EFFECTS OF MILK PROTEIN TYPE ON STRUCTURAL PROPERTIES OF ICE CREAM SYSTEMS

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ABSTRACT : We prepared ice cream model systems differing by their milk protein composition and by application or not of an additional heat treatment of protein solution before emulsification. The effects of protein composition and heat treatment on microstructural properties of ice creams, as evaluated by electron microscopy images, were studied and discussed in terms of particle size distribution and evaluation of the amount of adsorbed protein at the fat globule surface.
Key words : ice cream – milk proteins – protein denaturation - protein adsorption – fat destabilisation.

1. Introduction

The classical protein source used in ice cream manufacture is skim milk powder (SMP) at about 10-12% w/w and it contains a casein-to-whey proteins weight ratio (C/WP) of 80/20. In the present work, we used different milk protein mixtures at 2.25% w/w total protein concentration to prepare ice creams containing different protein composition (F0 for 0/100 C/WP; F20 for 20/80 C/WP; F40 for 40/60 C/WP), and different conformational states resulting from application or not of a heat treatment to protein solutions (F0 PHT ; F20 PHT ; F40 PHT), before homogenisation.

2. Materials and Methods

2.1. Ice cream manufacture

Ice creams were prepared using (all in weight percentage): 9% vegetable oil (palm kernel hydrogenated fat), 3% glucose, 14% sucrose and 0.5% emulsifier-stabiliser blend (Cremodan NS 30) containing saturated mono- and di-glycerides (0.3%), and polysaccharides (0.2% : locust bean gum, guar gum and κ–carrageenan). The protein sources used in this study were a whey protein isolate and an isolate of micellar casein. These isolates were used in mixture in order to obtain 2.25% total protein and 3 casein-to-whey proteins ratios (from 0/100, 20/80 and 40/60).

Ice cream mixes were prepared following two different thermo-mechanical treatments. The first process was a common process [1] where powder ingredients (emulsifier-stabiliser, protein, glucose syrup and sucrose) were dispersed in hot water (65 °C) under stirring. Then, fat (pre-melted at 40 °C) was added and the mixture was stirred for 15 min. The pre-mix was pre-heated in a plate exchanger (72 °C for 1 min), homogenised at 72 °C (110 + 40 bar) using APV Gaulin homogeniser (Evreux-France), pasteurised in a plate exchanger (86 °C for 30 s), cooled to 4 °C, and aged for 24 hours before analysis.

The second process involved a pre-heat treatment (PHT, 86 °C for 90 s) of 1% of the total protein concentration in 5% milk ultrafiltrate. After this additional heat treatment the resulting pre-denatured proteins were submitted to the same procedure as the one used in the first process, after being mixed with the other ingredients at 65 °C for 15 min.

After the aging step (4 °C for 24 h), all the ice cream mixes were whipped (100% overrun) at –5 °C in a scraped surface freezer (Hoyer KF 80, working with the following operating conditions : 70 $L.h^{-1}$ flow rate and -20 °C for 5 min). Ice creams were then stored at –30 °C for several weeks, and melted at 4 °C overnight before characterisation (excepted for the microscopy study).

2.2. Particle size distribution

Particle size distribution was determined by laser light scattering (Mastersizer MS 1000, Malvern Instruments, Orsay-France) after dispersion of the ice cream mixes or final melted ice creams in 1% (w/w) Sodium Dodecyl Sulphate (SDS) solution. This dissociating medium was used to disperse fat globule aggregates [1-6]. The presentation factor was selected after measurement of the refractive index (ABBE Atago 3T) of the dispersed fat relative to water (1.08), and the absorbance (0.1) at 633 nm of the dispersed phase (spectrophotometer Varian Cary 100 – Les Ulis- France). The samples were characterised using the particle size distribution shape.

2.3. Determination of adsorbed proteins

The melted ice creams were centrifuged at 5 000 *g* for 30 min and the cream layers formed on the top of the tubes (containing the protein coated fat globules) were carefully removed and submitted to protein (Kjeldahl) and fat (Mojonnier) determinations [1, 13]. The amount of adsorbed protein (P_{ads}) in the ice creams was expressed in mg of protein per 1 g of fat.

2.4. Electronic microscopy

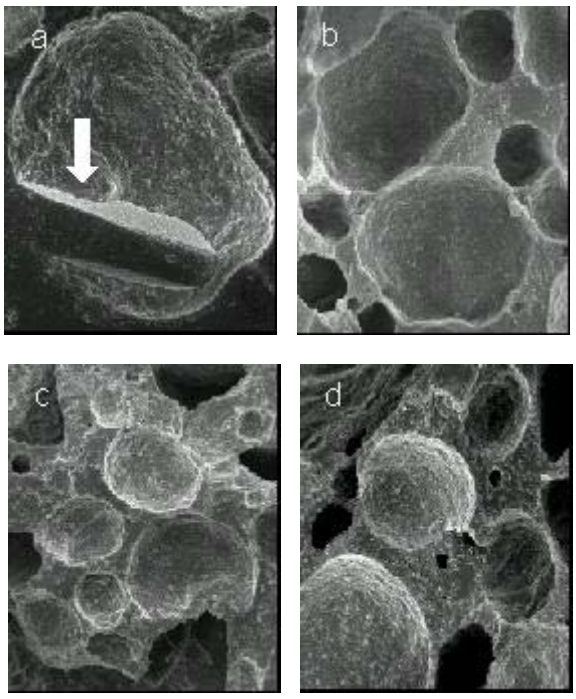
The microstructure of the different ice creams was observed by transmission and scanning electron microscopy (TEM and SEM, respectively).

Sample preparation for TEM involved several steps (cryo-fixation, freeze-substitution and fixation, dehydration and embedding, and sectioning) which were applied as detailed elsewhere [7]. Sections were viewed in the Hitachi 7100 TEM (Tokyo, Japan) at 75 kV. Digital images were acquired using Asis digital image capture software.

For SEM study, duplicate samples of each ice cream (3 mm^3) were clamped into a copper holder designed for the Emscope SP2000A Cryo-reparation unit (Ashford, Kent, UK). The copper holder was frozen and transferred under vacuum into the preparation chamber of the cryo unit where the frozen ice cream sample was fractured. The samples were sublimated for 20 to 25 m at –80 °C, a standard freeze drying temperature. When sublimation was complete the samples were coated with 30 nm of gold. The thin coat of the high atomic number element provides conductivity to prevent the sample from absorbing the electron beam and also results in more secondary electrons being created. Secondary electrons are the primary signal for SEM imaging. The holders were then transferred, frozen and under vacuum, into the Hitachi S-570 SEM (Nissei Sangyo, Tokyo, Japan). The images were captured digitally using Quartz PCI imaging software (Quartz Imaging Corp. Vancouver, BC).

3. Results and discussion

The SEM images observed from ice creams prepared using different milk proteins are shown in Figure 1. These images indicate that changing the protein composition and application or not of the protein solutions pre-heat treatment led to the formation of air bubbles characterised by different size ranges. From Figure 1a (F0), it appears that air bubble size is much higher than in the other ice creams (Figures 1c and 1e) containing casein (F20 and F40) and prepared without application of pre-heat treatment. This result indicates that the presence or absence of casein could be related to differences in air bubble sizes. Thus, the presence of casein gave an ice cream microstructure very similar to that obtained by using skim milk powder [8]. Moreover, application of the protein heat treatment before homogenisation seemed to cause a significant decrease in the air bubble size for ice cream prepared without casein (F0 PHT, Figure 1b) in comparison with sample F0 (Figure 1a), whereas in samples with casein (F20 and F40) this effect of protein pre-heat treatment did not affect the ice cream microstructure (Figures 1c, 1d, 1e and 1f).



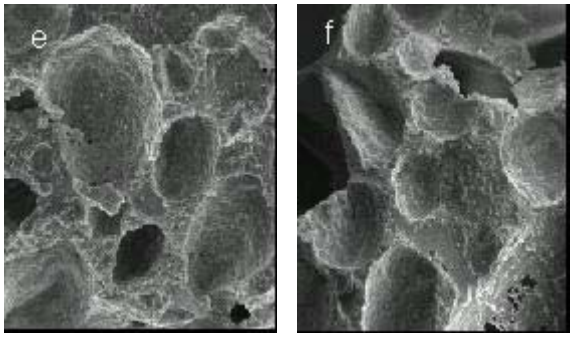


Figure 1 : Scanning electron microscopy images showing air bubbles in ice creams made with various protein mixtures: F0 (a) ; F0 PHT (b); F20 (c); F20 PHT (d); F40 (e) and F40 PHT (f). Notice the presence of a lactose crystal (arrow) inside an air bubble for ice cream F0 (a).

The SEM observations shown in Figures 2a and 2b indicate the presence of small particles (about 1 µm diameter) adsorbed at the surface of the air bubbles whatever the ice cream protein composition.

Images obtained from TEM clearly indicate that fat globules are adsorbed at the air bubble surface, and also dispersed in the continuous phase (Figure 3). The degree to which fat globules are adsorbed at the air bubble surface seems also to be affected by protein composition and application or not of pre-heat treatment, as seen in Figures 3a (F0) and 3b (F0 PHT). In ice cream prepared without casein and with the pre-heat treatment (F0 PHT), more fat globules are adsorbed to the air bubbles and those globules have a higher size (destabilised fat) than in ice cream F0.

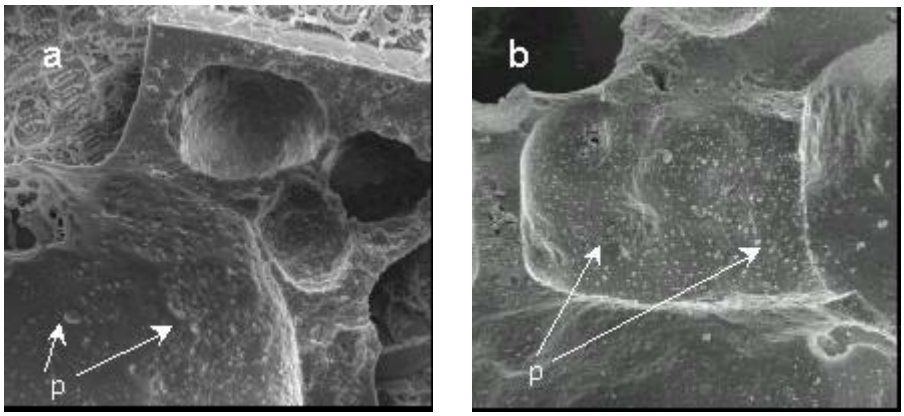


Figure 2 : Scanning electron microscopy images showing air bubbles stabilised by particles (p) in ice creams made with various protein mixtures : F20 (a) and F40 PHT (b).

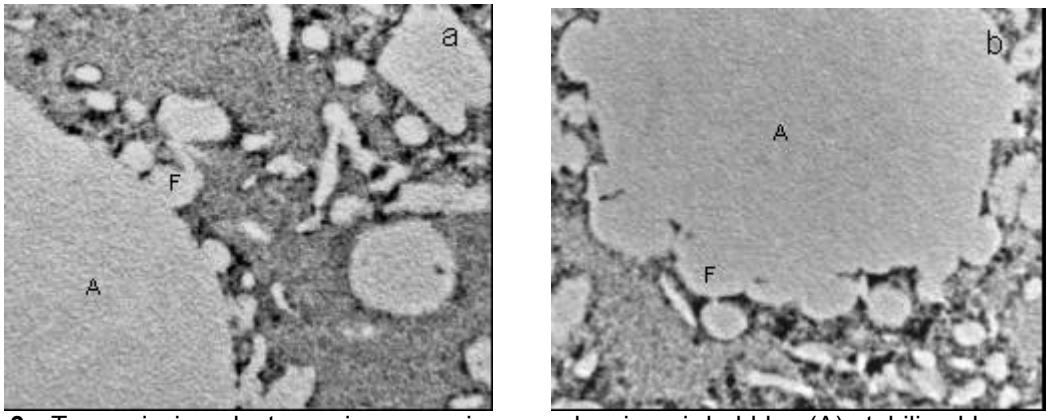


Figure 3 : Transmission electron microscopy images showing air bubbles (A) stabilised by coalesced fat globules (F) in ice creams made with various protein mixtures : F0 (a) and F0 PHT (b).

These observations could be explained in terms of fat globule size distributions, as determined from laser light scattering studies of similar ice cream samples. The curves represented in Figure 4 show that fat droplet size distributions observed from ice cream mixes and corresponding ice creams are monomodal (for F0, absence of caseins) and bimodal (presence of caseins. The differences between mixes and ice creams, were observed whatever the protein composition and application or not of the pre-heat treatment, but with a higher volume proportion of fat droplets having higher sizes in ice creams prepared by application of the pre-heat treatment of protein solutions (Figure 4b) than without pre-heat treatment (Figure 4a). Thus, the appearance of fat globules ranging from 4 to 30 µm in the ice creams that didn't exist in the corresponding mixes could be due to fat destabilisation under the effects of the whipping and freezing steps [2 ; 9-13], and could be responsible for stabilization of air bubbles in ice cream as shown in Figure 3 obtained by TEM.

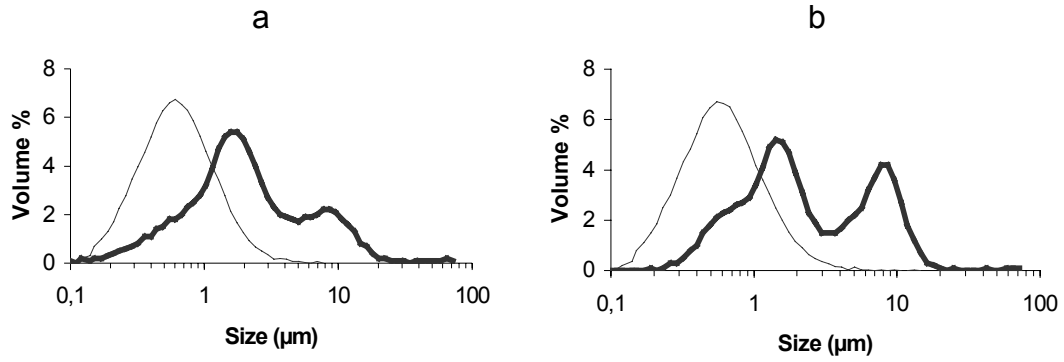


Figure 4 : Fat globule size distribution, as obtained by laser light scattering in ice creams (bold line) and corresponding ice cream mixes (simple line) made with various protein mixtures : F0 (a) and F40 PHT (b) after dispersion in dissociating medium (1% SDS solution).

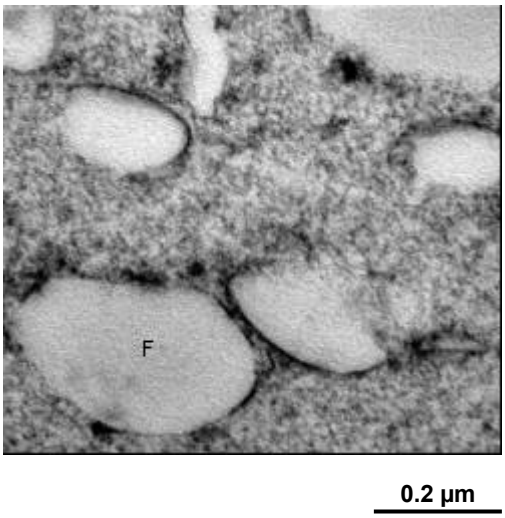


Figure 5 : Transmission electron microscopy image showing protein layer (in black) adsorbed at the fat globules surface (in white, F) in ice cream F20 as an example.

The TEM images also showed the presence of adsorbed protein layers around the fat globules and the presence of protein in the continuous phase (Figure 5).
The effect of protein composition on the proportion of adsorbed protein at the fat droplet surface is also illustrated by the TEM images (Figures 6a and 6b) where it is seen that a higher amount of proteins might be involved in adsorbed protein layers in ice creams containing casein (Figure 6b) than in those without casein (Figure 6a). This microscopy observation is in good agreement with previous study [14].

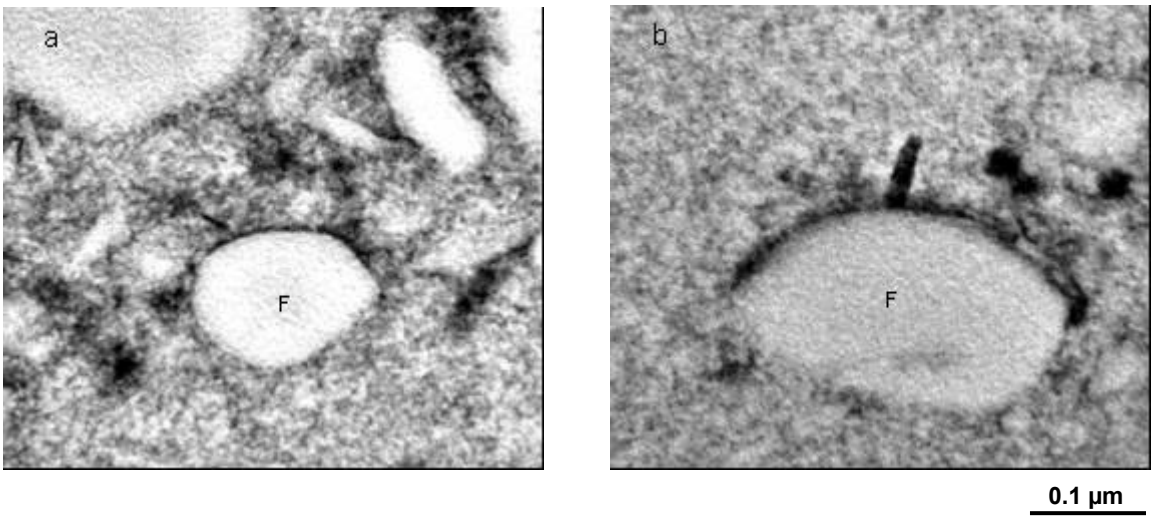


Figure 6 : Transmission electron microscopy images showing protein layer (in black) adsorbed at the fat globules surface (in white, F) in ice cream made with various protein mixtures : F0 (a) and F20 (b).

In the present study, this trend was confirmed by determination of the amount of adsorbed proteins following the methodology described in Materials and Methods. Actually, the amount of protein adsorbed at the oil-water interface was much higher in ice creams containing casein (F20 and F40) than in ice cream prepared with whey proteins only (F0) (Figure 7), as previously observed from milk proteins stabilised emulsions [15; 16] and from ice cream mixes [1-3].

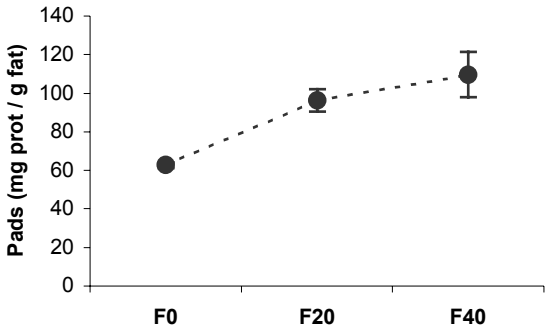


Figure 7 : Amount of protein adsorbed at the oil-water interface in ice creams prepared with different protein compositions.

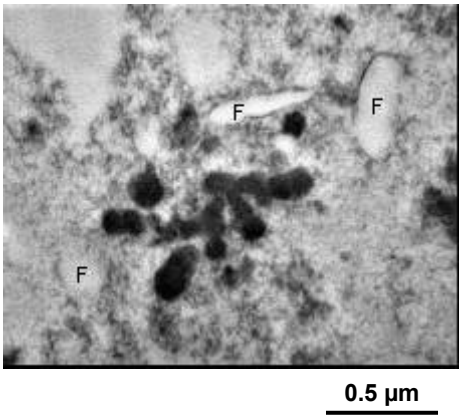


Figure 8 : Transmission electron microscopy image showing protein (in black) unadsorbed at the surface of fat globules (in white, F) in ice cream F40 PHT, as an example.

The presence of less adsorbed proteins in ice creams prepared by application to protein solutions of pre-heat treatment is reflected in TEM images (Figure 8) where much more protein aggregates were visible between fat droplets.

4. Conclusion

These examples of results underlined a significant effect of pre-heating proteins before homogenisation, with consequence not only on protein surface adsorption but also on microstructure of corresponding ice creams as revealed by SEM and TEM.

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