



Surface films formed by milk in hard water

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Dilute (1–10 vol. %) solutions of milk in hot London mains water formed films on the surface which increased with time. No film formed in solutions of milk in distilled water unless calcium chloride had been added. The mass of the surface film depended on the total mass of milk used, not on its concentration. These facts are consistent with an interaction between calcium (and other metal) ions and milk components within the bulk solution, the product then rising to the surface. Surface oxidation did not seem to be involved. This mechanism is quite different from that which forms films on infusions of black tea. The mass of the milk film increased in the order skimmed milk < homogenised full cream milk < non-homogenised full cream milk < single cream, and both proteins and fat were involved. This was confirmed by chemical analysis of the films, which were also examined by infrared spectroscopy and scanning electron microscopy. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Cooks know that an insoluble skin forms on the surface of boiled milk. It is not generally realised, however, that a sizeable film can even form on the surface of dilute solutions of milk in hot tap water. We came across this phenomenon when investigating the film on the surface of hot infusions of tea to which milk had been added (Spiro & Chong, 1997); to our surprise, most of this film was produced by the milk rather than by the tea. The present paper therefore reports our findings on films formed over hot milky water.

MATERIALS AND METHODS

According to analytical data supplied by Thames Water Utilities, the calcium content of the local London mains water increased gradually from 98 to 114 mg litre⁻¹ during the course of the research. The bicarbonate content was roughly constant at approx. 230 mg litre⁻¹ and the mean pH was 8.0–8.2. Fresh pasteurised milk (Express or Dairygate), skimmed, homogenised full cream or non-homogenised full cream, was bought in local shops; the single cream used was Eden Vale UHT.

The technique for obtaining surface films was analogous to that employed for collecting films on tea infusions (Spiro & Jaganyi, 1994a). Water (normally 800 ml) in a large beaker was heated to and kept at 80°C or 70°C in a thermostat bath. A known volume (often 40 ml) of milk was added, the mixture stirred,

quickly heated on a hotplate to 80°C or cooled to 70°C, and immersed again in the thermostat bath. Any initial film on the surface was removed with an aluminium scoop. The beaker was covered by a watchglass with the concave side facing the solution so that water vapour condensing on it ran back to the walls of the beaker with little disturbance to the surface film. After a known period of 'standing time' (usually 1 h), the film was collected with another spade-shaped aluminium foil scoop, washed off into a preweighed sintered-glass filter crucible, rinsed, and dried overnight, either in an air oven set at 80°C or in a vacuum oven, before being weighed. The pH of the final solution was measured with an EIL pH meter.

Microanalyses for carbon, hydrogen and nitrogen were usually carried out at Imperial College and for calcium and other elements by MEDAC at Brunel University. Fourier transform infrared (FTIR) spectra were taken with a Perkin-Elmer 1720 FTIR spectrometer using KBr pellets. Electron micrographs were taken with a JEOL T220A scanning electron microscope, and were coated with gold for structural analysis.

RESULTS AND DISCUSSION

Nature of the water used

Table 1 summarises the results obtained with different types of water when homogenised full cream milk was employed. Here and elsewhere the masses given are the

Table 1. Effect of water composition on mass (w) of milk film when 40 ml of homogenised full cream milk were mixed with 800 ml of water and allowed to stand at 80°C for 1 h

Water used	w (mg)	pH	Colour
London mains water	47.6	7.34	Yellowish white
London tap water + 0.05 M HCl	49.0	6.59	Yellowish white
Distilled water	0.0	7.17	—
Distilled water + 2.72 mM CaCl ₂	32.3	6.59	Butter yellow
Distilled water + 5.44 mM NaHCO ₃	0.3	7.65	—
Distilled water + 2.72 mM CaCl ₂ + 5.44 mM NaHCO ₃	34.1	7.16	Creamy white

means of at least two independent experiments. The reproducibility varied from almost perfect concordance between duplicates to variations of 10% or more, probably due in part to changes in milk composition over the months.

The first two lines in the table show that the film mass was not sensitive to pH. No film at all appeared when distilled water was used and hardly any on the addition of sodium hydrogen carbonate (NaHCO₃). However, addition of an amount of calcium ion corresponding to that in the mains water formed two-thirds as much surface film, and slightly more was produced when NaHCO₃ was present as well. Thus milk film formation depends essentially on the concentration of calcium and other ions also present in London mains water, but is largely independent of pH or the presence of NaHCO₃. Both temporary and permanent hard water will therefore produce milk scum. In contrast, black tea scum requires both calcium and bicarbonate ions to be present and the extent of its formation depends on pH (Spiro & Jaganyi, 1994b).

Variation with standing time

Figure 1 shows that the amount of milk film initially increased linearly with time, with a progressive slowing down of the rate at long times. The pH at the end of the experiment slowly increased with standing time, from 6.94 at 15 min to 7.34 at 40 min and 7.81 after 4 h. According to the chemical microanalytical data in Table 2, the percentage of calcium in the film was less after 4 h than after 1 h, even though the calcium content in the solution had decreased by only a few units % after 4 h.

Variation with concentration of milk and temperature

The more milk was added to a given volume of mains water, the more film was collected after 1 h. Figure 2 shows that, at 80°C, the increase was roughly proportional to the concentration of milk present, which means that the rate of film formation is approximately first-order in milk concentration. The results for the addition of 40 ml of milk at 80°C and 70°C lead to an Arrhenius activation energy of 67 kJ mol⁻¹. The reaction involved

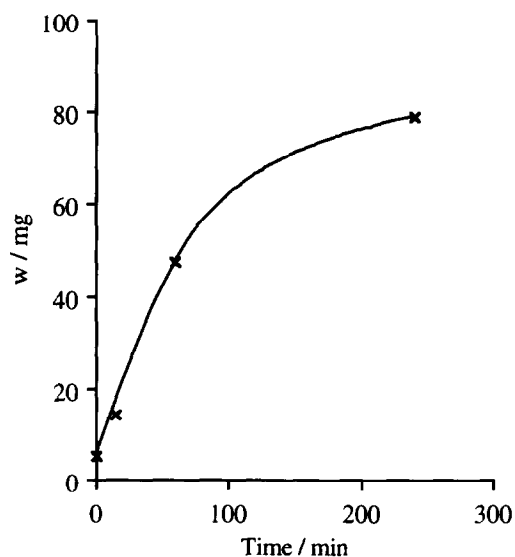


Fig. 1. Variation with time of the mass of film formed from a mixture of 40 ml of homogenised full cream milk in 800 ml London mains water at 80°C.

is therefore chemically controlled rather than diffusion controlled. Moreover, since the activation energy is much smaller than is involved in protein denaturation, such processes have either already taken place in the period before the standing time or are not involved in the rate-determining step of film formation.

Variation with dilution and the effect of gas flow

It is clear from Fig. 3 that dilution of a given quantity of milk with increasing amounts of London mains water had no significant effect on the mass of the surface film. Because this was a surprise, some experiments were repeated several months later, which accounts for the pairs of points at 200 and 800 ml of water, but the overall conclusion remained the same. These results are consistent with a mechanism whereby the product found in the film does not form at the surface (as does tea scum) but in the bulk solution, and then floats to the surface. From the discussion in the previous section:

$$\text{Rate} = d[\text{product}]/dt = k[\text{milk}] \quad (1)$$

Table 2. Analyses (in wt%) of milk films allowed to form for 1 h at 80°C in London mains water, together with analyses of two bulk milks dried in a vacuum oven at room temperature

Milk used	C (±0.3)	H (±0.3)	N (±0.1)	P	Ca ^{a,b} (±0.1)
Homogenised full cream	52.5	8.65	4.69	1.84	5.70
Homogenised (formed over 4 h)	56.6	8.69	5.43	1.56	3.92
Homogenised full cream (bulk)	49.7	7.58	4.17	0.77	1.06
Non-homogenised full cream	59.2	8.61	3.97	1.21	3.65
Skimmed milk	21.6	3.01	4.82	7.43	18.0
UHT single cream	64.4	9.6	2.63	0.88	2.20
UHT single cream (bulk)	63.5	9.96	1.72	0.32	0.37

^aThe calcium concentrations of the films may be higher than the figures tabulated because of the possibility of particles of calcium salts being retained in the pores of the filter crucibles when the dried films were removed for analysis (Spiro *et al.*, 1996).

^bThe magnesium concentrations were all negligible. The manganese concentrations of the films were 0.10 wt% for the full cream milks, 0.51 wt% for the skimmed milk, and not measured for the cream. The sodium concentrations of the films were approx. 0.07 wt% for the full cream milks, 0.18 wt% for the skimmed milk, and not measured for the cream.

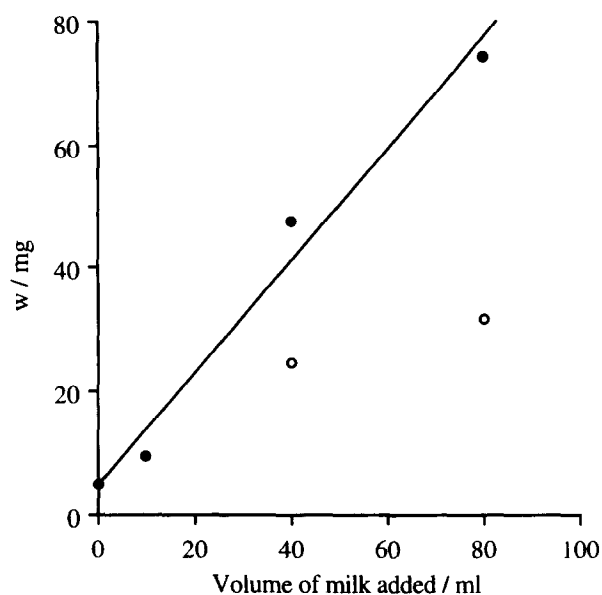


Fig. 2. Variation of the mass of film formed after 1 h when increasing volumes of homogenised full cream milk were added to 800 ml of London mains water kept at 80°C (●) or at 70°C (○).

where k is a first-order rate constant and square brackets denote concentrations in the solution. If the solution volume is written as V , the mass of film w formed by the product after a given time t is then given by:

$$w = [\text{product}]V = k[\text{milk}]Vt \quad (2)$$

Thus the mass of the surface film formed after a given time is proportional to the milk concentration if the volume of the solution is kept constant (Fig. 2), and proportional to $[\text{milk}]V$ or the total amount of milk present if the volume of the bulk solution varies (Fig. 3).

The process of film formation was disturbed by bubbling either nitrogen or oxygen through the solution, since the mass of film then more than halved. On the other hand, more film than usual was found when the experiment was carried out under an atmosphere of

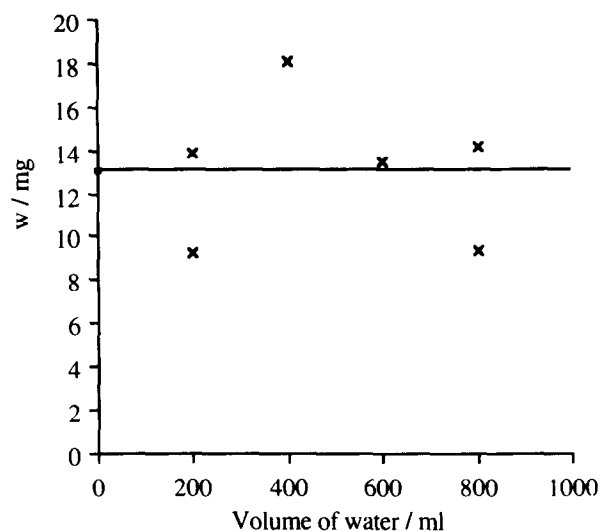


Fig. 3. Variation of the mass of film formed after 1 h at 80°C when 10 ml of homogenised full cream milk were mixed with increasing volumes of London mains water.

either nitrogen or oxygen, the latter forming only 31% more film than the former. This makes it unlikely that appreciable surface oxidation was involved, in contrast to the formation of tea scum where the mass of film was six times as large under oxygen as under nitrogen (Spiro & Jaganyi, 1994b).

Types of milk

Great variations in the amount of surface film were found when 40 ml of different types of milk were added to 800 ml of London mains water and the mixture allowed to stand for 1 h at 80°C. Thus, with non-homogenised full cream milk, three times as much surface film appeared as with the homogenised milk. Non-homogenised milk in London mains water at 70°C formed 83.4 mg of film, leading to an activation energy of 56 kJ mol⁻¹. The difference between these two types of milk therefore increases the lower the temperature. A summary of the results in Table 3 shows that almost

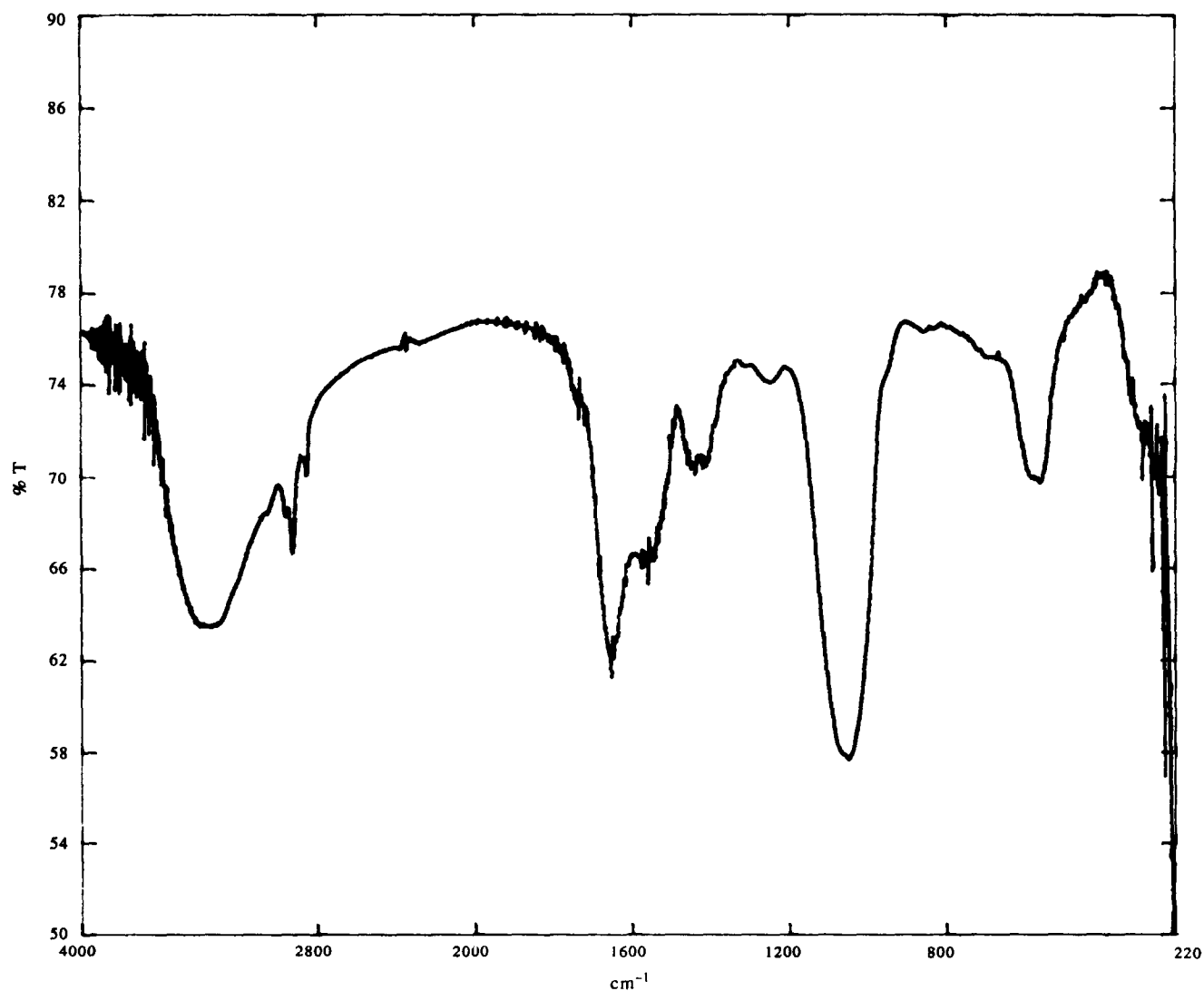
Table 3. Effect of water composition on mass (w) of milk film when 40 ml of non-homogenised full cream milk were mixed with 800 ml of water and allowed to stand at 80°C for 1 h

Water used	w (mg)	pH	Colour
London mains tap water	145.6	7.39	Yellowish white
Distilled water	1.45	7.12	—
Distilled water + 2.77 mM CaCl ₂	131.9	6.57	Yellowish white
Distilled water + 5.44 mM NaHCO ₃	0.55	7.86	—
Distilled water + 2.77 mM CaCl ₂ + 5.44 mM NaHCO ₃	140.7	7.20	Yellowish white

as much film was produced when distilled water containing 2.77 mM CaCl₂ and 5.44 mM NaHCO₃ was used, and not much less when only the CaCl₂ had been added to the distilled water. However, as in Table 1, distilled water alone or with added NaHCO₃ formed hardly any milk film.

According to the analytical data for nitrogen in Table 2, the non-homogenised milk film contains less protein than does the film formed by homogenised milk.

Both types of milk film contain between 1 and 2 wt% of phosphorus, which will have arisen largely from the incorporation of calcium phosphate with a minor contribution from the milk proteins (caseins overall contain about 20 times as much nitrogen as phosphorus, by weight). The fact that both films contain several times as much calcium as does the milk itself, points to the calcium ions in the hard water combining with milk constituents to produce the film, an aspect elaborated in a later section.

**Fig. 4.** FTIR spectrum of the film formed from a mixture of 40 ml of skimmed milk in 800 ml of London mains water kept at 80°C for 1 h.

With skimmed milk, only 6.1 mg of film were formed after 1 h at 80°C using London mains water. This is little more than the mass of calcium carbonate floating to the top in blank experiments with this water alone (Spiro *et al.*, 1996). However, as Table 2 shows, the very high percentage of calcium in the skimmed milk film (at least 18 wt%) is accompanied by an unusually large percentage of phosphorus, indicating that both calcium carbonate and calcium phosphate were present. This is hardly surprising since milk itself is saturated with calcium phosphate and the salt also acts as a cementing agent within casein micelles (Walstra & Jenness, 1984). The organic content of the film is correspondingly small, as attested by the relatively low percentages of carbon and hydrogen.

In contrast, UHT single cream containing 19 wt% fat produced the largest amount of film of all (238 mg in London mains water at 80°C after 1 h). According to its chemical analysis, it was largely composed of fat with only 2.2 wt% calcium, although the nitrogen and

phosphorus contents were higher than in the original cream. Some milk proteins, and probably some calcium phosphate, were therefore present as well, in part as constituents of the fat globule membrane (Oortwijn & Walstra, 1979).

Fourier transform infrared spectra

Figures 4 and 5 show the FTIR spectra of skimmed milk and non-homogenised milk films. The spectrum of the film formed over homogenised milk solution displayed characteristics similar to those in Fig. 5. The FTIR spectrum of vacuum-dried single cream is given in Fig. 6 and also closely resembles that in Fig. 5, indicating that the films over dilute full cream milk solutions are essentially formed from milk fat. This conclusion is supported by the fact that the spectrum over skimmed milk solution is quite different.

The wavenumbers of the peaks in these spectra are summarised in Table 4, together with their probable

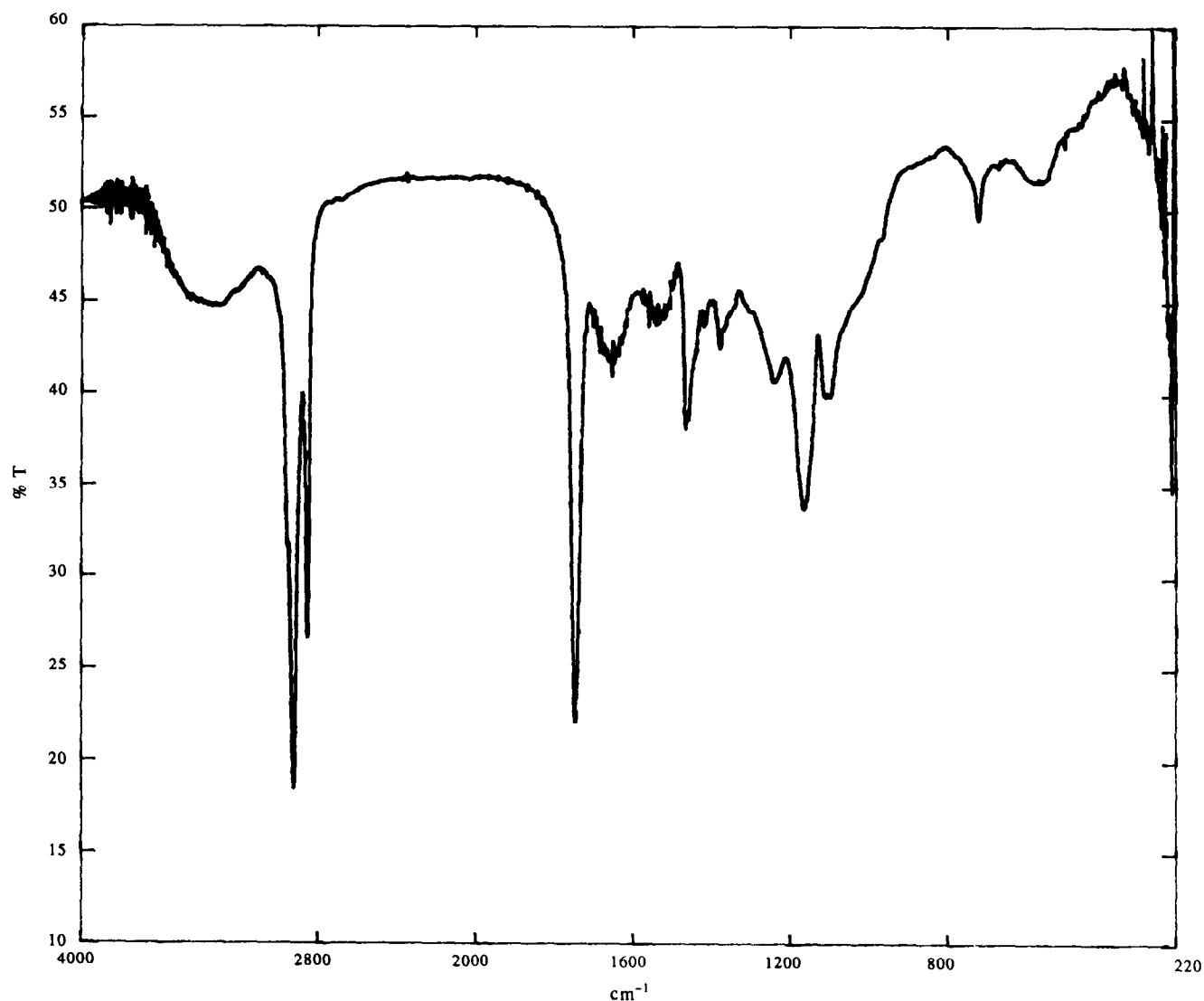


Fig. 5. FTIR spectrum of the film formed from a mixture of 40 ml non-homogenised full cream milk in 800 ml London mains water kept at 80°C for 1 h.

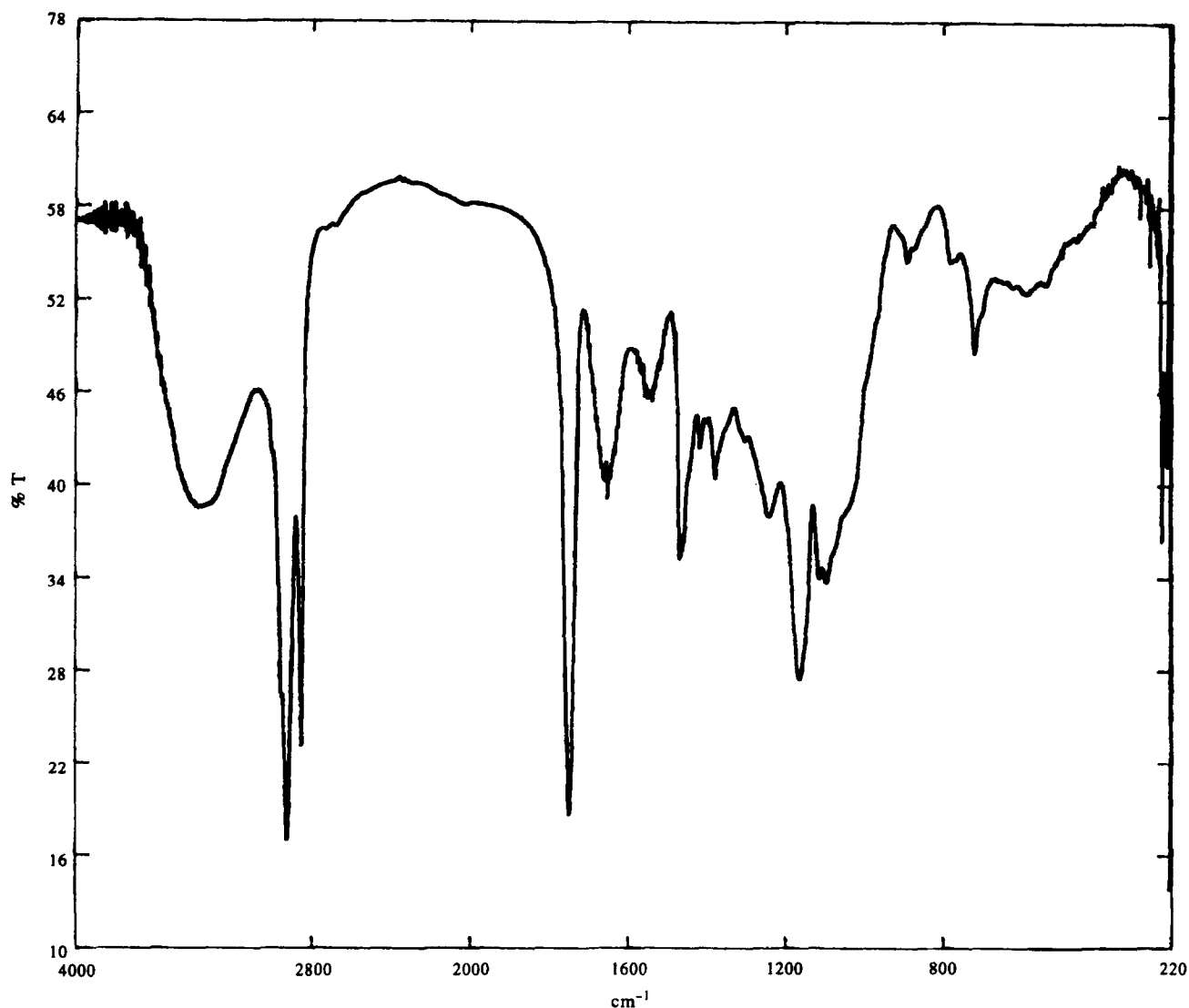


Fig. 6. FTIR spectrum of UHT single cream dried *in vacuo*.

Table 4. Peaks in the FTIR spectra (in cm^{-1})

Skimmed milk film	Non-homogenised milk film	Dried single cream	Assignment
3389 (s,b)	3337 (m,b)	3360 (m,b)	O-H stretch
2926 (w)	2925 (s)	2925 (s)	Asym. C-H stretch in $-\text{CH}_2-$
2854 (w)	2854 (s)	2854 (s)	Symm. C-H stretch in $-\text{CH}_2-$
	1746 (s)	1747 (s)	C=O stretch in $-\text{COOR}$
1654 (s)	1654 (m)	1654 (s)	C=O stretch in amide
1560 (w,d)			N-H bend in $-\text{CONH}-$
	1466 (m)	1467 (m)	C-H bend in $-\text{CH}_2-$
1438 (m,d)			Ionic carbonate
	1166 (s)	1166 (s)	C-O stretch
	1113 (m,d)	1097 (m,d)	Ionic phosphate
1051 (s,d)			Ionic phosphate
	722 (w)	722 (m)	$-\text{CH}_2-$ rock
563 (s,d)			Ionic phosphate

Peak designations: s, strong; m, medium; w, weak; b, broad; d, double.

structural assignments. Peaks close to 2925, 2854, 1747, 1466, 1166 and 722 cm^{-1} are also found in the FTIR spectrum of tripalmitin [1,2,3-propanetriol

tris(hexadecanoate)] (Pouchert, 1985), typical of the triglycerides in milk fat. However, the full cream milk films as well as cream itself also display peaks above



Fig. 7. Scanning electron micrograph of a film formed over a mixture of 40 ml homogenised full cream milk in 800 ml London mains water after 1 h at 80°C. The white bar at the bottom represents 100 μm .

3300 and at 1654 and 1100 cm^{-1} , attributed to hydroxy groups and protein and phosphate constituents. Some of these will have originated from the fat globule membranes. The film over dilute skimmed milk displays only weak methylene signals, whereas the peaks due to hydroxy, amide, phosphate and carbonate are more pronounced. This ties in with the analytical data in Table 2. The major component of this film would therefore appear to be casein micelles.

Scanning electron microscopy

An electron micrograph of a film formed over dilute homogenised full cream milk is shown in Fig. 7. Both flakes and bubbles can be seen; the latter may be trapped gas or, more likely, globules of milk fat.

General discussion

The milk products in our experiments were modified in three ways: they were heated, diluted, and subjected to the salts present in the diluting water. Heating will have ensured that the lipids in the fat globules were completely liquid and no longer partly crystalline. Dilution will have greatly decreased the concentrations of all

constituents; in particular, the overall calcium concentration will have dropped from approx. 29 mM to 1.4 mM in the experiments in which 40 ml of milk product were added to 800 ml of water. In the milk serum, the initial effect of dilution will have reduced the calcium concentration to 0.4 mM. This will have led to partial dissociation of the casein micelles and to increased solubility of calcium phosphate, although its solubility product will be lower at the higher temperature. The salts present in the London mains water (like those in the synthetic water) will have increased the overall ion concentration by 2.7 mM. If all this remained in the diluted milk serum, its concentration there would be at least 3.1 mM compared with around 9 mM in normal milk, much of it associated with other species (Walstra & Jenness, 1984). Our results showed that heating and diluting milk with distilled water produced only tiny amounts of film at the air-water interface, so that the crucial factor in film formation was the introduction of extra calcium and other salts.

Milk proteins are known to adsorb at the air-water interface. Dilute aqueous solutions of β -casein were recently found by neutron reflectivity to form a dense monolayer of protein molecules at the surface, at pH values well away from the casein isoelectric point of 5.3.

Near this pH, a second more diffuse layer appeared as well (Dickinson *et al.*, 1993; Atkinson *et al.*, 1995). In bulk solution, too, the decrease in electrostatic repulsion between such protein molecules near the isoelectric point can cause casein aggregation. Another powerful method of producing aggregation in solution is the addition of calcium and other multivalent ions which are able not only to screen the protein charges but also to form calcium bridges between casein molecules. Such calcium binding to caseins is stronger for α_{s1} -casein than for β -casein and relatively weak for κ -casein, reflecting the decreasing number of phosphoseryl groups per molecule (Dickinson, 1989). The kinetics of aggregation of dilute solutions of α_{s1} -casein by calcium ions have been studied by Dalgleish *et al.* (1981) using light scattering and centrifugation. However, there was no mention of aggregates rising to the surface. This is consistent with our finding that very little surface film was formed in experiments with skimmed milk in hard water.

Far more surface film was produced with full cream milk containing 3.9–4.0 g fat per 100 ml. Even in milk itself, the fat globules slowly rise to the surface since fat is lighter than the surrounding plasma. Walstra and Oortwijn (1975) have studied the rate of such creaming and concluded that it can be described by Stokes's equation provided the globules are not too small and the emulsion fairly dilute. The theory predicted that the proportion of globules of diameter d which reached the cream layer in a given time was proportional to d^2 as well as to a factor Q given by:

$$Q = (\rho_g - \rho_p) / \eta \quad (3)$$

where ρ_g is the density of the globules, ρ_p that of the surrounding plasma and η the viscosity of the plasma. Insertion of literature data (Walstra & Jenness, 1984) shows that the value of Q with a plasma of water at 80°C is 3.8 times greater than for a plasma of skimmed milk at 20°C. Thus, even simple creaming will have been more rapid under our conditions than for normal milk at room temperature.

Milk fat globules are surrounded and stabilised by a membrane composed of casein micelles, caseins and serum proteins (Walstra & Jenness, 1984). The protein load in our experiments will have been little affected by the dilution with water but increased as a consequence of the higher temperature (Oortwijn & Walstra, 1979). At 80°C, moreover, the fat lipids will have been liquid and not crystalline. Oil-in-water emulsions stabilised by α_{s1} -caseins are known to be flocculated by calcium ion concentrations of approx. 100 ppm, corresponding to that in London mains water, through calcium–casein binding. For this reason, complexation of calcium ions by citrate is essential for the stability of commercial cream liqueurs (Dickinson, 1989). The surface films obtained from hot dilute full cream

milk will therefore have been formed mainly from calcium-containing aggregates of protein-covered fat globules.

Approximately three times as much film was formed with non-homogenised milk than with homogenised milk, perhaps not surprisingly in view of the much larger size of the fat globules in the former (a volume-surface average diameter of 3–5 μm compared with 0.2–1.0 μm ; Walstra & Jenness, 1984). Although this size difference alone would have led one to expect an even greater difference in film mass, one must also bear in mind the changed nature of the protein covering the globules after high pressure homogenisation. In this process, casein micelles are broken up (Johnston & Murphy, 1995) and the original membranes covering the milk fat globules are largely replaced by casein molecules (Chazelas *et al.*, 1995). The binding of calcium and other ions to the globules will be affected accordingly. Chemical analysis of our films indicates an increased ratio of protein to fat from homogenised milk systems, consistent with the smaller size and larger surface area of the fat globules. It is also likely that milk proteins flocculated in the solution by calcium ions were brought up to the surface by rising fat aggregates and incorporated in the surface layer, so accounting for the relatively high nitrogen and phosphorus contents of the films.

Even more surface film was produced with UHT single cream containing 19.3 g fat per 100 ml. This cream will almost certainly have been homogenised but, since the pressure used is not known, neither is the size distribution of the fat globules. In general, homogenisation disrupts the original fat globules while the limited protein available for fat surface coverage then leads to the formation of fat clusters (McCrae & Lepoetere, 1996).

The work described above is relevant to the stability of milk-containing foods, especially those that incorporate large air–water interfaces such as whipped cream and ice cream. The films formed over hot dilute milk solutions also bear some similarity to type A fouling deposits formed by milk itself on hot surfaces during processes such as pasteurisation. These deposits, like our films, look soft and creamy and contain minerals, protein and fat; calcium ions are essential for their formation and the temperature dependence of fouling is far smaller than that of protein denaturation (Walstra & Jenness, 1984; Changani *et al.*, 1997). Unlike our films, however, fouling deposits increase greatly on lowering the pH, their protein content (judged from the percentage of nitrogen) is roughly twice as large, and they contain much less fat.

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REFERENCES

- Atkinson, P. J., Dickinson, E., Horne, D. S. & Richardson, R. M. (1995). Neutron reflectivity study of the adsorption of β -casein at the air-water interface. In *Food Macromolecules and Colloids*, eds E. Dickinson & D. Lorient. Royal Society of Chemistry, Cambridge, pp. 77-80.
- Changani, S. D., Belmar-Beiny, M. T. & Fryer, P. J. (1997). Chemical and engineering factors associated with fouling and cleaning in milk processing. *Exptl. Therm. Fluid Sci.*, in press.
- Chazelas, S., Razafindralambo, H., de Chassart, Q. D. & Paquot, M. (1995). Surface properties of the milk fat globule membrane: competition between casein and membrane material. In *Food Macromolecules and Colloids*, eds E. Dickinson & D. Lorient. Royal Society of Chemistry, Cambridge, pp. 95-98.
- Dalgleish, D. G., Paterson, E. & Horne, D. S. (1981). Kinetics of aggregation of α_{s1} -casein/ Ca^{2+} mixtures: charge and temperature effects. *Biophys. Chem.*, **13**, 307-314.
- Dickinson, E. (1989). Food colloids—an overview. *Colloids Surfaces*, **42**, 191-204.
- Dickinson, E., Horne, D. S., Phipps, J. S. & Richardson, R. M. (1993). A neutron reflectivity study of the adsorption of $\psi\alpha\sigma\epsilon\iota\nu\ \alpha\tau\ \phi\lambda\upsilon\iota\delta\ \iota\upsilon\tau\epsilon\rho\phi\alpha\psi\epsilon\sigma$. *Langmuir*, **9**, 242-248.
- Johnston, D. E. & Murphy, R. J. (1995). Some changes to the properties of milk protein caused by high-pressure treatment. In *Food Macromolecules and Colloids*, eds E. Dickinson & D. Lorient. Royal Society of Chemistry, Cambridge, pp. 134-140.
- McCrae, C. H. & Lepoetre, A. (1996). Characterization of dairy emulsions by forward lobe laser light scattering—application to milks and creams. *Int. Dairy J.*, **6**, 247-256.
- Oortwijn, H. & Walstra, P. (1979). The membranes of recombined fat globules. 2 Composition. *Neth. Milk Dairy J.*, **33**, 134-154.
- Pouchert, C. J. (1985). The *Aldrich Library of FT-IR Spectra*, Edition 1, Vol. 1, p. 621A.
- Spiro, M. & Chong, Y. Y. (1997). Kinetics and equilibria of tea infusion. Part 14. Surface films formed in hard water by tea brews containing milk. *Food Chem.*, **59**, 247-252.
- Spiro, M. & Jaganyi, D. (1994a). Kinetics and equilibria of tea infusion. Part 10. The composition and structure of tea scum. *Food Chem.*, **49**, 351-357.
- Spiro, M. & Jaganyi, D. (1994b). Kinetics and equilibria of tea infusion. Part 11. The kinetics of the formation of tea scum. *Food Chem.*, **49**, 359-365.
- Spiro, M., Chong, Y. Y. & Jaganyi, D. (1996). Kinetics and equilibria of tea infusion. Part 13. Further studies on tea scum: the effects of calcium carbonate, lemon juice, and sugar. *Food Chem.*, **57**, 295-298.
- Walstra, P. & Jenness, R. (1984). *Dairy Chemistry and Physics*. Wiley, New York.
- Walstra, P. & Oortwijn, H. (1975). Effect of globule size and concentration on creaming in pasteurised milk. *Neth. Milk Dairy J.*, **29**, 263-278.