min^{-1} to −10 °C, isothermal 20 min, ramp 2 °C min^{-1} to −80 °C, anneal by heating at 2 °C min^{-1} to −35 °C,

Table 2 Properties of sucrose and trehalose

	Glycemic index	MW	Freezing point depression	Relative sweetness	Calorie content (Kcal g^{-1})	Solubility w/w% 25 °C	Caries promoting	Relative cost	Molecular formula
Sucrose	59 ± 10	342	1	100	4.0	67/High	Yes	1	$C_{12}H_{22}O_{11}$
Trehalose	Unknown	378	0.9	45	3.62	>45 Med	No	3–4	$C_{12}H_{22}O_{11}.2H_2O$

Jenkins *et al.* (1981); Marie (1991); Portman & Birch (1995); Nakada *et al.* (1995a, 1995b) DuBois (2000); Nabors (2001).

holding for 60 min and then back to −80 °C at 2 °C min^{-1}, modulation on (amplitude ±0.318 °C every 60 s), isothermal 5 min, ramp 2 °C min^{-1} to 5 °C. Duplicate runs were made and results were averaged. Freezing points were calculated from the DSC freezing curves using the step transition tool in the TA Universal Analysis software. The temperature at which the steepest slope was observed on the curve for the initial freezing step was taken as the freezing point. Using the method of De Cindio *et al.* (1995) as applied by Goff *et al.* (2003) the amount of ice frozen at a given temperature was calculated, generating freezing curves for the different solutions tested. The T_g was determined by constructing tangents to the DSC curve baselines before and after the glass transition. The intersection of these tangents to the tangent at the inflection point gives the extrapolated onset, midpoint and endpoint temperatures. Two transitions were reported, the T'_g (lower transition temperature) and T'_m (higher transition temperature).

Nucleation assays were performed on 20% (w/w) solutions of sucrose and trehalose. A microscopic slide was covered with para-film® to ensure the drops remained in place. Twenty drops of similar volume of each solution were placed on opposite ends of the same slide to ensure they were subjected to the same freezing conditions. The slide was then placed on the cold stage to equilibrate at 0 °C. The temperature was lowered to −12 °C at a rate of 1 °C min^{-1} and held for 60 min. The total number of droplets nucleated (i.e. frozen) was counted every 5 min. Plotting the number of droplets nucleated vs. time gave an indication of the rate of nucleation. Triplicate runs were made and results were averaged.

For ice recrystallisation analysis, three containers of each ice cream formulation were transferred to a cabinet freezer at −20 °C. Each sample was subjected to a programmed heating and cooling cycle of 48 h, during which the freezer was kept at −20 °C for 12 h, then warmed at a rate of 0.83 °C h^{-1} to −10 °C, held for 12 h, and cooled at a rate of 0.83 °C h^{-1} back to −20 °C. This heating/cooling cycle was repeated twenty times. Micrographs were acquired from the uncycled and cycled samples using the method of Regand & Goff (2002). Ice crystal size distributions were characterised by the logistic dose–response model with a cumulative distribution of equivalent diameters, obtaining the ice crystal diameter at 50% of the cumulative distribution function of the sample (X_{50}) and the slope of the cumulative distribution at X_{50}. The equation of this function was

$$(y = a/(1 + (x/b)^n))$$

where a = asymptotic value (100%), b (or X_{50}) = inflection point and represents the diameter at 50% of the cumulative distribution function (i.e. median of the fitted data), and n = parameter related to the rate of change (slope) of the distribution (Flores & Goff, 1999a). The rate of crystal growth in the sample was calculated with the following equation, where the X_{50} values are taken from the averaged means of the three containers for each ice cream formulation.

$$\%\text{Rate of growth} = \frac{(X_{50}\text{ after cycling} - X_{50}\text{ before cycling}) * 100}{X_{50}\text{ before cycling}}$$

Statistical analysis of the data was carried out using Microsoft Excel 2000 (Microsoft Corporation, USA), ANOVA single-factor test. When significant effects were evidenced ($P < 0.05$) between sample treatments, T-test (LSD) was used to compare the means of each parameter.

Results and discussion

No difference in viscosity was observed between mixes (Table 3). This suggests that any possible inhibition of ice crystal growth would not be related to differences in viscosity of the freeze-concentrated unfrozen phases after freezing resulting from the different sugars, since, unlike polymers, the viscosity would increase almost linearly with concentration. Nucleation typically occurred between −17 and −18 °C for the 40% solutions and −13 and −14 °C for the ice cream mixes. There was no difference in the rate of ice nucleation in either the sucrose or trehalose solutions using the cold stage method. Over the 60 min period droplets nucleated randomly on the cold stage and were difficult to replicate with large standard deviations (Fig. 1).

Table 3 Apparent viscosity and experimental initial freezing points measured by DSC for solutions and ice cream mixes containing varying levels of sucrose (Suc) and trehalose (Tre)

	Solutions		**Ice cream formulation**					
	40% Sucrose	**40% Trehalose**	**15% Suc/0% tre**	**12% Suc/3% tre**	**9% Suc/6% tre**	**6% Suc/9% tre**	**3% Suc/12% tre**	**0% Suc/15% tre**
Apparent viscosity at 1 s^{-1} (Pa s)			1.52^a ± 0.23	1.55^a ± 0.09	1.42^a ± 0.19	1.46^a ± 0.04	1.55^a ± 0.21	1.50^a ± 0.13
Experimental freezing point (°C)	-5.0^b ± 1.2	-4.8^b ± 0.6	-2.4^c ± 0.7	-2.3^c ± 0.5	-2.4^c ± 0.2	-2.7^c ± 0.3	-2.4^c ± 0.4	-2.5^c ± 0.4

Means with different superscripts in the same row are significantly different. Means from duplicate readings from three separate batches (n = 6). (±SD).

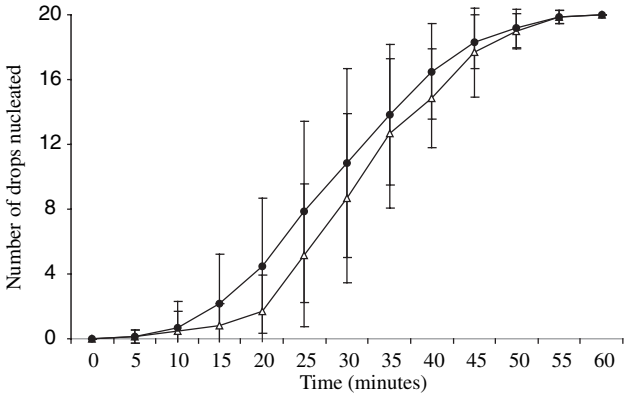

Figure 1 Nucleation rates of trehalose (●) and sucrose (Δ) solutions.

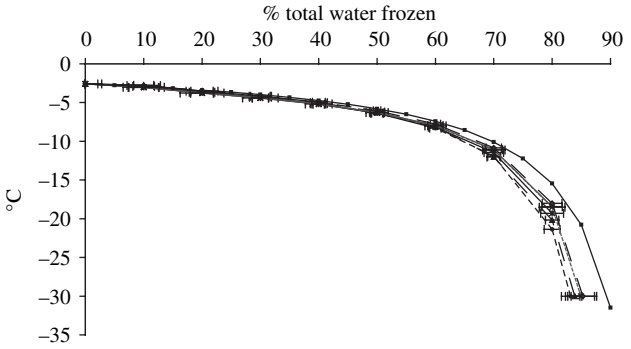

Figure 2 Theoretical (no symbols) and experimental freezing curves for ice cream mixes with 15% sucrose (◇), 12% sucrose/3% trehalose (Δ), 9% sucrose/6% trehalose (o), 6% sucrose/9% trehalose (*), 3% sucrose/12% trehalose (×) and 15% trehalose (−).

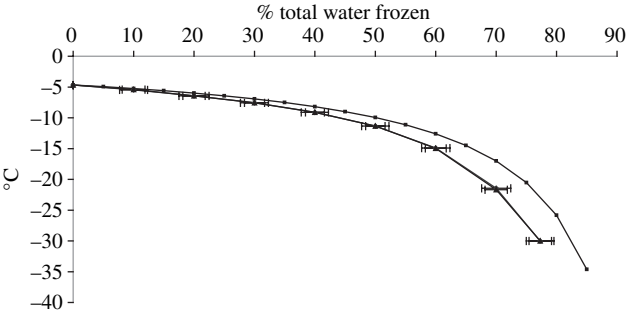

Figure 3 Theoretical (no symbols) and experimental freezing curves for 40% trehalose (Δ) and 40% sucrose (×) solutions.

Nonetheless, the sucrose system seemed to show a slower nucleation rate, especially within the first 20 min of the experiment.

The theoretical freezing points were calculated as −2.6 °C for all ice cream formulations and −4.7 °C for 40% sucrose and 40% trehalose solutions. As sucrose and trehalose have the same molecular weight, no differences in initial freezing points were expected. The 40% solutions and ice cream mix formulations had the same theoretical and experimental initial freezing points (Table 3), in agreement with previous findings (Livney *et al.*, 2003). However, it did prove difficult to measure the experimental initial freezing points using the DSC since a large sample variance was observed. The use of an osmometer would have allowed for more accurate estimation of initial freezing point (Baer & Keating, 1987).

Using the method of De Cindio *et al.* (1995), the amount of ice frozen at any given temperature was calculated, generating freezing curves for the different ice creams and solutions tested. As temperature decreased, water was frozen out of solution as ice crystals, which led to a concomitant increase in solute concentration. Calculated and measured freezing curves for the ice cream mixes were seen to deviate beyond 50–60% of total water frozen for the ice cream and the difference increased as the freezing process advanced (Fig. 2). Calculated and measured freezing curves for the 40% solutions were seen to deviate beyond 35–40% of water frozen (Fig. 3). Livney *et al.* (2003) reported similar findings in model ice cream systems. A comparison of the freezing curves from this study to those of the Livney *et al.* (2003) study shows that the experimental curves matched the theoretical more closely in this study. This can be attributed to the use of different DSC protocols, as the protocol used in their study may not have allowed for maximum formation of ice. Only the total amount of ice is determined using the DSC method, therefore, if all the water in the system is not converted into ice, inaccuracies may appear. An increase in the annealing time in this study from 30 to 60 min ensured a more complete freeze concentration step.

Two thermal transitions are reported in Table 4, a lower transition and a warmer transition (Goff *et al.*, 2003). We considered the lower temperature transition as T_g' (the glass transition of the maximally freeze-concentrated unfrozen phase) after annealing for 60 min at −35 °C (Goff *et al.*, 2003) and the midpoint of the warmer transition to represent the onset of melting of ice, T_m' (Roos & Karel, 1991a, 1991b; Knopp *et al.*, 1998). The small standard deviation for the glass transition values indicated the good reproducibility of the data. No devitrification (indicative of incomplete freeze concentration (Roos, 1995)) was observed in either the 40% solutions or ice cream mixes. The glass transition of the maximally freeze-concentrated 40% (w/w) trehalose solution (T_g' −39.5 °C) was found to be 7.5 °C higher than that of the 40% sucrose solution (−47 °C) (Table 4), the latter being in close agreement with previous reports (Goff *et al.*, 2003; Singh & Roos, 2005a). The T_g' value of trehalose (−39.5 °C) found in

Table 4 Glass transition and melting temperatures for 40% solutions and ice cream mixes containing varying levels of sucrose and trehalose (±Standard deviation)

	T_g' (°C)			T_m' (°C)		
	Onset	**Midpoint**	**Endpoint**	**Onset**	**Midpoint**	**Endpoint**
Solutions						
40% Suc	−50.4 ± 0.2	−47.0 ± 0.3	−44.7 ± 0.2	−34.3 ± 0.09	−32.4 ± 0.2	−31.1 ± 0.6
40% Tre	−42.6 ± 0.7	−39.5 ± 0.2	−37.1 ± 0.2	−31.2 ± 0.04	−29.6 ± 0.07	−28.6 ± 0.1
Ice cream mixes						
15% Suc/0% tre	−49.5 ± 0.8	−46.4 ± 1.0	−44.6 ± 1.5	−34.3 ± 0.1	−32.5 ± 0.4	−31.8 ± 0.4
12% Suc/3% tre	−48.6 ± 0.8	−45.4 ± 0.5	−43.5 ± 1.3	−33.9 ± 0.2	−32.4 ± 0.6	−31.8 ± 0.3
9% Suc/6% tre	−47.6 ± 0.6	−44.4 ± 0.4	−42.8 ± 0.3	−33.7 ± 0.1	−31.9 ± 0.04	−31.0 ± 0.3
6% Suc/9% tre	−46.6 ± 0.9	−43.5 ± 0.3	−42.6 ± 0.8	−33.6 ± 0.1	−31.8 ± 0.1	−30.8 ± 0.2
3% Suc/12% tre	−45.6 ± 0.9	−43.5 ± 0.5	−41.7 ± 1.0	−33.3 ± 0.08	−31.6 ± 0.1	−30.7 ± 0.3
0% Suc/15% tre	−44.5 ± 0.5	−42.0 ± 0.2	−41.1 ± 0.8	−33.1 ± 0.5	−31.4 ± 0.2	−30.5 ± 0.4

this study was 1.5 °C lower than that reported by Pyne *et al.* (2003). This discrepancy may be because of a number of factors, e.g. purity of substance, difference in annealing time, heating and cooling rate, etc. For the ice cream systems, T_g' values increased with increasing trehalose concentrations. The T_g' value increased approximately 4.5 °C from −46.4 ± 1.0 °C for the 15% sucrose sweetened ice cream to −42.0 ± 0.2 °C for the 15% trehalose sweetened ice cream (Table 4). An increase of 1 °C for the T_m' value was small in comparison (from −31.8 °C to −30.5 °C). A narrowing of T_g' was observed from ∼−5°C for the 15% sucrose (onset −49.5 ± 0.8 to −44.6 ± 1.5) to ∼−3.5 °C for the 15% trehalose (onset −44.5 ± 0.5 to endpoint −41.1 ± 0.8) sweetened ice creams. No narrowing of the transition was observed in the T_m' with increasing trehalose concentration. Since the T_g of trehalose is higher than sucrose, the increases were expected with increasing trehalose concentration. The transitions (T_g' and T_m') that were observed in the ice creams were not as strong as in the 40% solutions, since the ice creams contained only ∼21% disaccharide (including lactose).

Ice crystal size distributions in the ice creams were characterised by the values for the ice crystal equivalent diameter at 50% of the cumulative distribution (X_{50}, Table 5). The X_{50} before cycling (after hardening) for the 15% trehalose sweetened ice cream was significantly larger than the rest of the ice cream formulations, which was an interesting result since, based on the work of Tanadori *et al.* (2002) the opposite would have been expected. As shown by previous ice crystal growth studies (Hagiwara & Hartel, 1996; Flores & Goff 1999b) the X_{50} values increased for all ice creams because of the temperature cycling. No significant difference in X_{50} after cycling or % ice crystal growth rate was observed. The X_{50} after cycling lies between 71 and 85 μm, which represented a 132–180% growth in the size of the initial ice crystals because of the temperature cycling. This suggested that increasing trehalose concentration did not contribute significantly to the inhibition of ice crystal growth. However, it is worth noting that when the 15% trehalose mix was excluded and ANOVA was performed on the other five mixes a downward trend ($P = 0.094$) was evident. Despite the difference in T_g' values between the ice creams, the cycling temperature was probably too far above the glass transition temperature for any major effect to be exerted.

Tadanori *et al.* (2002) showed that, in solutions, trehalose was twice as effective in suppressing the growth of ice crystals than sucrose. When the trehalose concentration was 41.7 wt.%, the growth rate was 25% of that observed at a 20.8 wt.% concentration. However, the authors only reported results observed on individual ice crystals and no standard deviation was provided. The fact that trehalose was used at a concentration greater than 20.8% made it difficult to extrapolate their results to those shown in the present study.

Table 5 Mean ice crystal size (X_{50}) before and after temperature cycling and rate of growth of ice in ice creams with varying sucrose and trehalose contents (±Standard deviation)

	15% Suc/0% tre	**12% Suc/3% tre**	**9% Suc/6% tre**	**6% Suc/9% tre**	**3% Suc/12% tre**	**0% Suc/15% tre**
X_{50} before cycling (μm)	30.6^a ± 4.7	31.2^a ± 8.6	32.0^a ± 0.4	32.9^a ± 0.4	32.7^a ± 3.4	36.2^b ± 0.01
X_{50} after cycling (μm)	80.2^a ± 4.7	77.3^a ± 2.5	78.6^a ± 1.6	73.9^a ± 2.9	73.7^a ± 2.6	80.0^a ± 5.8
Ice crystal growth (%)	162.4^a ± 15.4	153.0^a ± 8.3	157.1^a ± 5.1	141.8^a ± 9.4	141.2^a ± 8.5	161.9^a ± 19.1

[a,b] Means with different letters in the same row are significantly different. Means from duplicate readings from three separate batches ($n = 6$) representing a total of 900 crystals per ice cream formulation.

Conclusions

Trehalose exhibited a higher T_g' when compared with sucrose, both in solution and in ice cream mix. Trehalose in ice cream mixes did not show any significant differences in ice crystal growth inhibition as compared with sucrose. The experimental freezing curve matched the theoretical closer than in previous studies, which was attributed to maximum formation of ice during the DSC runs.

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