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A LOW-TEMPERATURE SCANNING ELECTRON MICROSCOPY STUDY OF ICE CREAM.

I. TECHNIQUES AND GENERAL MICROSTRUCTURE

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Abstract

The objective of this study was to investigate techniques suitable for viewing the microstructure of ice cream in the frozen and fully hydrated state using low-temperature scanning electron microscopy (LT-SEM), and to examine the microstructure of the frozen product. Ice cream had four distinct structural phases: ice crystals, air bubbles, fat globules and serum. Air bubbles, 10 to 60 μm in diameter, were lined with fat globules, 0.5 to 2.5 μm in diameter. Ice crystals with a mean diameter of 40 μm showed a characteristic reticulate structure after sublimation. The appearance of the ice cream micrographs was greatly influenced by the degree of sublimation. Optimum sublimation time provided a clearly defined ice crystal socket with network structure remaining. Sublimation was influenced by composition, sample size, specimen holder, and temperature. Although sublimation can be achieved in both the preparation chamber and in the electron microscope, best results were obtained when the sublimation process was observed in the electron microscope.

Key Words: Ice cream, cryo-scanning electron microscopy, low-temperature scanning electron microscopy, sublimation, polysaccharides, freeze-concentration, ice crystals.

Introduction

Ice cream has a complex structure. It is both an oil-in-water emulsion, with most of the oil and water being in the crystalline state, and a fat-rich foam. The ice crystals and air bubbles form a coarser dispersion than the fat globules. The serum phase of ice cream surrounds the ice crystals and air bubbles and results from a freeze-concentration process as water is removed from solution in the form of ice (Berger, 1990). The examination of the microstructure of ice cream is complicated by its high water and fat content coupled with its highly temperature-dependent structure. Under conventional scanning electron microscopy (SEM), the high vacuum in the microscope renders the sample unstable due to volatilization of both fat and water (Brooker, 1990). Electron microscopy requires fat, water and volatile components to be removed from samples or chemically fixed before they can be viewed as dehydrated, partially defatted specimens at ambient temperatures. Kalab (1981, 1985) reviewed microscopic techniques for dairy foods and noted that most conventional SEM preparations were not suitable for viewing milk products based on fat, e.g., sour cream, whipping cream, ice cream. The obvious problem with fat-based dairy products is the loss of structural detail dependent on fat and, in the case of ice cream, further damage resulting from warming.

Low-temperature scanning electron microscopy (LT-SEM) stabilizes samples by quench-freezing in liquid nitrogen slush (-210 °C) and provides examination of intact biological material in a fully-hydrated frozen state. Samples are stable because below -130 °C the vapour pressure of the components nears zero and the ice recrystallization process is halted (Beckett and Read, 1986). This provides a rapid physical fixation and avoids the introduction of artifacts through chemical fixation and structural collapse. Information about ice crystal and air bubble size and distribution gained from the observation of intact samples is important for understanding the sensory properties of ice cream. Brooker (1988) examined ice cream using LT-SEM; in his unetched micrograph, the average maximum ice crystal
Figure 1. LT-SEM specimen holders. a) rivet style showing the openings for insertion of rivets (arrows); b) double-screw style showing movable supports (arrows) which were tightened against the cube of ice cream; c) spring-loaded support style showing movable support (arrow) that held the ice cream cube in place against the side support by the pressure of the spring.

diameter was ~90 μm, ranging from 60 to 130 μm. Air bubbles were lined with adsorbed fat and had a 60 μm diameter. In other foods, such as fat spreads, Brooker (1988) indicated that the water phase could be discerned because a characteristic network-like appearance results from solutes remaining at the ice crystal borders.

Other methods for examining ice cream involve transmission electron microscopy (TEM). Buchheim (1970) and Berger and White (1971) viewed freeze replicas in the TEM as follows: samples were frozen, fractured and/or etched, and a replica was prepared with platinum and carbon. The replica was removed and cleaned for examination. Berger and White (1971) studied the fat destabilization process during ice cream manufacture as it was influenced by fat composition and processing conditions. Buchheim (1970) was able to observe fat globules, casein micelles, ice crystals, air bubbles and the phase interfaces. Microencapsulation and fixation techniques used in TEM were employed by Goff et al. (1987) to examine ice cream mix. Although TEM provides the high resolution needed for studying the fat/serum interface, it is not suited for viewing frozen and fully-hydrated samples.

Arbuckle (1960) proposed light microscopy as a means of quality control of ice cream texture. Samples were sliced at low temperatures, embedded in immersion oil and examined in a hardening room at -40 °C. Smooth textured ice cream exhibited a large number of small, evenly distributed ice crystals and air bubbles surrounded by a uniform thickness of unfrozen material. "Coarse" textured ice cream had a number of large ice crystals and only a few small crystals. Severely temperature-shocked ice cream displayed large, flat plate-like ice crystals contributing to the coarse flaky texture. The "short-body" defect was characterized by small clustered ice crystals surrounding evenly distributed air bubbles. Arbuckle (1960) found that microstructural analysis by light microscopy was limited by maximum resolution of the microscope and cold fatigue suffered by operators, however, advanced equipment available now may have improved this situation (Donhowe et al., 1991).

The objective of the present study was to investigate LT-SEM techniques suitable for viewing the microstructure of ice cream in the frozen and fully hydrated state, and to examine the microstructure of the frozen product. The influence of processing parameters, viz., the incorporation of stabilizers into the mix, the freezing rates, and the storage times and temperatures of the product, on the microstructure of ice cream are reported in the following paper (Caldwell et al., 1992).

Materials and Methods

Ice Cream Manufacture

The ice cream mix utilized throughout this study for continuous freezing consisted of: 11% milk fat, 11% milk solids-not-fat (msnf), 12% sucrose, 4% 42 DE corn syrup solids (Casco, Inc., Etobicoke, Ont.), 0.23% natural and artificial vanilla (Bowes Ltd., Toronto, Ont.), 0.15% locust bean gum (LBG, Food Specialties, Halton Hills, Ont.) and 0.02% food-grade carrageenan (Food Specialties, Halton Hills, Ont.). Mixes were prepared utilizing fresh cream, skim milk and instantized, low-heat non-fat dry milk as sources of milk.
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solids. A control mix was prepared with no stabilizer. The 70 kg mixes were pasteurized at 75 °C for 15 minutes and homogenized at 17.2 MPa (2500 psig), 3.4 MPa (500 psig) second stage, on a Super Homogenizer (Cherry Burrell Corporation, Chicago, IL), cooled to 5 °C and aged 24 hours. Butterfat and total solids levels in the mixes were confirmed by the Pennsylvania modification of the Babcock test (Arbuckle, 1986) and by infrared drying (Sartorius Moisture Balance, Sartorius GmbH, Göttingen, Germany). A preliminary mixture of the control and stabilized batches was used initially to set conditions on the Vogt Instant Freezer (Model VAK-80, Cherry-Burrell Corporation, Chicago, IL). Ice cream was drawn with 85% overrun at -5 °C into 340 ml cups and immediately placed in a -25 °C hardening room.

Scanning electron microscopy

Ice cream preparation. The sample stubs for LT-SEM consisted of copper bases with closeable stainless steel shrouds. Three specimen stubs were utilized for the ice cream samples during this study. The standard specimen stub supplied with the EMscope SP2000A System (Emscope, Ltd., Kent, UK) was first used. The copper base of the specimen stub had two 4.5 mm diameter holes drilled into the base, 6 mm deep, into which stainless steel rivets were placed (Fig. 1a). Pairs of stainless steel rivets of 2 mm diameter were glued together at their rims. Ice cream samples were placed in the hardening room for approximately 1.5 hours after drawing from the barrel freezer. At this point, the ice cream was still soft enough to work with but hard enough to retain its structure. Ice cream was sliced on a cutting board and the glued rivet pairs were punched into the ice cream until it emerged through the top rivet. The rivets were then buried in ice cream to prevent sample dehydration and the ice cream was further hardened for several hours. Before viewing, the rivets were externally wiped, loaded into the chilled copper stub (-20 °C) and immersed in a liquid nitrogen-filled Dewar flask for transportation to the EMscope SP2000A Sputter-Cryo Cryogenic Preparation System (Emscope, Ltd., Kent, UK). The shrouded stub was taken from liquid nitrogen, quickly attached to the transfer device, and the assembly was immersed in slushed nitrogen (-210 °C) and delivered to the evacuated preparation chamber. The upper rivet was fractured using the blade in the preparation chamber, revealing a fresh surface. The fractured samples were transferred to the cold stage of the Hitachi S-570 SEM (Hitachi Ltd., Tokyo, Japan) and etched for 10 minutes at -80 °C after being heated from -160 °C. Subsequently, the etched sample was shrouded, transferred to the preparation chamber, sputter-coated with gold for 2.5 minutes at -150 °C and viewed at 10 kV accelerating voltage in the SEM.

The rivet system was not ideally suited for ice cream. The hardness of the ice cream was critical for entry into the rivets and the plugs of ice cream often exhibited air gaps. During fracture, gaps in the plug caused the surface to be low in the rivets producing weak signals. Consequently, a second specimen stub was constructed to enable larger and more varied sample size with greater ease of preparation (Fig. 1b). The sample could be taken from most ice creams regardless of their firmness, thus tempering the ice cream prior to sampling was not necessary. The copper base was gorged on one side and two movable supports were screwed into the lower portion of the base. The sample width ranged from 1 to 2 mm. The support length was 8 mm and one or two samples could be supported for viewing. The top layer (2 cm) of hardened (-25 °C) ice cream was cut and discarded to reveal fresh surfaces and samples were taken from locations 1.5 cm from the outside circumference of the ice cream. Small cubes of ice cream were prepared using a surgical blade tempered to -25 °C. Samples were secured in the stub via the screw supports and, if necessary, further secured with Tissue-Tek O.C.T. Compound (Miles Scientific, Naperville, IL) spotted at either end of the sample. The samples were further treated as with the riveted base samples but with an increase in sublimation time to 12 minutes.

A third specimen stub examined had a gorged area perpendicular to the length of the stub (Fig. 1c). The support was spring-loaded and held the cube of ice cream in place by the tension of the spring. The disadvantages of this stub were that only one sample could be loaded per stub and the spring-loaded support often was too strong, depending on the texture of the ice cream. Hence, only a small piece would remain after fracture.

Influence of sublimation time on microstructure. Sublimation was monitored over 30 minutes on uncoated samples. These samples were highly unstable because of their high moisture content (60%). Conditions for optimum viewing of uncoated samples by SEM involved a low accelerating voltage of 3 kV and a final lens aperture of 70 μm. Initially, the samples were photographed at -160 °C. When the microscope chamber reached -80 °C, timing began. Photographs were taken at 0, 5, 10, 15, 20, 25, and 30 minutes of sublimation at -80 °C at both 500 and 2500 magnifications of the control and stabilized ice creams.

Results and Discussion

Ice Cream Microstructure

The LT-SEM micrographs of ice cream revealed a
four-phase structure consisting of ice crystals, air cells, fat in an emulsified form, and a continuous serum phase containing dissolved and/or colloidal sugars, salts, proteins and stabilizers (Fig. 2). A thin serum interface separated the ice crystals from air bubbles (Fig. 3a). At lower magnifications, air bubbles were spherical and smooth and contained fat globules whereas ice crystals were more rectangular with a network structure (Fig. 2). The structure denoted by "C" in Fig. 2 is the space once occupied by an ice crystal prior to freeze-etching, i.e., ice sublimation. This socket possessed a characteristic network structure; higher magnification of the ice crystal socket revealed a reticulate structure and the presence of small spheres of approximately 0.2 to 0.4 μm (Fig. 3a). The denser/darker base of the socket was thought to be residual ice and the small spheres on the base were regarded as salts and/or solids normally dissolved in water. Berglund et al. (1990) were able to observe the
SEM Study of Ice Cream. I. Microstructure

Figure 2 (page 4 top-left). A typical micrograph depicting the four phase structure of batch-frozen ice cream: partially freeze-etched ice crystal socket (C), air bubble (A), fat globules (F), and serum phase (S). The ice crystals depicted here are considered large due to the batch freezing and the heat-shocking treatment given to this sample. Bar = 25 μm. From Goff and Caldwell (1991) with permission.

Figure 3 (page 4, right). The ice crystal socket in ice cream. Ice crystals were etched, leaving residual ice and reticulate structure in the sockets. a) Serum phase (S) separating ice crystal socket (C) from air bubble (A). Small spheres (salts/solids) on residual ice (arrow). b) Three dimensional shape of ice crystal socket (C). The serum interface (S) illustrates the depth of the receding ice phase in the socket. Bar = 2 μm (both figures are at same magnification).

Figure 4 (page 4, bottom left). Air bubble (A) in ice cream showing fat globule (arrow) associated with the air/serum interface and surrounded by serum phase (S). The serum phase separated the air bubble from unetched ice crystals. Bar = 5 μm.

Water distribution in frozen bread doughs prepared by LT-SEM because the aqueous phase formed reticulate patterns as a result of etching. Robards and Sleytr (1985) believe reticulation in ice crystals to be caused by the sublimation of water from an aqueous phase and that it represents solutes within the ice crystals. Sublimation of the ice crystals revealed their three-dimensional structure by the depth of sublimation of the crystal into the serum phase (Fig. 3b). The receding ice crystal was distinguished from the bordering serum because the serum was denser in appearance with fat globules in its matrix. After initial hardening the continuously frozen ice cream had a mean maximum ice crystal diameter of approximately 40 μm. Ice crystals in excess of 55 μm diameter contribute to coarse-textured ice cream (Arbuckle, 1986).

Spherical air bubbles surrounded by fat globules and the serum phase constituted the foam portion of ice cream (Fig. 4). Air cell diameters ranged from about 10 to 60 μm. Fat globules protruded into the air bubble and seemed disproportionately distributed at the air bubble/serum interface, but were present to a lesser extent throughout the serum. Air bubbles always showed the presence of many fat globules at the interface, thus allowing for easy differentiation from ice crystals. The fat globule diameter ranged from 0.5 to 1.5 μm (Fig. 5). During homogenization fat globule size is reduced and subsequently the surface area is increased by five to six times (Darling and Butcher, 1978). Casein, partially denatured whey proteins, and added surfactants interact to coat the newly exposed fat surface (Goff et al., 1987, 1989). During freezing and aeration of ice cream mix, it has been suggested that the weak membranes on the fat globules are not able to withstand the high shear forces and agglomeration and partial coalescence of the fat occurs (Lin and Leeder, 1974; Goff and Jordan, 1989). SEM studies of whipped cream have revealed similar structure for the incorporation of air (Buchheim, 1974; Schmidt and van Hooydonk, 1980; Brooker et al., 1986).

Influence of Ice Sublimation on Sample Microstructure

Ice cream samples were viewed, uncoated, during sublimation. Sublimation of ice to the vapour state was achieved by raising the sample temperature under vacuum in the microscope from -160 °C to -80 °C. Etching aids in differentiation of ice crystals in a solution due to the characteristic reticular network which remains at the crystal boundary as a result of eutectic crystallization (Brooker, 1988). Figures 6 and 7 illustrate the progress of sublimation within the samples. Before sublimation, sockets were filled with ice. However, it seemed more difficult to focus the sample image, presumably due to the high moisture content. The absence of gold
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Figure 6. Sublimation of ice from ice cream at -80 °C in SEM at low magnification. Sublimation times = a) 10; b) 15; c) 20; and d) 30 minutes, respectively. Ice crystal (C), serum phase (S) and air bubble (arrow) are marked in Fig. 6b. Arrow points to an ice crystal from lower layer in Fig. 6d. Bar = 25 μm (all four figures are at same magnification).

Sputter-coating caused increased levels of charging. After 10 minutes of sublimation, image outlines were still impaired by the moisture content of the sample (Fig. 6a). Differentiation of ice crystals from the serum phase was difficult at low magnifications. At higher magnifications, however, the surfaces of ice crystals and serum can be differentiated much easier because the ice forms smooth sheets whereas the serum is textured with fat and other components (Fig. 7a). After 10 to 15 minutes sublimation, the ice crystal sockets were outlined and usually by 15 minutes the sockets appeared to be sharp (Figs. 6b and c and 7b and c). Beyond 20 minutes, it was more difficult to find unaltered fields as the samples appeared slightly deformed due to beam damage. After
Figure 7. Sublimation of ice from ice cream at -80 °C in SEM at a higher magnification. Sublimation times: a) 10; b) 15; c) 20; and d) 30 minutes, respectively. Ice crystal (C), serum phase (S) and air bubble (A) are marked in Fig. 7a. Bar = 10 μm (all four figures are at same magnification).

30 minutes, all the residual ice had been etched away and the reticulate structure and spheres normally associated with the ice crystal socket were indistinguishable from the bottom serum border (Fig. 6d and 7d). Often ice crystal sockets would appear in the layer below (Fig. 6d). The ice crystal sockets were so deeply etched that it was difficult to positively differentiate the ice sockets from air crevices (Figs. 6d and 7d).

Optimum sublimation time provided sharp outlines of the ice crystal sockets and the remaining network structure. Sublimation time was influenced by sample size, sample holder and sublimation chamber (preparation chamber versus SEM). Although sublimation of ice can be achieved in both, best results were obtained when the sublimation process was observed in the SEM since the process could be stopped at the appropriate
degree of etching. Often air bubbles in ice cream were saturated with water vapour and polyhedral ice formed at the air bubble interface. Brooker (1988) also observed polyhedral ice within air bubbles in ice cream. Initially the air bubble in Fig. 8a was lined with polyhedral shapes but after 30 minutes sublimation these had disappeared (Fig. 8b). Fig. 8a also illustrates a clump which consisted of both serum and ice. They were discerned because of the roughness of the serum phase. The ice portion etched away leaving only the serum mass as sublimation proceeded (Fig. 8b).

In conclusion, the microstructure of ice cream was best examined by designing a specimen holder that would accommodate whole cubes of frozen product. Ice cream had four distinct structural phases: ice crystals, air bubbles, fat globules and serum. Fat globules 0.5 to 2.5 μm in diameter, seemed disproportionately distributed at the air bubble/serum interface. The air bubbles were 10 to 60 μm in diameter. Ice crystals with a mean diameter of 40 μm under these freezing conditions showed a characteristic reticulate structure after sublimation and their image was greatly enhanced by sublimation. Optimum sublimation time provided a clearly outlined ice crystal socket with network structure remaining. Sublimation was influenced by composition, sample size, specimen holder, and temperature. Although sublimation can be achieved in both the preparation chamber and the SEM, best results were obtained when the sublimation process was observed in the SEM.

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References


Figure 8. Distinguishing the ice component of ice cream.
a) Polyhedral ice (arrows) lining air bubble. Block of ice (I) and serum (S) contaminated ice crystal.
b) Polyhedral ice disappeared from air bubble (arrows) and only serum (S) remained in the block. Sublimation times a) 10 minutes; b) 15 minutes.
Bar = 10 μm (both figures are at same magnification).
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Discussion with Reviewers

R.W. Martin, Jr.: Do you have any plans to identify the material present in the network following sublimation? Do you think the appearance of such material would change with different freezing velocities, e.g., liquid propane or freon cooled in liquid nitrogen?

Authors: Although it would be of interest to identify the material remaining in the ice crystal socket after sublimation, we do not have the equipment to do this at present. We do not believe that the reticulate structure would change if the ice cream samples were frozen for SEM using different cryogens. We think that this structure may have during the preparation of the ice cream and thus was not an SEM artifact.