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Ice Recrystallization Inhibition in Ice Cream as Affected by Ice Structuring Proteins from Winter Wheat Grass

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ABSTRACT

Ice recrystallization in quiescently frozen sucrose solutions that contained some of the ingredients commonly found in ice cream and in ice cream manufactured under commercial conditions, with or without ice structuring proteins (ISP) from cold-acclimated winter wheat grass extract (AWWE), was assessed by bright field microscopy. In sucrose solutions, critical differences in moisture content, viscosity, ionic strength, and other properties derived from the presence of other ingredients (skim milk powder, corn syrup solids, locust bean gum) caused a reduction in ice crystal growth. Significant ISP activity in retarding ice crystal growth was observed in all solutions (44% for the most complex mix) containing 0.13% total protein from AWWE. In heat-shocked ice cream, ice recrystallization rates were significantly reduced 40 and 46% with the addition of 0.0025 and 0.0037% total protein from AWWE. The ISP activity in ice cream was not hindered by its inclusion in mix prior to pasteurization. A synergistic effect between ISP and stabilizer was observed, as ISP activity was reduced in the absence of stabilizer in ice cream formulations. A remarkably smoother texture for ice creams containing ISP after heat-shock storage was evident by sensory evaluation. The efficiency of ISP from AWWE in controlling ice crystal growth in ice cream has been demonstrated.

Key words: ice recrystallization, ice cream, ice structuring protein, antifreeze protein

INTRODUCTION

For >30 yr, biologists have recognized the presence of specific proteins in living organisms that help them to overcome extreme low temperature environments (DeVries et al., 1970). These proteins have been identified in fish and insects, over-wintering plants, and in bacteria and fungi (Griffith and Ewart, 1995). They have been called antifreeze proteins and antifreeze glycoproteins. Recently, the term ice structuring proteins (**ISP**) has been adopted, as it describes better their mechanisms of action (Clarke et al., 2002). It has been suggested that ISP affect ice crystal morphology and growth by an adsorption-inhibition mechanism (Brown et al., 1985; DeVries, 1986). This theory states that the inhibition of the ice crystal growth derives from local ice surface curvature effects induced by the adsorption of these proteins at the ice-solution interface, and it is explained thermodynamically by the Gibbs-Thomson (Kelvin) principle (Yeh and Feeney, 1996).

This ability of ISP to retard ice recrystallization suggests possibilities for their use as a natural ice modulator in the cold storage of frozen products such as ice cream (Goff et al., 2002). It is well known in the ice cream manufacturing industry that product formulations, processes, and storage and distribution conditions must all try to minimize ice crystal size and minimize rate of recrystallization of ice (Hartel, 1998; Adapa et al., 2000). The strong and direct relationship between ice crystal size and development of a coarse and/or icy texture is well known. The potential to include ISP as a means of recrystallization control greatly enhances the opportunity to provide smoother ice cream to the consumer and, therefore, a higher quality product. An examination of the patent literature from the last few years shows that industrial researchers have been active in this area as well, notably, on the ice cream front [Unilever; Byas (1998), Fenn (1998), and Lillford (1998) and Pillsbury; Clemmings et al. (1997) and Clemmings (2000)]. Using a plant extract, cold-acclimated winter wheat grass extract (**AWWE**), as the source of ISP brings a considerable advantage in availability and consumer acceptance vs. the use of similar active compounds derived from other sources such as gene transfer technology (Lillford and Holt, 1994; Feeney and Yeh, 1998).

In our previous studies (Regand and Goff, 2005), an increasing effect on the retardation of ice recrystallization was observed as the concentration of AWWE was increased in sucrose solutions quiescently frozen. This effect reached a plateau at approximately 0.13% total protein from the extract, after which the addition of

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Table 1. Solutions prepared to evaluate ice recrystallization inhibition by AWWE (cold-acclimated winter wheat grass extract) as affected by common ice cream ingredients

	AWWE[1]							
	0% Formulation				+25% Formulation			
	A1	B1	C1	D1	A2	B2	C2	D2
Water	75.20	74.23	74.00	73.85	52.25	51.70	51.13	50.99
Sucrose	24.80	12.27	10.00	10.00	22.75	11.10	9.18	9.18
SMP[2]		13.50	11.00	11.00		12.20	10.10	10.10
CSS[3]			5.00	5.00			4.59	4.59
LBG[4]				0.15				0.14

[1]Containing 4% TS from which 12% is protein.
[2]SMP = Skim milk powder.
[3]CSS = Corn syrup solids.
[4]LBG = Locust bean gum.

more AWWE did not reduce ice recrystallization further. The proteinaceous nature of the component responsible for this effect has been confirmed; ice recrystallization was not retarded when AWWE was previously treated with a nonspecific protease (A. Regand and H. D. Goff, unpublished data). An extract of the same winter wheat variety that was not cold-acclimated was also shown to have no ice recrystallization inhibition effect (Regand and Goff, 2005). The active proteins present in the AWWE have been identified as ISP and were concentrated and partially characterized in the same study (A. Regand and H. D. Goff, unpublished data).

Our objectives in this study were as follows.

- To determine the influence of other ingredients [i.e., skim milk powder (**SMP**), corn syrup solids (**CSS**), locust bean gum (**LBG**) as stabilizer] on the ice recrystallization inhibition activity of AWWE in quiescently frozen sucrose solutions, both before and after heating.
- To analyze the concentration effect of AWWE on the ice crystal growth in conventional ice cream formulations stored under 2 different constant temperatures (–35 and –18°C) and under heat shock.
- To determine the effect of pasteurization and stabilizer removal on the ice recrystallization inhibition behavior of AWWE in ice cream.
- To examine resulting products from the ice cream application by sensory evaluation.

MATERIALS AND METHODS

Ice Recrystallization in Sucrose Solutions– Ingredient Addition and Pasteurization Effect on ISP Activity

To study the effect of ice cream components (i.e., milk proteins, polysaccharides, and sugars) on the recrystallization properties of ISP from winter wheat, the solutions shown in Table 1 were prepared. Solutions were designed to have the same freezing point depression (–2.02 ± 0.05°C) and constant sugar:protein:stabilizer ratios [when SMP (Parmalat, Toronto, ON, Canada), CSS (42DE, Casco Inc., Toronto, ON, Canada) and/or LBG (Danisco Canada, Toronto, ON) were added]. Ingredients, except AWWE [Ice Biotech, Flamborough, Canada; AWWE contained 4.33% TS, of which 12.05% were proteins (or 0.52% wet basis)], were added to the specified volumes of water at 75°C while stirring for 15 min (resembling pasteurization treatment). Then, after cooling the mix down, AWWE was incorporated into the solution, and the evaporated water was added back. To measure the effect of pasteurization on AWWE activity, 2 more solutions (A2 and D2) were prepared, in this case by adding the AWWE before pasteurization.

Five microliters of each solution were placed on a slide, covered with a cover slip, and loaded on a cold stage (Linkham Instruments, Surrey, UK) that was attached to a light microscope (Olympus BH, Tokyo, Japan). The cold stage was programmed to quench cool the slide to –50°C and then warm it to –10°C at 10°C/min. Samples were held at –10°C for 10 min, warmed to –4°C, cooled to –6°C, and warmed again to –5°C where the temperature was held for 1 h. Images were acquired every 10 min of the last period at –5°C. Image processing and analysis of the samples were carried out using the Image Processing Tool Kit as described in Regand and Goff (2003). Ice crystal size distributions were characterized by a logistic dose-response model with a cumulative distribution of equivalent circular diameters (Flores and Goff, 1999). The X_{50} values were calculated as the theoretical median value of the fitted data to the model at 50% of the cumulative distribution. The percentage growth of the X_{50} values from 0 to 60 min for each cycle at each concentration was calculated. The ISP activity was determined as the reduction in percentage of ice crystal growth relative to a sample

without AWWE. The determination of statistically significant differences between rates of growth for each sample was carried out using the ANOVA single factor and the least significant difference test.

Additional experiments were performed with the objective to measure the ice crystal growth kinetics under constant temperature for an extended period of time. Samples A and D, with or without 0.25% total protein from AWWE, were prepared. The temperature cycling protocol included taking the samples to –50°C, warming them up to –10°C, holding them at that temperature for 10 min, warming them up to –5°C, and maintaining this temperature for 6 h. Image processing and analyses were carried out as in previous experiments. The accreted ice crystals on these images were quantified as the number of linear segments used to divide joint crystals during the processing of the acquired images.

Ice Recrystallization in Ice Cream

ISP Concentration Effect on the Ice Recrystallization in Ice Cream Conventional ice cream mixes containing 10% anhydrous milk fat (Gay Lea, Guelph, ON, Canada), 11% SMP, 10% sucrose, 5% CSS, 0.1% mono- and diglycerides, and 0.15% LBG were prepared in triplicate. Zero, 0.0025, and 0.00375% of total protein from AWWE were added to these mixes prior to pasteurization. These concentrations of AWWE were selected considering preliminary experiments, where the range of optimal concentration of added AWWE (significant inhibition on ice recrystallization without compromising texture or flavor in ice cream) was defined. Mixes were pasteurized (74°C; 15 min), homogenized (17.2/3.4 MPa, APV Gaulin V15-8T, Everett, MA), cooled to 4°C overnight, and frozen in a scraped-surface heat exchanger (Taylor Batch Freezer, B733-32; Tekni-Craft, Rockton, IL). 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. The containers were kept at this temperature until analysis by microscopy.

For temperature cycling, 3 containers of each formulation were subsequently transferred to a cabinet freezer at –20°C. Each sample was subjected to a programmed heating and cooling cycle during which the freezer was kept at –20°C for 12 h, then heated at a rate of 0.83°C/h to –10°C, held there for another 12 h, and cooled at a rate of 0.83°C/h back to –20°C. This heating-cooling cycle was repeated 16 times on every sample; each cycle was 48 h long. For constant temperature storage, 3 containers of each formulation were transferred to a cabinet freezer at –18°C, and the temperature was held constant for 32 d (same total length time as the cycling protocol).

For image capture and analysis, ice cream containers were transferred from –35 to –24°C and then immediately prepared for analysis by microscopy. All mechanical devices that were used for further treatment of the samples were precooled to –24°C. Cubes of approximately 1 cm^3 were taken from the core section at the center of the container using a sharp knife. A thin slice (~1 mm thick) was subsequently cut with a sharp blade and placed on a drop of iso-amyl-butanol (previously cooled to –24°C) on a standard glass microscope slide. The microscope slide was covered with a coverslip and placed above liquid nitrogen in an insulated Styrofoam container and transported immediately to the cold stage, which was previously programmed to a constant temperature of –17°C.

Images were acquired using a Leica light microscope (DMRXA2, Wetzlar, Germany) equipped with a green filter. Bright field images were acquired from the uncycled, cycled and constant-temperature stored (–18°C) samples. Several different fields were photographed from 2 different containers to obtain at least 300 crystals per container. Measurements of ice crystal size were performed on a Macintosh computer using the public domain NIH Image program Object Image 2.10 (developed at the US National Institutes of Health and available at http://rsb.info.nih.gov/nih-image/) by manually tracing the perimeter of the crystal with a computer mouse; the area of each crystal was automatically calculated by the software. Microsoft Excel 2000 was used to determine the equivalent circular diameter of the crystals and for further statistical analysis. The X_{50} values, percentage of ice crystal growth, and ISP activity were calculated as described in the previous section.

Stabilizer Removal Effect on ISP Activity in Ice Cream. To determine the feasibility of the removal of stabilizers from the formulation of ice cream containing ISP, conventional ice cream mixes were prepared containing 0% LBG and 0.0025 and 0.00375% of total protein from AWWE. Mixes were frozen, stored, and analyzed as described in the previous section.

Pasteurization Effect on ISP Activity in Ice Cream. In this case, to determine the pasteurization effect, conventional ice cream mixes, containing 0 and 0.15% LBG and 0 and 0.00375% total protein from AWWE, were prepared by adding the AWWE after pasteurization. Mixes were frozen, stored, and analyzed as described in the previous section.

Sensory Evaluation of Ice Creams With and Without AWWE. A trained panel of 7 ice cream experts was asked to evaluate the different ice cream formulations after being stored under the previously defined conditions. In the first of the 2 tests performed, a 9-point evaluation for iciness (perception of large crystals)

Table 2. Ice crystal growth (%) in quiescently frozen solutions

Formulation[1]	0%[2]	0.13%[2]	0.13%[2] Pasteurized
A	82.81[b,A]	23.62[b,C]	34.01[b,B]
B	59.92[a,A]	24.57[b,B]	NS[3]
C	50.50[a,A]	28.77[ab,B]	NS[3]
D	58.16[a,A]	32.35[a,B]	60.80[a,A]

[a,b]Values with the same letters in the same column are not significantly different ($P > 0.05$).

[A,B]Values with the same letters in the same row are not significantly different ($P > 0.05$).

[1]See Table 1.

[2]Total protein from AWWE (cold-acclimated winter wheat grass extract).

[3]NS = Not studied.

Table 3. Ice structuring protein activity in quiescently frozen solutions

Formulation[1]	0.13%[2]	0.13%[2] Pasteurized
A	71.47[c,A]	52.87[b,B]
B	58.99[b]	NS[3]
C	43.03[a]	NS[3]
D	44.37[a,A]	–4.54[a,B]

[a–c]Values with the same letters in the same column are not significantly different ($P > 0.05$).

[A,B]Values with the same letters in the same row are not significantly different ($P > 0.05$).

[1]See Table 1.

[2]Total protein from AWWE (cold-acclimated winter wheat grass extract).

[3]NS = Not studied.

of the ice cream samples containing 0, 0.0025, and 0.00375% total protein from AWWE was performed. The following values for standardized controls were initially agreed to by the panelists in the training session: control (0% AWWE), stored at –35°C = 1; control (0% AWWE), stored at –18°C = 3; control (0% AWWE), temperature cycled = 6; grossly heat-shocked ice cream sample = 8. The second test was a discriminative paired-comparison test used to confirm the first evaluation. In this case, each sample, prepared with a different concentration of total protein from AWWE, was compared separately with its specific control (0% AWWE). In both tests, the samples were coded, and their order was randomized.

RESULTS AND DISCUSSION

Ice Recrystallization in Sucrose Solutions Containing Ice Cream Ingredients

The addition of ISP from AWWE had a significant impact on ice crystal growth in sucrose solutions containing ice cream ingredients compared with those without (Table 2), demonstrating its efficacy in controlling ice recrystallization. As expected, sample composition also had a large and significant impact on ice recrystallization. The percentages of ice crystal growth of solutions containing sucrose and other ingredients (SMP, CSS, and LBG), without AWWE, were significantly ($P < 0.05$) lower than ice crystal growth observed in solutions containing only sucrose (Table 2). Even by controlling one of the most important formulation factors that influences ice recrystallization, the ice phase volume, critical differences in moisture content, viscosity, ionic strength, and other properties caused a modification of the heat and/or mass transfer rates with the subsequent reduction in ice crystal growth (Hartel, 1998). In food systems, it is a combination of counter-diffusion of large molecules and the rate of removal of the heat energy released by crystallization that controls ice crystal growth rates (Blanshard et al., 1991). Conditions that maximize counter-diffusion and heat transfer result in the fastest ice crystal growth rates.

This principle also applies to the mechanism of action of ISP in retarding ice recrystallization. As discussed in Regand and Goff (2005), for ISP to be active in retarding ice crystal growth, ISP molecules need to migrate from the bulk solution to the crystal interface, orient into the proper conformation, and then diffuse around the ice crystal surface to find an appropriate location for incorporation into the ice lattice. Simultaneously, other solute molecules (sugars, other proteins, polysaccharides, salts, etc.) must diffuse to allow ISP adsorption (or growth of the ice crystal), which explains the reduction in ISP activity with the addition of other ingredients to the sucrose solution observed in Table 3. Nevertheless, all formulations containing 0.13% total protein from AWWE showed significantly ($P < 0.05$) lower ice crystal growth values than their respective formulations without AWWE. The activity of the ISP in the retardation of ice crystal growth in solutions was as high as 44% for the most complex mix.

In the present experiments, we also found that when the AWWE was pasteurized, the effect of ISP in retarding recrystallization was eliminated in complex formulations and was slightly reduced in sucrose solutions, which is not desirable. (For an industrial application, it is more convenient to add all of the ingredients to the ice cream mix and then pasteurize it.) This effect could be due to the high concentration of proteins and other solids added with the AWWE (0.13% total protein included in 1% TS from AWWE), which promoted the interaction of ISP with themselves or with the other components present in the extract during the heat treatment and, thus, loss of activity. However, it has been also demonstrated in this study (see subsequent) that the pasteurization of ice cream mixes containing

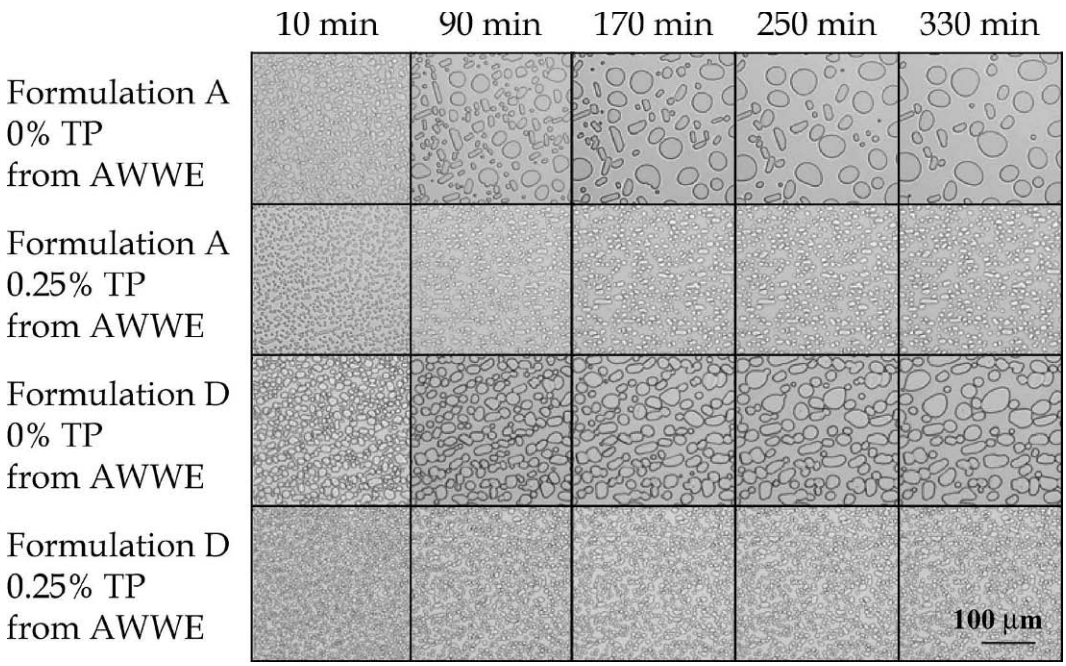

Figure 1. Bright field images acquired every 80 min (starting at 10 min) at –5°C from sucrose (Formulation A) and ice cream (Formulation D) solutions containing 0 and 0.25% total protein (TP) from cold-acclimated winter wheat grass extract (AWWE).

ISP from AWWE does not affect the activity in reducing ice crystal growth at the recommended concentrations (0.0025 to 0.00375% total protein included in 0.02 to 0.03% TS from AWWE).

In a previous study (Regand and Goff, 2005), it was demonstrated that the major effect of ISP in retarding ice recrystallization occurs during longer temperature cycles at low frequency. To better understand the ISP mechanism of action, the ice crystal growth kinetics of 4 solutions held at constant temperature for a longer period of time were studied. Under constant temperature conditions, the main recrystallization mechanisms occurring are accretion and Ostwald ripening. Accretion has been considered a substantial contributing mechanism in ripening of ice crystals in ice cream (Donhowe and Hartel, 1996b). In our study, it appeared (Figure 1) that at the early stages, the crystals joined together and formed network-type structures. The numbers of total and accreted ice crystals with time are shown in Figure 2. After around 90 min, the degree of aggregation appeared to decrease, and the total and accreted ice crystals reached a constant value. The difference between the total and accreted ice crystals with the addition of AWWE to sucrose and the ice cream solution is remarkable. However, it is still unknown whether the number of ice crystals was initially the same. Later, in the control samples, most of them were lost by the different recrystallization mechanisms, before time zero in our experiments.

Once the number of accreted features has reached a plateau, it can be assumed that the remaining ice crystals will grow mainly via migration (Sutton et al., 1996). Based on approximations for the ice-sucrose system, the differences in the melting point between ice crystals of different sizes at –5°C can be calculated. Hartel (1998) has shown that although ice crystals of 1-μm radius have only about 0.05°C difference in melting temperature as compared with an infinitely large crystal, during long times of storage, significant changes in ice crystal size distribution can still occur. The rate of increase in mean crystal size depends on the mechanism that controls crystallization under these conditions. For well-separated, noninteracting crystals, the change in average length follows (Jain and Hughes, 1978):

$$r^n = r_o^n + Rt \quad [1]$$

where r is the mean radius at any time t, r_o is initial mean crystal size, R is the rate of recrystallization, and n is a parameter that depends on the mechanism of recrystallization. When bulk diffusion of molecular species limits the rate of ripening, n = 3, and when surface integration process controls ripening, n = 2. The general mechanism for recrystallization processes can be determined, in part, by determining the value of n from Equation 1, which matches the experimental results (Hartel, 2001). For crystallization of ice during storage

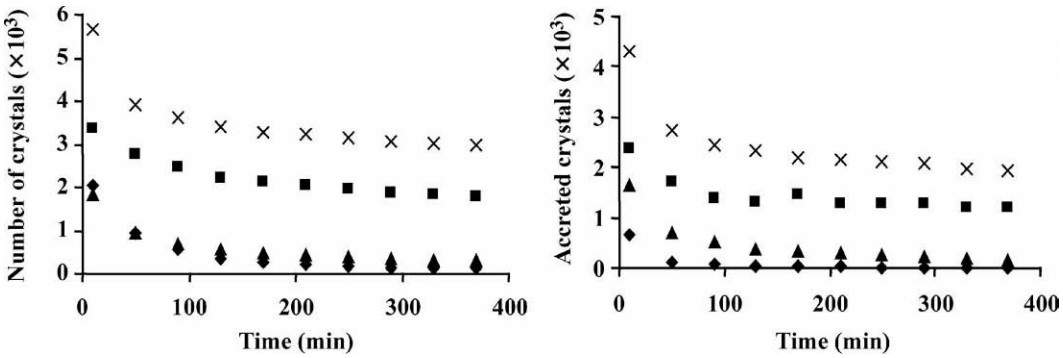

Figure 2. Total and accreted ice crystals measured from acquired images at –5°C of sucrose solution (Formulation A; ◆), sucrose solution containing 0.25% total protein (TP) from AWWE [cold-acclimated winter wheat grass extract (AWWE); ■], ice cream solution (Formulation D; ▲) and ice cream solution containing 0.25% TP from AWWE (×).

of ice cream, it has been found that n = 3. This result indicates that it is the mobility of water molecules through the ice cream matrix that limits the rate of recrystallization in ice cream (Donhowe and Hartel, 1996a, b).

Figure 3 shows the ice crystal growth kinetics for the 4 solutions analyzed in this study. Fitting the experimental data (values were only considered when the accretion was constant) to Equation 1, *n* is equal to 3.08 in the control ice cream solution and 1.42 in the control sucrose solution, which agrees with the values reported in the literature (Donhowe and Hartel 1996a, b). The ice crystal growth in the ice cream solution is mainly controlled by the diffusion of water molecules to the ice and the counter-diffusion of the other solids from the ice surroundings (Hartel, 2001). With the addition of 0.25% total protein from AWWE, the ice crystal growth behavior changes radically. Once the accretion has reached its maximum, the ice recrystallization is practically stopped. No Ostwald ripening was observed in either of the 2 samples.

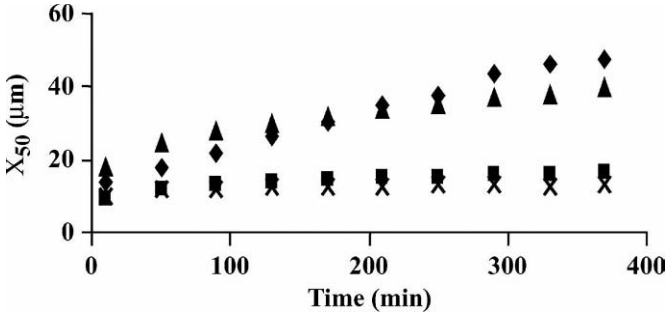

Figure 3. Mean ice crystal equivalent diameter (X_{50}) from acquired images at –5°C of sucrose solution (Formulation A; ◆), sucrose solution containing 0.25% total protein (TP) from AWWE [cold-acclimated winter wheat grass extract (AWWE); ■], ice cream solution (Formulation D; ▲) and ice cream solution containing 0.25% TP from AWWE (×).

The fact that longer times at constant temperatures are required during the temperature cycling for the ISP to be active (Regand and Goff, 2005) could be related to the inability of the ISP to retard, at the early stages, the accretion present in the sample. After a certain time has passed, however, accretion could also be completely reduced.

It has been proposed that the kinetics of the orientation of the ISP molecules into their preferred binding orientation might well govern their activity (Knight et al., 1991; Sicheri and Yang, 1996; Chapsky and Rubinsky, 1997). If we suppose that, to inhibit ice crystal growth, ISP molecules must be bound on the ice surface in a specific orientation and if we further assume that ISP molecules can and do adsorb in other, non-optimal orientations as well (Wen and Laursen, 1992; Chapsky and Rubinsky, 1997), then ordering of adsorbed ISP molecules into their preferred binding orientation through surface diffusion or reorientation would be a necessary step for achieving maximal inhibition.

Ice Recrystallization in Ice Cream

Ice cream samples were prepared by adding 0, 0.0025, and 0.00375% total protein from AWWE. Three different formulations were subjected to the same freezing process and stored at 3 different conditions: constant temperature at –35°C, constant temperature at –18°C, and heat shock. The ice crystal equivalent diameters (X_{50}) for each formulation after storing under the defined conditions are shown in Table 4. The samples stored at –35°C were considered as the initial values for the ice crystal growth calculation. In these samples, no crystal growth after hardening is expected, because the temperature at which they were stored is close to

Table 4. Ice crystal equivalent circular diameters (X_{50}) of ice cream samples containing 0, 0.0025, and 0.00375% total protein (TP) from AWWE (cold-acclimated winter wheat grass extract) added before (Past) and after (No past) pasteurization, with (Past, No past) and without (No stab) stabilizer, after being frozen in a scraped-surface heat exchanger and stored at constant temperature at –18 and –35°C and under temperature cycling for 1 mo

	X_{50}								
	–35°C			Temperature cycling[1]			–18°C		
TP from AWWE	Past	No past	No stab	Past	No past	No stab	Past	No past	No stab
0%	41.2[a,A]	NA[2]	42.2[a,A]	80.9[a,A]	NA	87.1[a,A]	NA	58.8	58.6[a,A]
0.0025%	40.7[a]	NS[3]	NS	64.4[b]	NS	NS	53.0[a]	NS	NS
0.00375%	41.1[a,A]	42.5[A]	41.3[a,A]	62.6[b,A]	68.7[B]	81.0[a,C]	52.1[a,A]	57.7[A]	60.3[a,A]

[a,b]Values with the same letters in the same column are not significantly different ($P > 0.05$).

[A–C]Values with the same letters in the same row are not significantly different ($P > 0.05$) for each storage condition.

[1]Sixteen cycles of 48 h from –10 to –20°C.

[2]NA = Not applicable.

[3]NS = Not studied.

the glass transition of ice cream and, in this state, practically all of the physicochemical phenomena, including recrystallization, are reduced to zero (Hartel, 1998).

After 1 mo, ice cream samples stored at constant temperature at –18°C showed smaller ice crystal sizes than the samples that had been stored under temperature cycling from –10 to –20°C. This result could be explained by 2 factors. First, ice recrystallization at constant temperatures (Ostwald ripening and accretion) is slower at lower temperatures (Hartel, 1998); the temperature range of the cycling period included warmer temperatures than –18°C (i.e., –10°C). Second, temperature fluctuations promote ice crystal growth via ice recrystallization mechanisms as "melt-diffuse-grow" and "melt-regrow" (Regand and Goff, 2002). The reduction in the water and solute mobility at lower temperatures (samples stored at –18°C) could also affect the diffusion of ISP and the counter-diffusion of other solids from the ice-water interface and, therefore, reduce the ability of ISP to retard the ice crystal growth. This consideration and the fact that there was only a small increment in the actual size of the ice crystals from the hardening step to the end of the storage period at –18°C resulted in a nonsignificant ($P > 0.05$) retardation of ice recrystallization by the ISP present in these samples. However, in the heat-shocked samples, the higher temperatures (i.e., –10°C) and the long periods of time at constant temperature (i.e., 12 h) allowed ISP to migrate to the crystal interface, orient into the proper conformation, and then diffuse around the ice crystal surface to find an appropriate location for incorporation into the ice lattice. In these samples, ISP significantly ($P < 0.05$) inhibited ice crystal growth when 0.0025 and 0.00375% of total protein from AWWE were added (Table 5).

In contrast to the quiescently frozen solutions, in heat-shocked ice creams where the amount of other solids from AWWE added was considerably lower (0.02 and 0.03%), pasteurization increased ISP activity to 27% (the reduction in the ice recrystallization was only 36% when the AWWE was not pasteurized). These results emphasize the importance of the concentration of raw AWWE added. When low amounts of other solids from AWWE are present, ISP are more available, and pasteurization can actually improve the ISP activity, probably because of a more homogeneous distribution of the ISP in the mix (Table 5).

Another interesting finding was that with the total removal of stabilizer, ISP activity was reduced from 46 to 10% (79% of the original) in heat-shocked ice creams. A synergistic effect between ISP and stabilizer is suggested. During temperature fluctuations, the localization of ISP in the surroundings of the ice-water interface is possibly favored by the reduction of mobility in the serum phase because of the addition of the stabilizer (Regand and Goff, 2003). For samples stored at –18°C, the differences in ice crystal growth were not significant ($P > 0.05$); however, the same trends were observed as for the heat-shocked samples.

In both of the sensory tests, the trained sensory panel was able to detect significant differences in texture (iciness) derived from the presence of ISP in the ice creams, confirming the results obtained by the microscopy assay (Table 6).

CONCLUSIONS

The addition of other ingredients (SMP, CSS, LBG as stabilizer) to sucrose solutions significantly ($P < 0.05$) reduced ice crystal growth. Critical differences in mois-

Table 5. Ice crystal growth (ICG) in ice cream containing 0, 0.0025, and 0.00375% total protein (TP) from AWWE (cold-acclimated winter wheat grass extract) added before (Past) and after (no Past) pasteurization, with (Past, No past) and without (No stab) stabilizer, after being frozen in a scraped-surface heat exchanger and stored under temperature cycling and at constant temperature at –18°C for 1 mo

	ICG					
	Cycled[1]			–18°C		
TP from AWWE	Past	No past	No stab	Past	No past	No stab
	(%)					
0%	96.43[a,A]	NA[3]	106.48[a,A]	42.88[a,A]	NA	38.90[a,A]
0.0025%	58.22[b]	NS[4]	NS	30.22[a]	NS	NS
0.00375%	52.18[b,A]	61.64[B]	96.11[a,C]	26.63[a,A]	35.63[A]	46.04[a,A]

[a,b]Values with the same letters in the same column are not significantly different ($P > 0.05$).

[A–C]Values with the same letters in the same row are not significantly different ($P > 0.05$) for each storage condition.

[1]Sixteen cycles of 48 h from –10 to –20°C.

[2]NA = Not applicable.

[3]NS = Not studied.

ture content, viscosity, ionic strength, and other properties caused a modification of the heat or mass transfer rates with the subsequent reduction in ice crystal growth. Although the ISP activity was also reduced as low as 43% in the presence of these other molecules, the retardation of ice recrystallization with the addition of AWWE was still significant ($P < 0.05$) in all solutions. The increase in viscosity and TS with the added ice cream ingredients reduced the kinetics of diffusion-mediated processes, such as the adsorption to the ice-water interface that has been proposed as the main mechanism of action for ISP. Pasteurization eliminated ISP activity in complex mixes and reduced it significantly ($P < 0.05$) in sucrose solutions. A combined effect of the interaction of ISP with themselves or other components rather than only heat denaturation is suggested because of the lack of reduction of ISP activity by pasteurization in ice cream.

Ice crystal growth kinetics experiments detected a remarkably higher number of ice crystals in samples containing AWWE, implying substantial ISP activity in retarding ice recrystallization before the time zero of our experiment. In these solutions, accretion and Ostwald ripening were practically stopped by the presence of ISP after certain time at constant temperature conditions. The stereospecific ice binding property upon which the inhibitory activity of ISP has been proposed to depend suggests a possible role for surface rearrangement of ISP molecules in the ice crystal growth kinetics.

In conventional ice cream formulations frozen under commercial conditions, recrystallization inhibition with the addition of AWWE was also observed. High values for reduction of crystal growth (e.g., 46%) in relation to the growth without AWWE were detected in heat-shocked ice creams. In ice creams containing 0.02 and

Table 6. Sensory evaluation by 7 trained panelists of ice cream samples containing 0.0025 and 0.00375% total protein (TP) from AWWE (cold-acclimated winter wheat grass extract) stored at –35°C, –18°C, and under cycling conditions. The 9-point test for iciness was evaluated using the following scale: 9 = extremely icy to 1 = extremely smooth ice cream

	Panelists								
Samples	1	2	3	4	5	6	7	Average	Control[1]
0.0025% TP									
–35°C	2	2	2	1	1	1	2	1.57[a]	1[a]
Cycled	3	3	4	3	4	3	6	3.71[b]	6[a]
–18°C	5	4	3	3	5	2	3	3.57[a]	3[a]
0.00375% TP									
–35°C	1	2	1	2	1	1	1	1.29[a]	1[a]
Cycled	5	3	2	5	3	3	2	3.29[b]	6[a]
–18°C	4	4	4	4	6	2	2	3.71[a]	3[a]

[a,b]Values with the same letter in the same row are not significantly different ($P > 0.05$).

[1]0% AWWE.

0.03% TS from AWWE, pasteurization improved ISP activity, which represents an important advantage in their industrial application. Sensory evaluation verified the improved textural characteristics of the ice cream after the addition of 0.0025 or 0.00375% of total protein of AWWE in heat-shocked samples. Ice structuring proteins may prove to be valuable ice cream ingredients with appropriate formulation testing and product development.

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