Functionality of Soluble Proteins in Ice Cream

Confidential Report for Idaho Milk Products

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Idaho Milk Products has developed a new functional milk protein product (IdaPlus 1085) enriched in soluble caseins through the partial removal of calcium phosphate. As a result, superior emulsification and foaming properties have been demonstrated. This paper discusses the potential application of this ingredient in ice cream formulations.

By way of introduction and background, Appendix 1 contains an excerpt from Goff, H. D. 2016. Milk Proteins in Ice Cream. In "Advanced Dairy Chemistry – 1B – Proteins. Applied Aspects, 4th edn." P.L.H. McSweeney and J. A. O'Mahony, eds. Springer, New York. pp. 329345, which presents a recent review of the structure of ice cream and the functional properties of proteins in ice cream (emulsification, aeration and water holding capacity). The discussion that follows here builds on this background information.

Also, by way on introduction, it is important to recognize the sequential nature of ice cream processing. Fat is first emulsified through homogenization. The very large increase in surface area of fat that occurs results in a large adsorption of proteins to stabilize this newly-formed fat globule surface area. Milk proteins are very good at emulsion stabilization, and soluble caseins will improve this functionality as they lead to more complete and cohesive coverage of the fat globule, as opposed to large and bulky casein micelles. However, in ice cream processing we need the fat globules to undergo partial coalescence (fat destabilization or agglomeration) to establish a fat aggregated network, which provides properties of dryness, shape retention, melt resistance, creaminess upon consumption, etc. to the frozen product (similar to the structure formed from the whipping of heavy cream). If the fat emulsion is too stable, it will not undergo appropriate fat partial coalescence. Hence it has been the traditional role of emulsifiers (egg yolk or monoglyceride) to displace and desorb some of the milk proteins, rendering the fat globule more prone to fat-fat interactions. So enhanced emulsion stabilization is not a desirable property in ice cream per se. It is also recognized in the literature that proteins which create a strongly-cohesive membrane, such as whey proteins, sodium caseinate and b-casein, are harder to displace from fat globules by emulsifiers compared to proteins with a higher content of micellar casein, due to the nature of the adsorption and film formation once adsorbed. On the other hand, heat-aggregated whey proteins have been shown to behave more similarly to casein micelles, resulting in higher fat destabilization when used in ice cream compared to unaggregated whey protein (Relkin et al., 2006).

The second major functionality of the proteins is in air bubble formation and stabilization during whipping. For this, we need a high content of soluble, surface active proteins in the aqueous phase to produce small, stable bubbles with complete protein coverage. Hence this becomes a secondary effect of added emulsifiers, to ensure that surface active proteins are available for aeration and not all associated with the fat interface. The surface activity of the proteins give rise to both emulsification and foaming, so typically good emulsifying proteins are also good foaming proteins. Here the enhanced content of soluble caseins should be a positive attribute, since

they will enhance air bubble formation, assuming they are available in the aqueous phase. Once formed, secondary fat adsorption helps to stabilize the bubbles. It should be noted here that lower fat content in ice cream mix will also mean higher protein content in the aqueous phase, which should lead to better foam formation, but not necessarily better foam stability. During the freezing process, air bubbles tend to be distorted, which can lead to coalescence and channeling and eventually to shrinkage. It is thus important that the integrity of the individual bubbles is maintained. Foam stability in ice cream is thus reliant on the formation of a small, intact bubble with a strong interface.

The third functionality of proteins is in water holding capacity/protein-protein interaction, and or protein-polysaccharide interaction and thus network formation in the freeze-concentrated unfrozen phase after freezing. Again, the enhanced level of soluble casein should be a positive attribute, leading to enhanced ice crystal stability and better shelf-life.

Our research group has completed several projects that may help to shed light on the functionality of soluble caseins in ice cream systems. Zhang and Goff (2004) studied the effects of EDTA-induced partial dissociation of casein micelles and the presence of emulsifiers on the aerating properties of milk proteins and the composition of air–serum interfaces in both dairy foams and ice cream. It was observed that added EDTA increased soluble caseins and improved the whip ability of milk protein solutions. EDTA induced more non-micellar caseins to adsorb at both fat–serum and air–serum interfaces while the addition of mono- and di-glycerides (MDG) caused fat globules to also directly adsorb to air–serum interfaces. The presence of EDTA in the absence of MDG increased the stability of the ice cream emulsion to shear (too much adsorbed protein) and greatly decreased the fat partial coalescence and the proportion of fat globules involved in air–serum interfacial adsorption. This they concluded that enhanced aeration can only occur if protein adsorption to fat is minimized or blocked.

Augustin and Clarke (2008) studied the effects of citrate addition to milks on the stability of milk foams. Citrate addition (0.1–0.5 mol added citrate kg−1 milk solids non-fat) improved the whipping properties of milks reconstituted from conventional low-heat (72°C for 30 s) and high heat (85°C for 30 min) powders. This effect was attributed to the role of citrate in dissociating casein micelles. Similar effects of citrate addition on foaming were obtained when the salts were added to skim milk concentrate prior to drying. Thus, they concluded that the citrated milk powders may deliver added functionality for enhancing the foaming properties of milks at both low and high application temperatures.

Subsequently, Prof. Augustin approached us to examine the functionality of citrated concentrated milk in ice cream. We conducted an investigation with two visiting exchange students from France (2013), but the results were not published. In 10% fat ice cream, the soluble caseins over-stabilized the emulsion creating a more continuous thin layer of proteins around the fat globules. This prevented the formation of a partially-coalesced fat network necessary for good air stabilization. Consequently, no improvement in foamability and a decrease in meltdown stability was observed. It was also observed that emulsifiers did not have the same impact on fat destabilization, and a higher level of emulsifier was needed, due to the more coherent protein film at the fat interface. In 2% fat ice cream, the air stabilization was dependent on level of citrate addition. If the quantity of soluble casein is too high, foam stability was reduced, similar to that observed with sodium caseinate - good at foamability but less so at foam stability. However, when micellar casein was also present, air stabilization was enhanced, suggesting that the combination of soluble and micellar casein led to sufficient protein interaction to enhance air bubble stability.

I was also involved in research at University College Cork in 2011 on the applications of concentrated milk from high hydrostatic pressure processing (HP) in ice cream formulations (Huppertz et al., 2011, 2012). HP treatment

had little effect on the size of the milk fat globules but increased the viscosity of the ice cream mix considerably. Transmission electron micrographs showed the presence of a network of micellar fragments, arising from HP-induced disruption. Ice cream from HP-treated mix showed a higher resistance to melting than ice cream from untreated mix. Although sensory analyses were not performed, the ice cream was also noted to be much smoother in texture. The network of micellar fragments is believed to be responsible for the increased viscosity and reduced melting and is hypothesized to occur because of calcium induced aggregation of caseins on decompression. We concluded that restructuring casein micelles in ice cream mix with HPP is an interesting technology for improving textural properties of ice cream and related products, and therefore it is worthwhile considering whether similar effects can be achieved using structured milk protein ingredients treated by other technologies, so as to circumvent the requirement of HP processing.

A recent trend in the ice cream industry has been the development of low-fat, low-calorie, high-protein, high-overrun frozen desserts (e.g., Halo-Top). In such products, the protein is responsible for aeration and air stability, with high overrun and in the absence of fat, and viscosity and water holding capacity in the unfrozen phase. Both structural requirements should also contribute to good texture.

From the above discussion, we can conclude that milk protein concentrates with enhanced soluble casein content should be able to deliver enhanced functionality to ice cream systems, but not in all cases. In normal to high-fat systems, the critical factor is over-stabilization of the fat globules, due to the enhanced emulsification properties of the proteins, which leads to a decrease in fat structuring directly, and also leads indirectly to a lack of protein for aeration at the whipping stage. Hence, the addition of small-molecule emulsifiers would need to be optimized to control protein adsorption to fat and ensure that proteins are available for foaming. In low-fat systems, the critical question is the balance of micellar to non-micellar caseins such that in addition to air bubble formation they also lead to air bubble stability to protect against distortion during freezing, channeling and the potential for product shrinkage. This consideration is even more relevant in high overrun products. In high-protein products, in addition to the above, solubility also has to be optimized so the protein does not deliver a chalky mouthfeel.

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Appendix 1. Excerpt from Goff, H. D. 2016. Milk Proteins in Ice Cream. In “Advanced Dairy Chemistry – 1B – Proteins. Applied Aspects, 4th edn.” P.L.H. McSweeney and J. A. O’Mahony, eds. Springer, New York. pp. 329-345.

Milk proteins contribute three important structural functions to ice cream. They emulsify the fat phase during homogenization to produce a stable emulsion in the mix state. Their subsequent interaction with emulsifiers during the ageing process reduces the adsorbed protein level, thus producing a fat emulsion that is able to partially-coalesce in the whipped and frozen ice cream and produce desirable fat structure. Proteins present in the serum phase of the mix during whipping contribute to the development of an air bubble interface that is capable of maintaining small and stable air bubbles. Unabsorbed proteins also increase mix viscosity, particularly in the unfrozen serum phase after cryo-concentration, which leads to enhanced body and texture and reduced rates of ice recrystallization.

ICE CREAM STRUCTURE

The texture of ice cream is perhaps one of its most important quality attributes. It is the sensory manifestation of structure; thus, establishment of optimal ice cream structure is critical to maximal textural quality. It is also critical to processing parameters (e.g., dryness on extrusion), shelf-life, and quality parameters during consumption (e.g., shape retention during melting) (Muse and Hartel, 2004). Structurally, ice cream is comprised of thee discrete phases – fat globules, some of which may be partially-coalesced, air bubbles, and ice crystals – these are embedded into a freeze-concentrated unfrozen matrix of soluble sugars, proteins, minerals salts, stabilizers and water (Fig. 1). Both the fat phase and the air phase also have interfacial layers associated with them. An understanding of the functional role of proteins in ice cream depends on conceptualization of this structure. The structure of ice cream begins with the mix as a simple emulsion, with a discrete phase of partially crystalline fat globules surrounded by an interfacial layer comprised of proteins and surfactants (Fig. 2). The continuous, serum phase consists of the unabsorbed casein micelles in suspension in a solution of sugars, unabsorbed whey proteins, salts and high molecular weight polysaccharides. Ice cream is a complex food colloid in that the mix emulsion is subsequently foamed, creating a dispersed phase of air bubbles, and is frozen, forming another dispersed phase of ice crystals (Fig. 3). Air bubbles and ice crystals are usually in the size range of 20 to 50 µm (Caldwell et al., 1992). The serum phase is freeze-concentrated. In addition, the partially-crystalline fat phase, at refrigerated temperatures, undergoes partial coalescence during the concomitant whipping and freezing process, resulting in a network of agglomerated fat, which partially surrounds the air bubbles and gives rise to a solid-like structure (Fig. 4) (Goff, 2002, 2006). Given this context, the functional role of protein can be examined, considering their behavior at the fat interface, the air interface and in the serum phase.

FUNCTIONAL ROLES OF MILK PROTEINS IN ICE CREAM

Emulsification

The interfacial behavior of milk protein in emulsions is well documented, as is the competitive displacement of proteins by low molecular weight surfactants (Chen and Dickinson, 1993; Euston et al., 1995, 1996; Granger et al., 2005a,b). In ice cream, the emulsion must be stable to withstand mechanical action in the mix state but must undergo sufficient partial coalescence to establish desirable structural attributes when frozen. These include dryness at extrusion for fancy molding, slowness of melting, some degree of shape retention during melting and smoothness during consumption. This implies the use of small molecule mix state but must undergo sufficient partial coalescence to establish desirable structural attributes when frozen. These include dryness at extrusion for fancy molding, slowness of melting, some degree of shape retention during melting and smoothness during consumption. This implies the use of small molecule surfactants (emulsifiers) to reduce protein adsorption and produce a weak fat membrane that is sensitive to shear action (Goff et al., 1987, 1989; Gelin et al., 1994, 1996a, b; Goff, 2002). Fig. 2 demonstrates the action of emulsifiers in ice cream by showing protein adsorption to fat globules in the absence of emulsifier (Fig. 2A), in which considerable casein micelle adsorption can be seen, and

in the presence of emulsifier (Fig. 2B), which shows little or no casein adsorption. The loss of steric stability from the globule, which was contributed by micellar adsorption, accounts for its greater propensity for partial coalescence during shearing. Partial coalescence is responsible for establishing a threedimensional aggregation of fat globules that provide structural integrity (Fig. 4). This is especially important if such integrity is needed when the structural contribution from ice is weaker (i.e., before hardening or during melting). Variables that affect the destabilization of fat in ice cream have been well studied (Kokubo et al., 1998; Sourdet et al., 2003; Mendez-Velasco and Goff, 2012a,b).

With respect to protein contribution to fat globule integrity, it is obvious from the studies to date that a weak surface layer is most desirable. Segall and Goff (1999) examined the susceptibility of ice cream emulsions to partial coalescence during shear when the emulsion was prepared with varying concentration and type of protein, while still retaining sufficient quiescent emulsion stability. The membranes of fat globules stabilized by an excess of whey protein (from whey protein isolate) are generally too stable to undergo partial coalescence (Sourdet et al., 2002; Zhang and Goff, 2005). However, the membranes of fat globules stabilized by limited whey protein isolate were more susceptible than those made from sodium caseinate or casein micelles, while those made from partially hydrolyzed whey proteins did not show sufficient quiescent emulsion stability (Segall and Goff, 1999). The problem with this approach, though, is that when casein was added after homogenization to the limited whey protein-stabilized emulsion, further casein adsorption to the whey protein stabilized membrane was rapid, unless suitably controlled (Segall and Goff, 2002a,b). Heat aggregation of whey proteins plays an important role in producing a fat globule interface that is more susceptible to partial coalescence (Sourdet, 2002, 2003; Granger et al., 2005a; Relkin et al., 2006). However, the heat-aggregated proteins by themselves may not be sufficient to establish a stabilizing membrane to get good homogenizing effects (Granger et al., 2005a). Thus, an understanding of protein structures and protein-surfactant interactions at the fat interface is critical to develop the optimal level of partial coalescence in the finished product.

Aeration

Milk proteins are well known for their foaming properties (Xinyi et al., 2010) and during the manufacture of ice cream, air is incorporated to about 50% phase volume. Thus, it should be unsurprising that milk proteins contribute to stabilizing the air interface in ice cream. Foam formation and stabilization in ice cream has recently been reviewed (Xinyi et al., 2010). Continuous air interfaces in ice cream can always been seen by scanning electron microscopy (Fig. 3B). During sublimation, these interfaces remain intact, suggesting that a combination of protein, emulsifier and fat forms a continuous layer separating the air bubble from direct contact with ice. This air interface is very important for overall structure and structural stability (Turan et al., 1999; Sofjan and Hartel, 2004). Loss of air, usually due to a lack of functional protein and the development of air channels, can lead to a defect known as shrinkage, the occurrence of which is fairly common and very significant for quality loss and unacceptability of the product (Dubey and White, 1997; Turan et al., 1999). The process of whipping heavy cream includes an initial protein adsorption to the air interface and a subsequent adsorption of fat globules and their associated membrane to the existing protein membrane of the air bubble (Stanley et al., 1996; van Camp et al., 1996). Globular fat adsorption to air interfaces is known to stabilize air bubbles from rapid collapse (Stanley et al., 1996; Chang and Hartel, 2002a,b). Proteins at the fat interface have also been fat globules and their associated membrane to the existing protein membrane of the air bubble (Stanley et al., 1996; van Camp et al., 1996). Globular fat adsorption to air interfaces is known to stabilize air bubbles from rapid collapse (Stanley et al., 1996; Chang and Hartel, 2002a,b). Proteins at the fat interface have also been shown to play an important role during the aeration of emulsions (van Camp et al., 1996; Zhang and Goff, 2005). Incorporation of air into ice cream in commercial whipping/freezing equipment is rapid, occurring within seconds, and at the same time, viscosity of the surrounding matrix increases exponentially due to freezing, such

that air bubbles after formation become physically trapped in a semi-solid matrix, making their collapse quite difficult.

Goff et al. (1999a) examined air interfaces in ice cream and fat-air interactions using transmission electron microscopy with freeze-substitution. The structures created by increasing levels of fat destabilization in ice cream (achieved through increased emulsifier concentration in the mix and batch versus continuous freezing) were observed as an increasing concentration of discrete fat globules at the air interface (as in Fig. 3B) and increasing coalescence and clustering of fat globules both at the air interface and within the serum phase (Fig. 4). Air interfaces at the highest levels of fat destabilization were not completely covered by fat globules. There was no evidence of a surface layer of free fat in the work of Goff et al. (1999a). Further, air interfaces in ice creams showing low level of fat adsorption to the air interface, due to very stable fat globule membranes Fig. 6 shows a very similar, continuous membrane to those from a formulation containing adsorbed fat, offering further suggestion that the air bubble membrane itself is comprised of protein, with discrete and partially-coalesced fat globules subsequently adsorbed.

Proteins adsorbed to the air interfaces in ice cream are difficult to quantify. Zhang and Goff (2004) used immune-gold labeled b-casein and b-lactoglobulin in ice cream and visualized protein adsorption to the air interfaces by transmission electron microscopy. They showed that the air interfaces were stabilized to a great extent by soluble casein and whey proteins, when available, compared to casein micelles. Modifications of micellar casein to enhance soluble casein, for example by EDTA addition, also enhanced protein adsorption to the air interface (Zhang and Goff, 2004).

Solution Behavior

Milk proteins interact with water and the subsequent hydration is responsible for a variety of functional properties, including rheological behavior. Thus, freeze-concentration of proteins in ice cream must lead to a sufficient concentration to have a large impact on the viscosity of the unfrozen phase and its subsequent effect on ice crystallization, ice crystal stability and solute mobility (Flores and Goff, 1999; Regand and Goff, 2002). The cryo-concentration of casein micelles in the unfrozen serum phase in ice cream can easily be seen in Fig. 4. This has a large impact on all the other structural elements in ice cream (Sofjan and Hartel, 2004). Jonkman et al. (1998), who studied the effect of ice cream manufacture on the structure of casein micelles, found that the micelles per se were not affected by the process. Although the stability of the micelle was expected to be affected by low temperature, this was offset by an increasing concentration of milk salts in solution during freeze-concentration, such that the micelle remained intact in a similar state to that found in the mix (Fig. 4).

Polysaccharides are also added to ice cream mix to enhance solution viscosity and to impact on ice crystallization behavior. Commonly used polysaccharides can be incompatible in solution with milk proteins leading to a microscopic or macroscopic phase separation (Syrbe et al., 1998; Vega et al., 2005), a phenomenon that has been studied in milk and ice cream mixes (Garnier et al., 1995; Bourriot et al., 1999; Schorsch et al., 1999a, b, 2000). Goff et al. (1999b) examined the interaction between milk proteins and polysaccharides in frozen systems using labelled polysaccharides and fluorescence microscopy, and demonstrated a clear phase separation between the two, leading to discernable networks created by freezing from both locust bean gum and milk proteins (Fig. 6). The same phenomenon can be seen by transmission electron microscopy of ice cream, where when in solution with polysaccharides, the casein aggregates into distinct networks (Fig. 7). Flores and Goff (1999) demonstrated that milk proteins had a large impact on ice crystal size and stability. It thus appears that microscopic phase separation of the milk protein induced by polysaccharides, and “aggregation” of casein into a weak gel-like network, promoted also by freeze-concentration, may be at least partly responsible for ice crystal stability and for body and texture of the ice cream during consumption. It is well known that k-

carrageenan controls macroscopic phase separation between casein micelles and polysaccharide stabilizers in ice cream or ice cream mixes, although it has recently been shown that they are still phase separated at a microscopic scale (Vega and Goff, 2005; Vega et al., 2004, 2005). Spagnuolo et al. (2005) demonstrated that k-carrageenan interacts directly with casein micelles and k-carrageenan helices interact with each other, to form a weak structural network that holds the individual protein rich or polysaccharide-rich phases intact in a water-in-water type "emulsion", so they do not lead to macroscopic phase separation.

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FIGURES

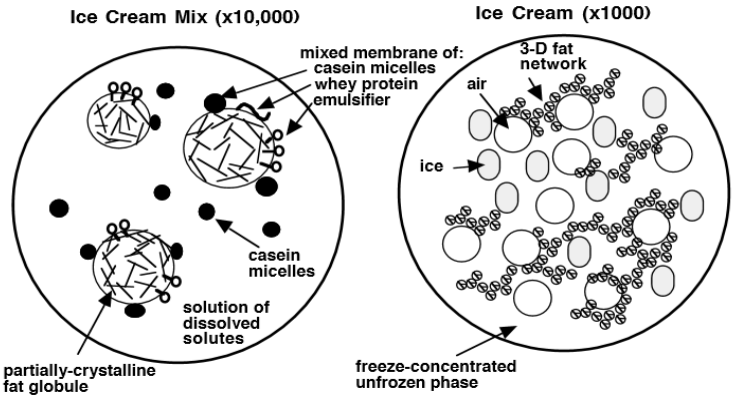

Fig. 1. Highly schematic illustration of the structure of ice cream mix and ice cream. Ice cream mix contains the partially crystalline fat globules and casein micelles as discrete particles in a solution of sugars (including lactose), salts, dispersed whey protein and stabilizers, etc. The surface of the fat globule demonstrates the competitive adsorption of casein micelles, globular, partially denatured whey proteins, b-casein and added emulsifiers. Ice cream contains the ice crystals, air bubbles and partially-coalesced fat globules as discrete phases within an unfrozen serum containing the dissolved material (including lactose). The partially-coalesced fat agglomerates adsorb to the surface of the air bubbles, which are also surrounded by protein and emulsifier, and link the bubbles through the lamellae between them.

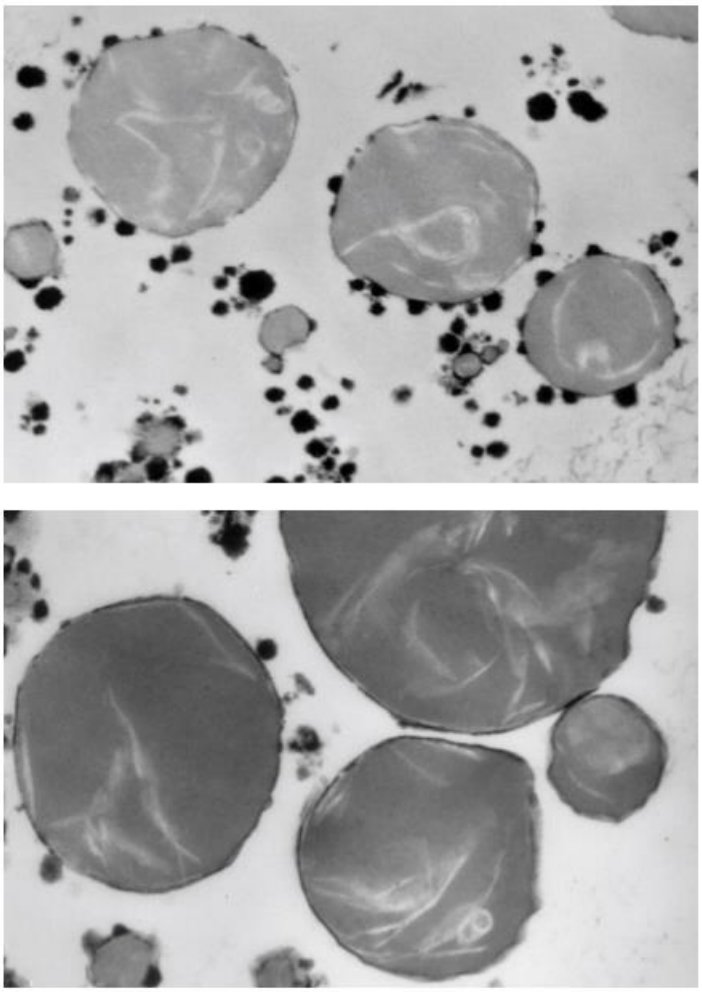

Fig. 2. Transmission electron micrographs showing the structure of fat and protein in ice cream mix in the absence (A) or presence (B) of emulsifier. f = fat, c = casein micelle, bar = 1 µm. For preparation method, see Goff et al. (1987).

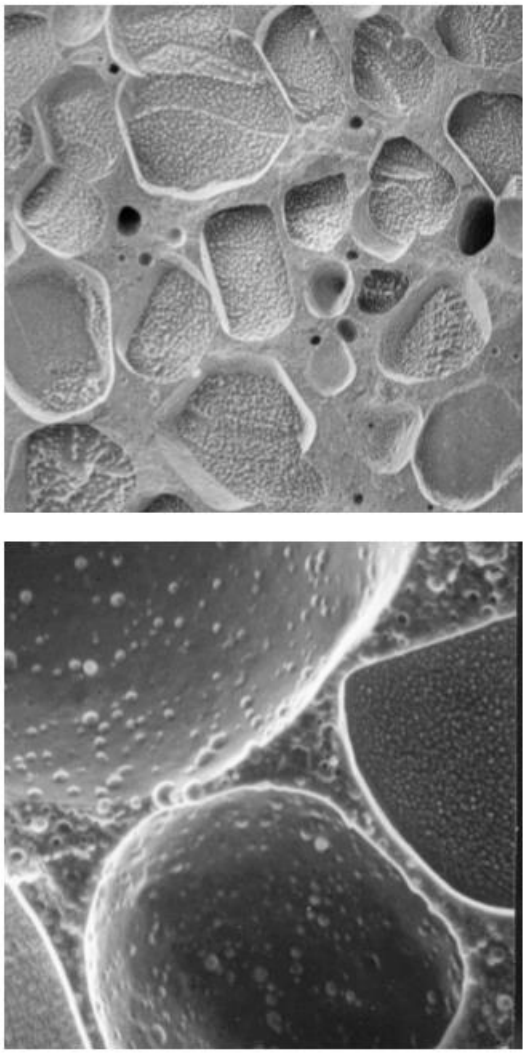

Fig. 3. Cryo-scanning electron micrographs of ice cream structure. (A) low magnification showing region dominated by ice crystals. Bar = 30 µm. (B) higher magnification showing close-up of air bubble interior and fat globules. Bar = 10 µm. a = air bubble, f = fat globule, i = ice crystal, s = unfrozen serum phase, For preparation method, see Caldwell et al. (1992).

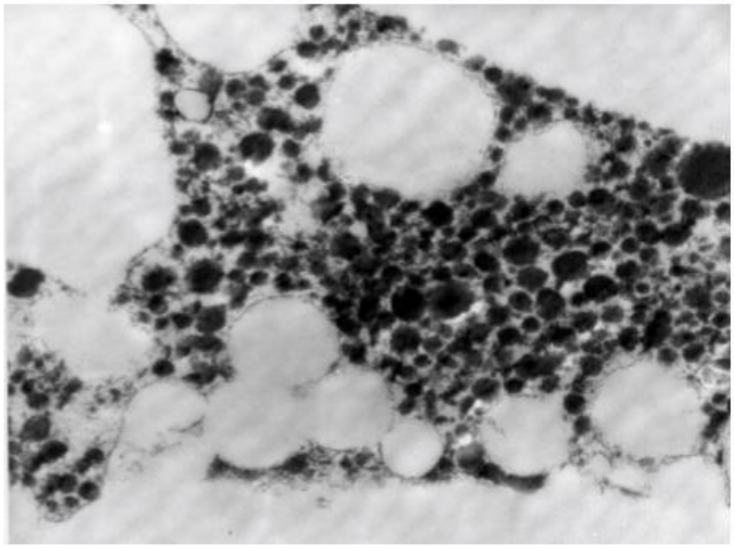

Fig. 4. A transmission electron micrograph prepared by freeze substitution showing the structure of partially coalesced fat, the freeze-concentration of casein micelles in the unfrozen phase and the interaction of fat at the air interface. a = air bubble, f = fat globule, i = ice crystal, s = serum phase packed with casein micelles, bar = 1 µm. For preparation method, see Goff et al. (1999a).

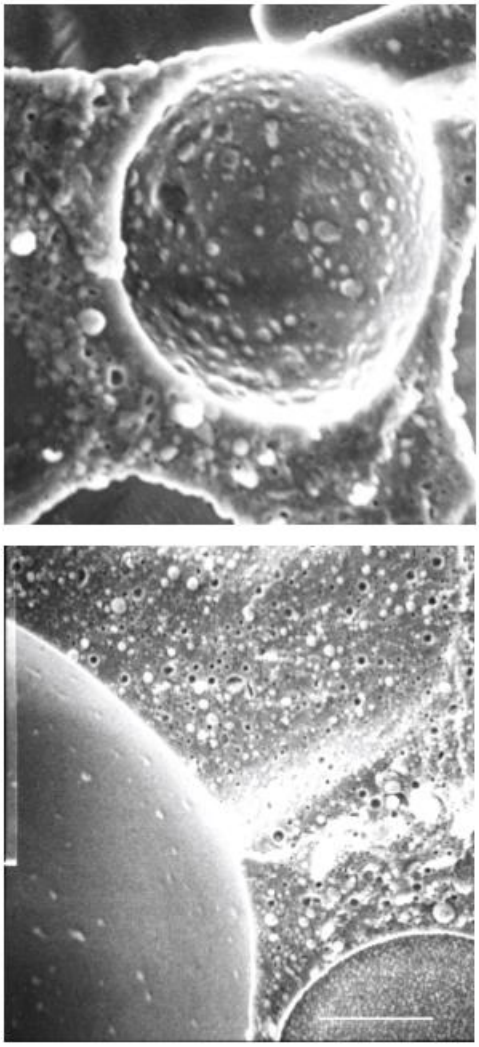

Fig. 5. Cryo-scanning electron micrographs of the air interface in ice cream showing a high level of fat adsorption (A) or a low level of fat adsorption (B). In both cases, the air interface surrounding the fat is comprised of protein. a = air bubble, bar in A = 30 µm, bar in B = 10 µm. For preparation method, see Caldwell et al. (1992).

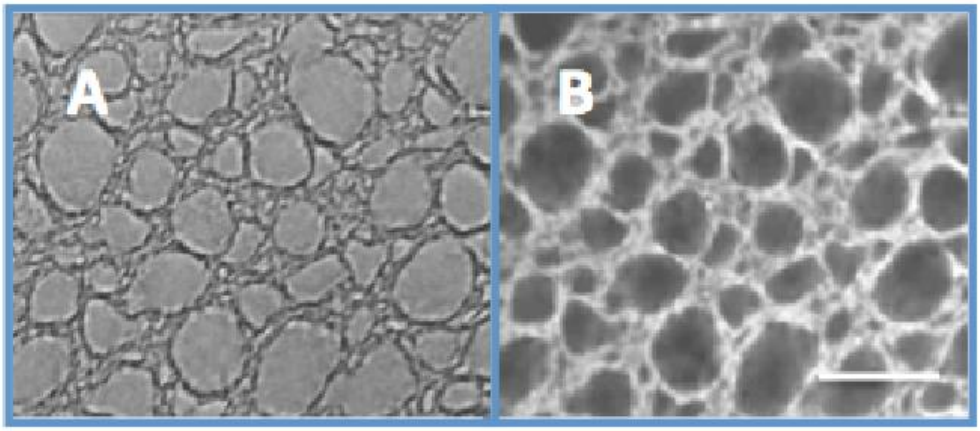

Fig. 6. Confocal scanning laser micrographs of frozen and melted non-fat ice cream mix, showing phase separation of protein and locust bean gum (tagged with a fluorescent marker). The structure resulted from the formation of ice; however, melting has caused the ice to disappear but the protein and polysaccharide structure to remain. (A) phase contrast image, showing the structure that results from cryo-aggregated protein. (B) fluorescent image from the same field as A, showing the structure that results from cryo-gelled, fluorescent-labeled locust bean gum. Bar = 50 µm. For preparation method, see Goff et al. (1999b).

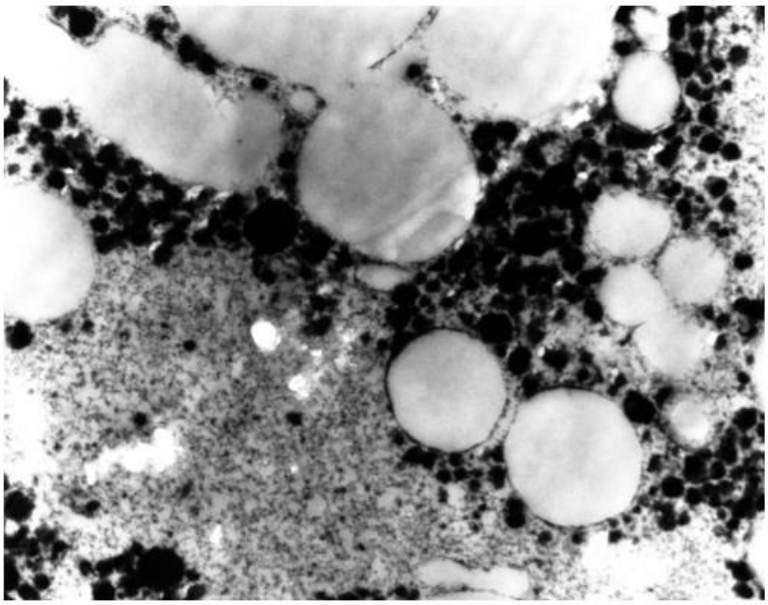

Fig. 7. A transmission electron micrograph prepared by freeze substitution showing the phase separation and aggregation of casein micelles resulting from the combination of partial coalescence of the fat and addition of polysaccharide. f = fat globule, c = casein micelle, p = polysaccharide network devoid of casein micelles, bar = 1 µm. For preparation method, see Goff et al. (1999a).