Bacterial Extracellular Ice Nucleator Effects on Freezing of Foods

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ABSTRACT

Extracellular ice nucleators (ECINs) were incorporated into foods and subjected to subzero freezing. Time-temperature profiles, ice-formation patterns and textures were examined by thermocouple, microscopy and texture analyzer. Onset temperatures (initial freezing), enthalpies and freezing rates were measured by DSC. Addition of ECINs to liquid foods elevated ice nucleation temperatures and promoted freezing. Solid or semisolid products frozen with ECINs resulted in a fiber-like texture. These effects were more apparent at −10°C or higher. Differential scanning calorimetry revealed onset temperatures were increased 11°C by addition of ECINs, but length of time to complete the phase transition was extended at constant cooling rates. Results indicated that ECINs can be used instead of whole bacterial cells for efficient freezing and textural modification.

Key Words: ice nucleation, bacterial, extracellular, freezing rate, texture

INTRODUCTION

FREEZING IS ONE OF THE BEST METHODS FOR LONG-TERM PRESERvation for a range of processed foods (Reid, 1983). However, ice crystal growth within the delicate food matrix often causes undesirable textural changes (Lawrence et al. 1986). Supercooling occurs when sample temperatures drop below their freezing points prior to solidification. Ice nucleation is the process where initial ice crystals are formed and begin to grow. Supercooling and ice nucleation rates directly affect ice growth patterns and energy-costs.

Use of active bacterial cells for ice nucleation in the freezing of foods could reduce the degree of supercooling by raising the temperatures of ice nucleation, and thus change the texture of frozen foods (see reviews by Watanabe and Arai, 1994; Li and Lee, 1995). Arai and Watanabe (1986) applied *E. ananas* cells to freeze proteinaceous foods, such as egg white and soy protein. Fiber-like textures were obtained, which were unique relative to corresponding products processed without the bacterial cells. It was also demonstrated that the application of *E. herbicola* cells could shorten freezing times and lead to considerable energy-savings (Ryder and Lee, 1988; Li and Lee, 1997; Li et al., 1997).

Bacteria-induced ice nucleation has been a major contributing factor to frost injury in plants. This trait is thought to rise from expression of a single ice nucleation gene, *Ina* (see reviews by Lindow, 1983; Gurian-Sherman and Lindow, 1993; Wolber, 1993). Purified ice nucleation proteins, however, only exhibit activity at temperatures below −6°C, which is about 4°C lower than whole bacterial cells (Fall and Wolber, 1995). This is probably due to a requirement for phospholipid (Govindarajan and Lindow, 1988). Active ice-nucleation structures may be located at the outer membrane of *Pseudomonas syringae* as lipoglycoprotein complexes (Lindow et al., 1989; Kozloff et al., 1991). Phelps et al. (1986) reported that *E. herbicola* could release ECINs into the culture medium. Enrichment of these ECINs by our lab and others (Kawahara et al., 1993) indicated that they exist as membrane vesicles. In addition to protein, lipid and saccharide are essential for activity at temperatures slightly below zero.

The application of ECINs, rather than whole cells, in food freezing could be advantageous regarding safety concerns. Use of enriched ECIN preparations for freezing of food systems has not been reported. Our objective was to test the potential applications of ECINs for this purpose.

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MATERIALS & METHODS

Preparation of cells and ECINs

Erwinia herbicola subsp. *ananas* (Cat. No. 11530) was obtained from American Type Culture Collection (ATCC) (Rockville, MD). Bacteria were routinely grown at 18°C in yeast extract as described (Obata et al., 1990). ECINs were purified by centrifugation, filtration and density gradient centrifugation (Li and Lee, 1997b). Purified ECINs were suspended into a 20 mM Tris buffer (pH 7.8) and stored at −20°C before use.

Food materials

Foods including milk, juices, ice cream, yogurt, soybean curd, ground beef, frankfurter, fish fillet, and eggs were obtained from local markets. Sucrose, egg white protein and starch were purchased (Sigma Chemical Co., St. Louis, MO).

Emulsion preparation

Emulsions of safflower oil / water at ratios of 25% to 75% were prepared. Tween 80 was used at 2% based on oil content. A 30-sec burst of sonication was applied to 40 mL of sample after addition of ECIN preparations (10 g protein/mL in final concentration). These emulsions were very stable at room temperature and at 4°C for more than 1 mo. Phase separation did not occur.

Freezing curves

Samples were subjected to freezing in a custom-designed freezer (Scien Temp Lo-Cold Model, Scien Tem Co., Adrian, MI). For the freezing of water, the freezer temperature was set at −6, −12 or −18°C; freezing curves were recorded at −6°C with various foods, and emulsions were frozen at −7°C. The temperature of each sample (10 mL) was monitored with a Type T copper-constantan thermocouple (Omega Engineering Inc., Stamford, CT), placed at the geometric center of a 15-mL test tube and connected to a WB-AAI data acquisition system (Omega Engineering Inc., Stamford, CT). This was interfaced with an IBM computer through the QuickLog PC Data Acquisition and Control Software (Strawberry Tree, Sunnyvale, CA). Four separate measurements were taken for each sample.

Microscopy

Samples of distilled water (100 L) with or without ECINs were placed on a glass slide and overlaid with a thin plastic cover. Preparations were set on a thermally controlled stage with a hole in the center to allow light to pass through (Model TS-4 Controller, Sensortek, Inc., Clifton, NJ). This stage was observed under a dissection microscope (×10 magnification) and photographed with a Polaroid camera. Samples were cooled at 1−/min. ECIN-containing water froze at −3 to −4°C; the controls did not freeze until −10°C. Samples containing ECINs, such as yogurt, rice flour paste and soybean curd (100g each), were placed in aluminum dishes (dia 64 mm), and frozen at −6°C, observed and photographed.

Texture profile analysis

Texture profiles of foods after freeze texturing and thawing were evaluated with a TA-XT2 texture analyzer (Texture Technologies Corp., Scarsdale, NY). A rounded plate probe (5 × 20 mm), custom designed and fabricated, was used to exert force in the middle of each sample (100g in an aluminum dish). Samples were tested in a Texture Profile Analysis mode, which consisted of two passes with a 2 sec pause between passes. Probe speed was 2 mm/sec, and the distance of each probe pass was 75% of the product height. Data were processed with an XT-RA Dimension software package (Stable Micro System, Haslemere, Surrey, UK). Resultant time-force curves were recorded and textural parameters of each product were obtained from the curves. Hardness, the force necessary to attain a given deformation, was given as the final peak of the TPA curve. Fracturability, the force at which the material fractures, was the height of the first major break in the TPA curve; a sample with a high degree of hardness and low cohesiveness would fracture.

Differential scanning calorimetry

A DSC-30C (Mettler Instruments, Princeton, NJ) with a thermal data analysis unit was used. The calorimeter was fitted with an Intracooler II sub-ambient accessory and a dry box. The DSC head and dry box were purged continuously with dried nitrogen. Samples (2 to 5 mg) were put in a sealed aluminum pan and an empty pan was the reference. Nucleation patterns were evaluated using a scanning mode and parameters of freezing processes were monitored using several constant cooling rates.

RESULTS & DISCUSSION

Determination of experimental parameters

To apply ECINs for the freezing of food products, valid freezer temperatures and application dosages were required. Two sets of experiments were designed to examine these effects. ECINs were added to distilled water at 70 units/ mL (0.7 μg protein/ mL). Water added to test tubes served as controls. Freezer temperatures were set at −6, −12 and −18°C, and freezing curves of each sample were recorded. Four replicates were tested and nucleation temperatures were obtained from freezing curves. Nucleation temperatures were the lowest temperatures reached during the supercooling process (Table 1). At −6°C, the control did not freeze. In contrast, ECIN-containing samples nucleated at −3.7°C and froze. At −12°C, both samples nucleated and froze; however, nucleation temperatures of ECIN-containing samples were markedly higher than controls (raised by ~7°C). At −18°C, differences between controls and ECIN-containing samples became less apparent. Similar results were obtained with 10% egg white solutions, and there were no differences between controls and ECIN-containing samples at −12°C and above (data not shown). These experiments demonstrated that ECINs did not exert an effect above −12°C. Below −10°C, differences in nucleation temperatures between controls and ECIN-containing samples became less apparent. For this reason, our freezer temperatures were −6°C for this study, unless otherwise indicated.

Results demonstrated that freezer temperature settings had significantly affected ice nucleation activity of cells and ECINs in bulk volume, which has not been reported. Bacterial cells have been used in the freezing of food materials (Watanabe and Arai, 1987; Li and Lee, 1997; Li et al., 1997). Bacterial cells exhibit ice nucleation activity in the range of −2 to −5°C. The highest temperature at which ice nuclei form as initiated by bacterial cells is defined as the threshold temperature, which is strain specific (Wolber, 1993). ECINs described here also exhibited activity in this temperature range, but much smaller amounts of total materials were required. In nature, most ice nucleators only exhibit activity below −10°C. The effect of ECINs was most apparent at temperatures near 0°C (Table 1). At low temperatures (e.g. −12°C), endogenous non-ECIN food system components may also function as ice nucleators, rendering negligible the effects of ECINs.

Table 1—Nucleation of water at various freezer temperatures[a].

Freezer temp	Nucleation temp (°C)	
(°C)	Control	+ ECINs
−6	Dnf[b]	−3.7 ± 0.9
−12	−7.6 ± 3.8	−0.6 ± 0.2
−18	−1.9 ± 2.6	−0.4 ± 0.1

[a]Each sample contained 700 units of ECINs (70 g protein) in 10 mL. Results are averages of 4 separate tests.
[b]Dnf: did not freeze (1 of 4 samples nucleated at −6.1°C).

This explains why most of the work reported for the freezing of food materials with bacterial ice nucleators has been conducted at freezer settings of −10°C or higher, usually around −6°C (Watanabe and Arai, 1994; Li and Lee, 1995). Freezer temperature also has a direct effect on cooling rates.

As mentioned, nucleation temperature was defined as the lowest temperature reached during supercooling, which was measured at the geometric center of the test tube. This should be a very close estimate of the bulk sample nucleation temperatures. The periphery of the test tube may be supercooled faster than the center, due to faster heat removal. Nucleation could occur anywhere in the test tube, end supercooling and cause a negative peak on a freezing curve (the peak point was defined as nucleation temperature). That temperature was apparently an average of various local nucleation events and crystal growth.

Application dosages were investigated by adding various amounts of ECINs into distilled water and recording freezing curves at −6°C (Fig. 1). The ECINs raised the nucleation temperatures of water. This relationship was not linear. The general trend was that the more ECIN added, the higher the nucleation temperatures obtained. As ECIN levels were increased from 1 to 1000 units/mL, nucleation temperatures increased from about −4 to −2°C. ECINs were therefore routinely added at a level of 100 units/ mL, or about 1 g protein/ mL (1 ppm).

This nonlinear relationship is another important property of bacterial ice nucleators: frequency. Under defined conditions, a critical amount of material must be present to initiate nucleation (Wolber, 1993). Fall and Wolber (1995) indicated that a bacterial load of 10^6 CFU/mL was

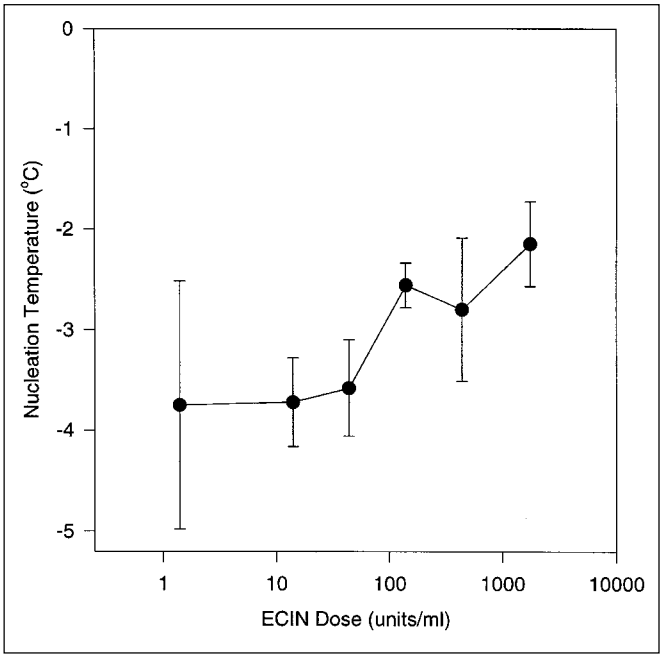

Fig. 1 — Effect of ECIN levels on nucleation temperatures of water. Results are averages of 4 separate tests. Sample size: 10 mL; Freezer temperature: −6°C.

required to produce active ice nuclei at −2°C. In order to obtain the best enhancement of freezing, using *Erwinia ananas,* 10^6 CFU/mL was required (Watanabe and Arai, 1987), but only 1 g ECIN protein/mL was needed. The advantage of using ECINs to enable freezing in food systems at temperatures slightly below zero is apparent.

Freezing curves and nucleation temperatures

The effect of ECIN addition on the freezing curve shape was studied, using a sample volume of 10 mL, and freezing curves of water, milk, juice, ice cream and ground beef (Fig. 2). As before, distilled water did not freeze at −6°C, and it remained supercooled for 200 min (Fig. 2a). Addition of ECINs readily nucleated water at around −3°C and the whole freezing process was completed in about 160 min (temperature of ice reached freezer temperature). Similarly, freezing of milk (Fig. 2b) and apple juice (Fig. 2c) could be achieved at −6°C, while controls remained in the unfrozen state under the same conditions. With the addition of ECINs to vanilla ice cream and starch gels (Fig. 2d, e), freezing occurred at −6°C, while some controls remained unfrozen even after 240 min. Some controls achieved nucleation at the freezer temperature (−6 ±1°C), taking longer to freeze than ECIN-containing samples. Nucleation in solid foods such as ground beef seemed to be less important. When held at −6°C, we observed controls and ECIN-containing samples nucleated at around −1.5°C and no significant differences (Fig. 2f).

Effects of ECINs were summarized (Table 2) for nucleation temperatures of various foods. Their most important effect was to elevate nucleation temperatures in liquid and semisolid foods.

Ice nucleators naturally present in the food systems do not cause effective nucleation at temperatures higher than −10°C. However,

Table 2—Effect of ECINs on nucleation temperatures (°C) of various foods [a].

	Sample	Control	+ ECIN
Liquid	Water	Dnf [b]	−0.6 ± 0.1
	Sucrose (10%)	Dnf	−1.3 ± 0.3
	Egg white (9%)	Dnf	−1.7 ± 0.1
	Safflower oil (20%)	−4.3 ± 0.5	−0.8 ± 0.2
	Raw egg white	−5.4 ± 0.6 (2 of 4)[c]	−4.3 ± 0.1
	Whole milk	Dnf	−1.9 ± 0.3
	Skim milk	−2.9 ± 0.3 (2 of 4)	−0.9 ± 0.3
	1% milk	Dnf	−1.6 ± 0.1
	2% milk	Dnf	−1.2 ± 0.2
	Heavy cream	Dnf	−1.5 ± 0.1
	Non-dairy cream	Dnf	−0.6 ± 0.1
	Apple juice	Dnf	−6.0 ± 0.7 (3 of 4)
	Grape juice	Dnf	−6.8 ± 0.9 (3 of 4)
Semi-Solid	Vanilla ice cream	Dnf	−4.7 ± 0.3
	Chocolate ice cream	Dnf	−5.2 ± 0.4
	Starch gel (5%)	Dnf	−0.6 ± 0.2
	Starch gel (10%)	Dnf	−1.8 ± 0.3
	Yogurt (banana)	Dnf	−1.5 ± 0.2
	Yogurt (strawberry)	Dnf	−3.3 ± 0.2
Solid	Soybean curd	−3.8 6 0.5	−3.1 6 0.3
	Ground beef	−1.5 6 0.7	−1.5 6 0.2
	Frankfurter	Ns[d]	Ns
	Rice flour paste	Ns	Ns
	Tilapia fish fillet	Ns	Ns
	Salmon muscle fillet	Ns	Ns

[a] Freezer temperature set at -6°C. A sample of 10 mL (liquid) or 10g (solid) was used.
[b] Dnf: did not freeze.
[c] 2 of 4 samples froze at -5.4±0.6°C.
[d] Ns: no supercooling observed. Both nucleation and freezing occurred at 0°C.

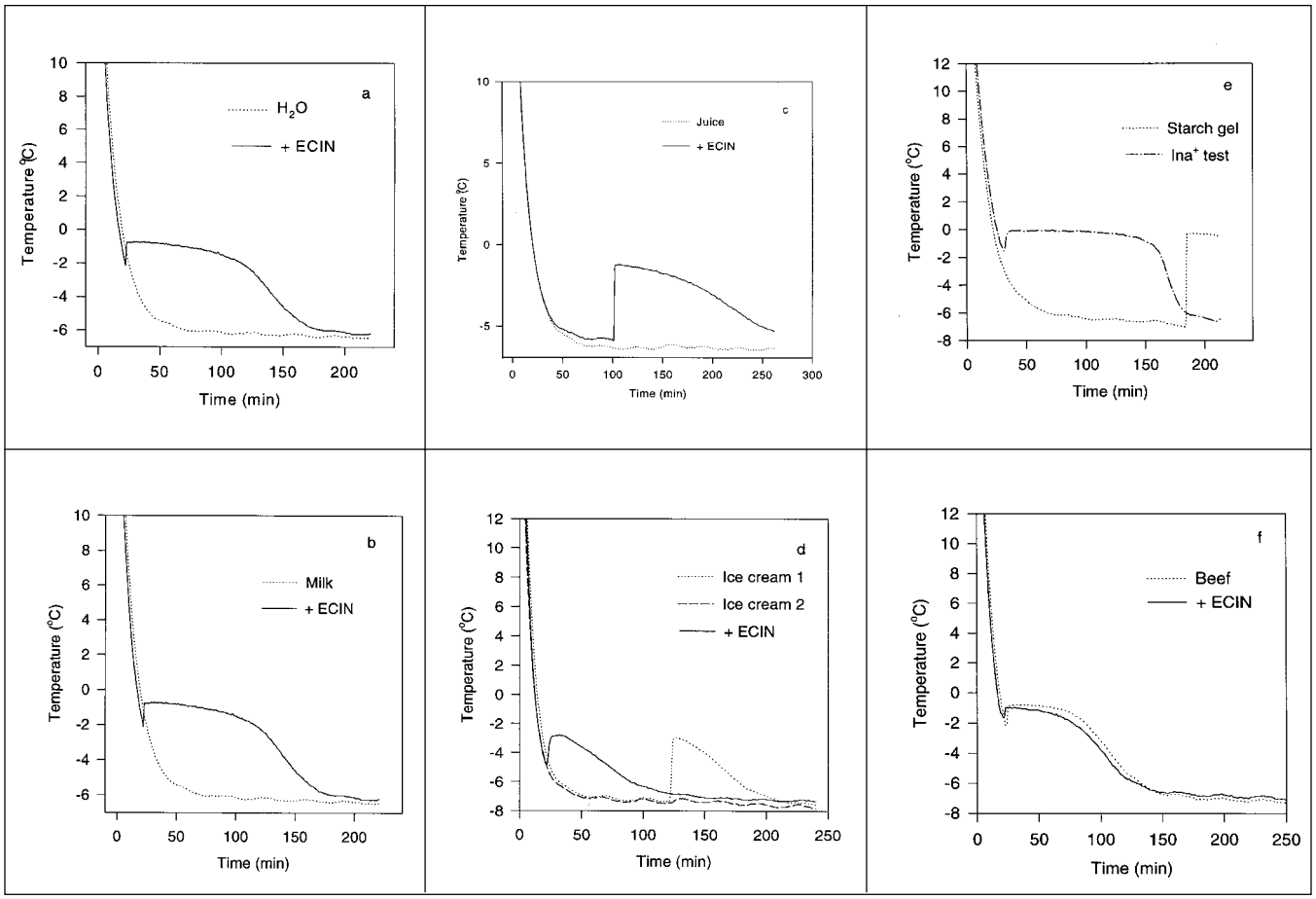

Fig. 2 — Freezing curves (a-f) of foods in the absence or presence of ECINs.

these food systems invariably froze in the presence of whole cells or ECINs. Addition of whole cells of *E. ananas* or *E. herbicola* cells promoted freezing by elevating nucleation temperatures (Watanabe et al., 1990; Li and Lee, 1997; Li et al., 1997). Similar food systems such as oil/water suspensions, soy protein isolate solutions and salmon muscle were used. Some model food systems such as, glucose, NaCl and KCl solutions have also been tested in the presence of whole cells (Watanabe and Arai, 1987). Use of ECINs rather than whole cells could provide great advantages in terms of energy requirements, providing a potential means to implement freezing operations above −10°C, which otherwise would not be possible. Total freezing times were also markedly reduced (Li and Lee, 1997; Li et al., 1997).

Freezing of emulsions

Emulsification is very important in foods and freezing of emulsions is inhibited by the presence of oil. We prepared model emulsions of safflower oil in water, with oil to water ratios of 10% to 75% (v/v). The freezing of these emulsions was examined at a freezer temperature of −7°C. Nucleation temperatures from each freezing curve were summarized (Table 3). When the oil content of these emulsions was adjusted to 25% or less, emulsions froze at −7°C, although nucleation temperatures were 5 to 6°C lower than ECIN-containing samples. When oil content was raised above 40%, controls rarely exhibited nucleation and remained supercooled over the test period.

With ECIN-containing emulsions, results were extremely different. Addition of ECINs to emulsions, even at oil levels of 75%, eliminated supercooling. In these samples, nucleation and freezing both occurred at around −0.5°C (Table 3). Thus, even residual amounts of water present in emulsions could freeze with the aid of ECINs. Total freezing times varied from 30 to 150 min (Fig. 3), and differences were obviously due to water content (25% to 75%).

With a bulk volume (10 mL) of emulsions, increases in nucleation temperatures also occurred using ECINs (Table 3). Emulsions with oil to water ratios higher than 40% were resistant to freezing at −7°C. However, with ECINs these systems readily froze, even at 75% oil. Freezing of emulsions in the presence of ice nucleators had only been reported in micro-size droplets (2-5 mg) and with bacterial cells (Charoenrein et al., 1991; Charoenrein and Reid, 1989; Clausse et al., 1991; Ozilgen and Reid, 1993). Bacterial ice nucleators significantly raised nucleation temperatures in all of the emulsions tested.

It was noted that increasing the percentage of oil also decreased the nucleation temperatures of emulsions in bulk volume in the absence of ECINs. One possible reason is that decreasing water content resulted in decreased water droplet volume in emulsions, and thus a lower probability for finding a critical nucleus. The other possibility is that the interaction of ice nucleators would also be reduced. The aggregation of ice nucleators may be important in activating the effectiveness of ice nucleation sites (Li and Lee, 1997, Unpublished data).

ECIN-induced freeze texturing

Addition of ECINs elevated nucleation temperatures, and also markedly affected ice-formation patterns. This was shown by placing 100 μL of water on a thermally controlled stage and cooling. Water alone remained supercooled at temperatures as low as −10°C. Samples containing ECINs began to freeze at −3°C. The ice thus formed was observed at −15°C under a dissection microscope (Fig. 4). Ice crystals formed in the absence of ECINs seemed to be smooth, with no directionality, and consisted of very fine particles. In contrast, when ECINs were present, ice crystals became ordered, with a defined directionality and uneven surfaces (Fig. 4).

When frozen with ECINs, ice-formation patterns also changed the textural patterns of some foods. After soybean curd was dipped in ECIN suspensions (test) or in an equal amount of water (control), both samples were held at −6°C overnight. Samples were thawed and photographed (Fig. 5A). Freezing in the presence of ECINs produced a fiber-like texture in the curd. The control curd dipped in water alone did not exhibit a well-defined directional structure and appeared to be sponge-like. Very similar results were observed with yogurt and rice flour paste (Fig. 5B, C).

After freezing, rice flour paste was warmed to room temperature and texture profile analysis was performed. Resultant time-force curves (Fig. 6A, B) showed that application of ECINs produced a higher degree of hardness and that the sample fractured at a force of 992g. The control had a lower degree of hardness (1750g vs 2130g), and

Table 3—Effect of ECINs on nucleation temperatures (°C) of oil/water emulsions[a]

Emulsion[b] (oil/water, v/v, %)	Control	+ ECIN
10	−5.1 ± 2.7	−0.3 ± 0.1
25	−6.5 ± 0.4	−0.5 ± 0.1
40	(1)[d]	−0.4 ± 0.2
60	Dnf[c]	−0.5 ± 0.0
75	Dnf	−0.5 ± 0.0

[a] Freezer temperature set at -7.0±0.5°C. Results are averages of 4 separate tests.
[b] Emulsions (40 mL each) were prepared with safflower oil and water at indicated ratios by 30s sonication in 40 mL with Tween 80.
[c] Dnf: did not freeze.
[d] (1) 1 of 4 controls nucleated at -7.1°C and the other 3 remained unfrozen.

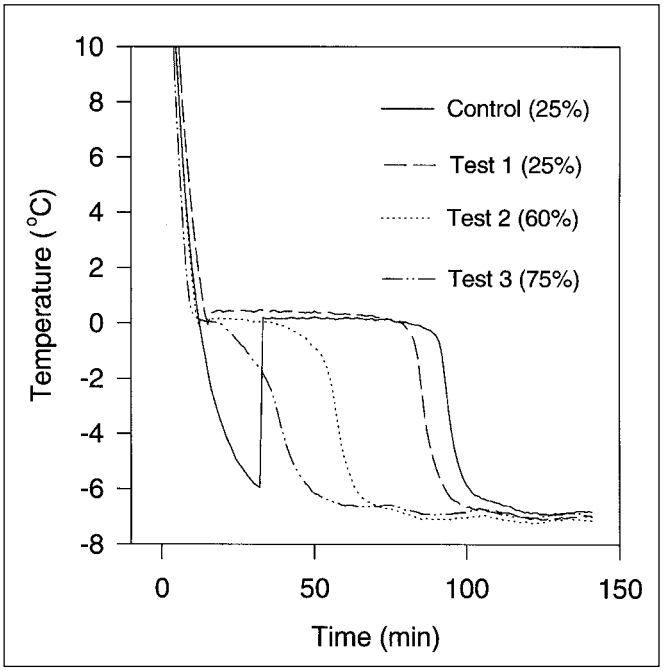

Fig. 3 — Freezing curves of emulsions (oil/water) in the absence and presence of ECINs.

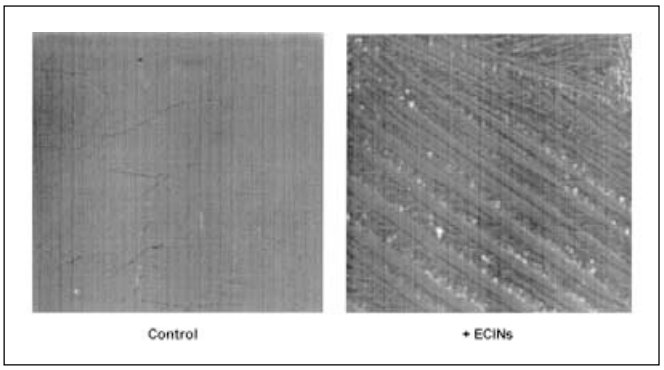

Fig. 4 — Ice-formation patterns in the absence (control) and presence of ECINs.

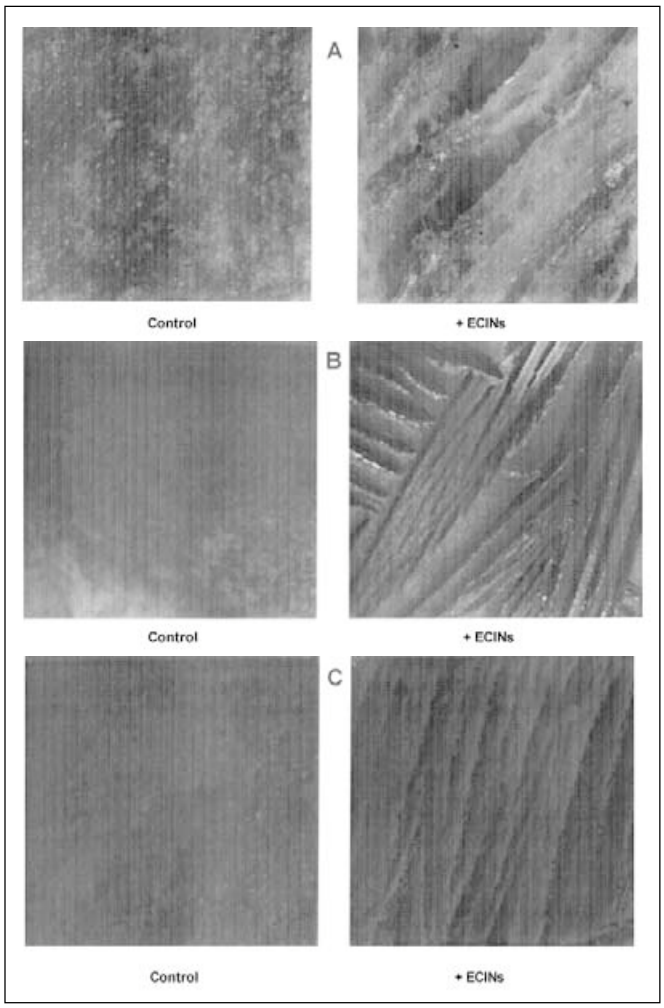

Fig. 5 — Effect of ECINs on the morphological characteristics of selected foods after freezing with or without ECINs (magnification 20×). (A) Soybean curd; (B) Yogurt; (C) Rice-flour paste.

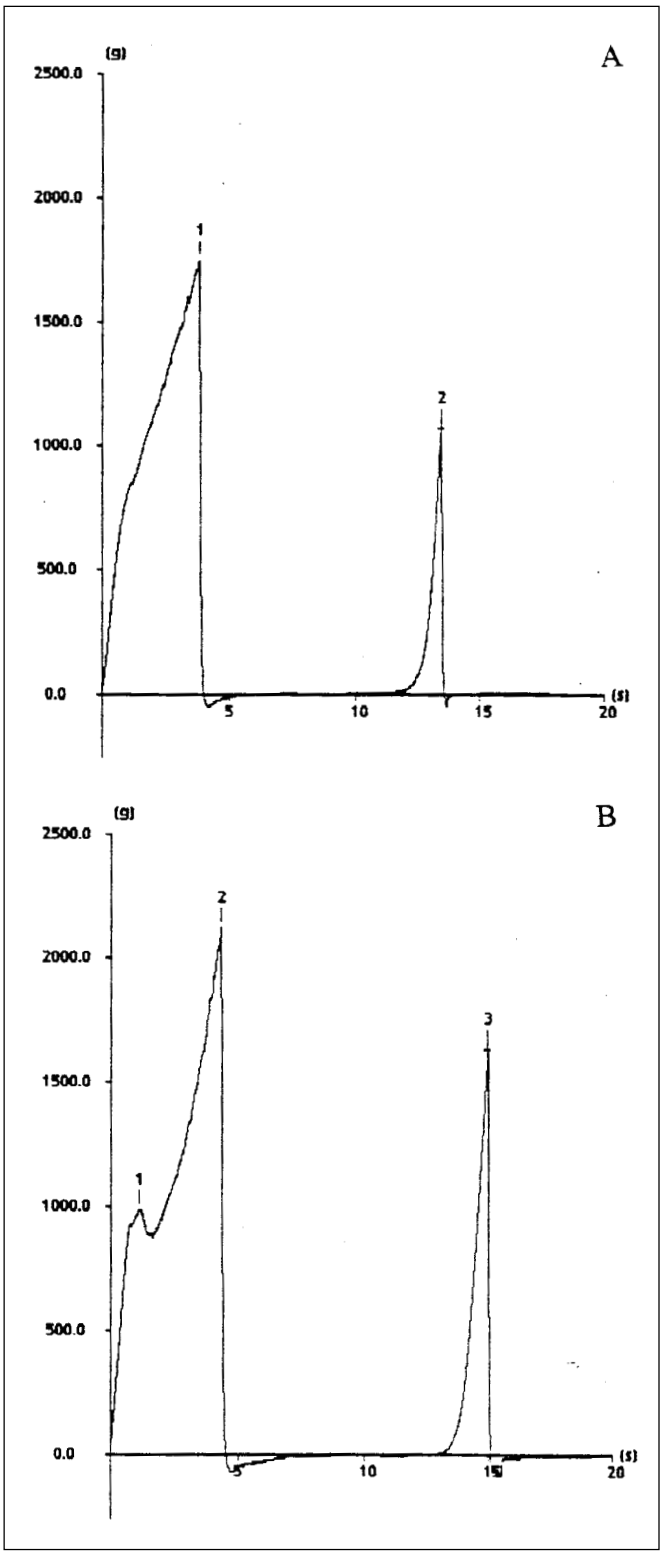

Fig. 6 — Textural profile analysis of rice flour paste. Samples were freeze-textured with or without ECINs. Peak values: control (A) peak 1, 1750g; peak 2, 1076g; +ECINs (B) peak 1, 992g; peak 2, 2130g; peak 3, 1636g.

was not fracturable, as with a sponge-like structure.

ECINs did not apparently affect nucleation temperatures for solid foods, such as soybean curd, ground beef and frankfurters (Table 2). This was probably due to their low thermal conductivity, which prevents heat removal from the center, thereby inhibiting supercooling. The temperature at the center could not attain a certain degree of supercooling before nucleation. Thus, supercooling temperatures measured at the geometric centers of materials did not differ between controls and ECIN-containing materials. The texture attributes of such foods, however, showed marked differences.

With ECINs, unique ice-formation patterns were observed (Fig 4). In solid foods, such as rice flour paste, strawberry yogurt and soybean curd, a fiber-like texture was produced (Fig. 5). Some proteinaceous materials such as egg white, bovine blood, soy protein isolate and milk, had also been textured by freezing with whole cells of *E. ananas* at −5°C (Arai and Watanabe, 1986). However, the mechanisms for such freezing-induced texture patterns in the presence of bacterial ice nucleators have not been explained.

Textural modification of proteinaceous materials by freezing is several centuries old (Lawrence et al., 1986). Fibrous structures of some materials, such as tofu, alkali-extracted red meat or poultry proteins were produced by controlled rates of ice crystal growth. With slow cooling, rates of crystal growth are higher relative to nucleation rate, causing small numbers of large crystals to form (Lawrence et al., 1986). Consequently, in a proteinaceous slurry, this would produce relatively thick fibers. Increasing the cooling rate, so that the nucleation rate was higher than the rate of crystal growth, would form greater numbers of smaller crystals. In a proteinaceous slurry, this would result in a greater number of fine fibers.

Thermal parameters

Patterns of ice formation are closely related to the mechanism of ice nucleation, rates of crystal growth, cooling rates and the presence of other materials. To better understand the effects of ECINs on ice-formation patterns, we further examined the thermal parameters of freezing by DSC. Two representative thermograms for milk (Fig. 7) showed that initial freezing temperatures were markedly affected by addition of ECINs. Onset temperatures were −14.3°C for the control, and −5.4°C with the ECIN-containing sample and shapes of the two curves greatly differed. After freezing was initiated, the exothermal peak for the control spanned from −14.3°C to about −25°C. This difference of 11°C corresponded to about 1 min at a cooling rate of -10°C/min. The exothermal peak in the ECIN-containing sample, however, spanned from −5.4°C to −25°C, a difference of about 20°C, within a time period of 2 min where the phase transition from liquid to ice occurs. Thus, when ECINs were present, milk froze at a subzero temperature 9°C higher than controls. Moreover, it took about double the time to complete the phase transition. Similar results were obtained with other products, such as juice (Fig. 7C, D), water, and sucrose solutions.

Thermograms were examined for a 20% (w/v) sucrose solution at cooling rates of −2 and −10°C/min (Table 4). Onset temperatures for controls were considerably lower (a difference of 13.3°C) than for ECIN-containing samples; and phase transition times for ECIN-containing samples were much longer than that of controls. At a slow cooling rate (−2°C/min), phase transition times can be 5-fold greater than controls. At −10°C/min, the observed difference was less than 2-fold. Enthalpy values, which reflect the latent heat released during phase transitions, indicated that with ECINs, the ratio of ice to unfrozen water was slightly less than controls.

Our results provide an explanation for texture formation effects, as related to ice nucleators. DSC results showed that ECINs induced ice nucleation at about 10°C higher than controls (Fig. 7 and Table 4). However, phase transition times were extended; thus, rates of ice crystal growth were much higher than rates of ice nucleation, resulting in the fiber-like structure. For samples prepared without ECINs, ice nucleation occurred at lower temperatures; however, at the same cooling rate, it took much less time to complete phase transition, and thus the rate of ice nucleation was much higher than the rate of ice crystal growth. The resultant ice-crystals were fine and textures were generally smooth and sponge-like.

Table 4—DSC parameters for freezing of sucrose solution (20% w/v).

Cooling rate (°C/min)	Sample	Onset temp (°C)	Phase transition time (min)	Enthalpy (J/g)
−2	Control	−18.6	1.3	252
	+ECINs	−5.2	5.5	235
−10	Control	−18.9	1.0	254
	+ECINs	−5.6	1.5	240

CONCLUSIONS

THE ADDITION OF ECINS TO FOODS RAISED NUCLEATION TEMPERATURES, and made some foods freezable as high as −6°C, which otherwise could not be achieved. This was clearly demonstrated on the freezing curves of selected foods. The DSC results further showed that ECINs incorporated into foods also extended the phase transition time, indicating that the ice-formation patterns were probably changed. The changed ice-formation patterns could result in different textures

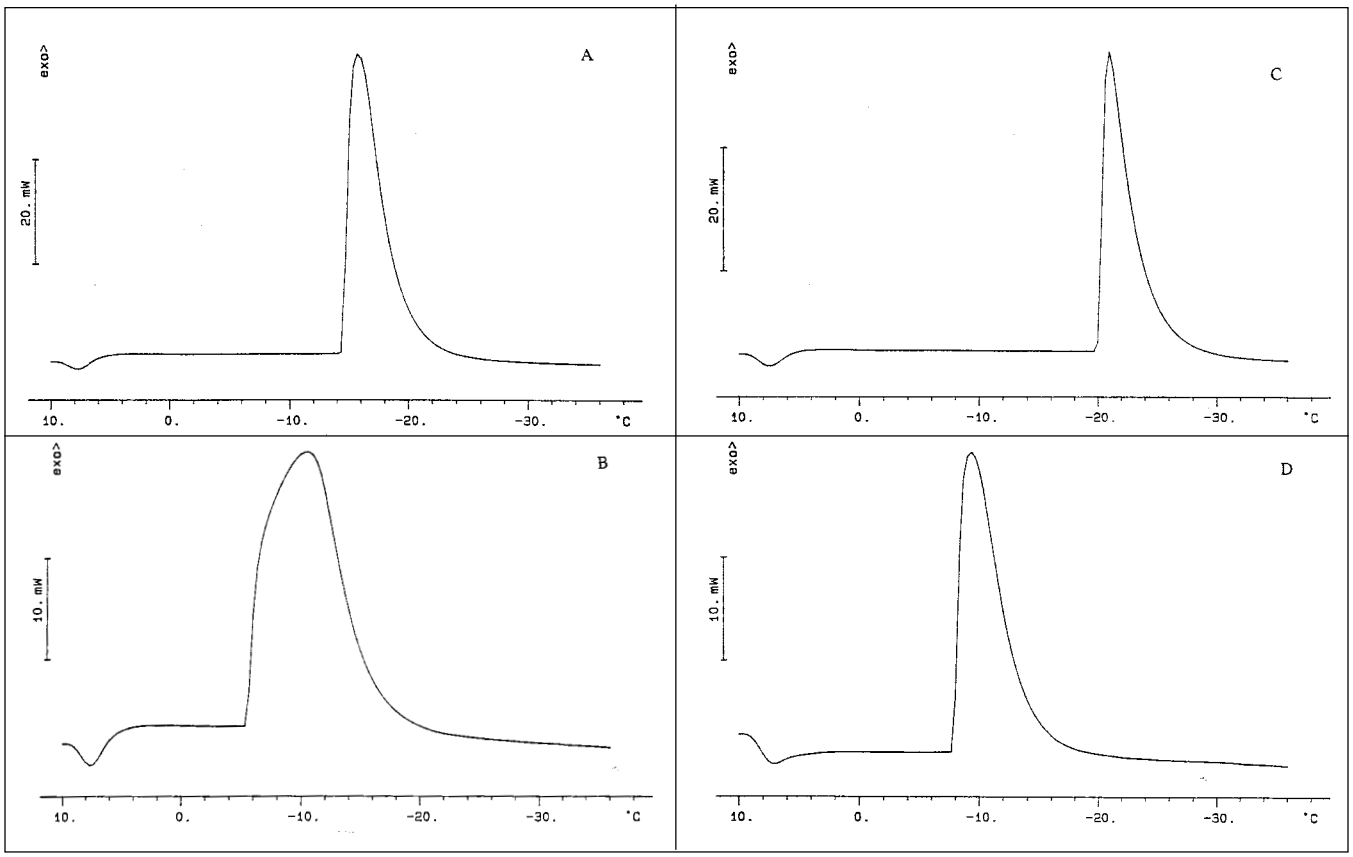

Fig. 7 — Cooling curves of milk and juice in the presence and absence of ECINs by DSC. Cooling rate: −10-C/min; Sample size: ~3 mg. Onset temperatures – Milk: control (A) −14.3°C; +ECINs (B) −5.4°C. Juice: control (C) −19.7°C; +ECINs (D) −7.5°C.

of frozen foods, which were observed by temperature-controlled microscopy. The effects of ECINs on a pilot plant level and feasibility of commercial use need further study. A large-scale production of ECINs, and their complete compositional analysis, would be necessary. Sensory analysis of freeze-textured foods would also be important in additional applications.

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