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Kinetics and equilibria of tea infusion, Part 14. Surface films formed in hard water by black tea brews containing milk

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Added milk greatly increased the mass of film formed on the surface of black tea brewed in hot London mains water. The amounts of film collected after adding different types of milk (skimmed, homogenised full cream, non-homogenised full cream, and UHT single cream) to black tea brews increased in the sequence listed. No film formed when distilled water was used, and very little even in hard water if the tea concentration was large. Chemical microanalysis and Fourier transform infrared examination of the films indicated that they were composed of milk constituents modified by tea polyphenols and aggregated by calcium ions. The role of the tea polyphenols is consistent with their being sequestered in casein micelles as well as bound to the protein membranes of milk fat globules. The presence of milk prevented the formation of most of the dark tea scum observed on black tea infused in hard water. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

In Britain and many overseas countries, tea is traditionally drunk with a small amount of milk. According to Morton (1979), this addition of milk has greatly reduced the risk of habitual British tea drinkers developing oesophageal cancer. A major organoleptic effect produced by milk is to render the resultant drink less astringent on the tongue than black tea without milk (Sanderson *et al.*, 1976). These effects on health and palatability have been ascribed to association between the various polyphenolic tea compounds (tannins) and proteins in the milk, but only recently has a more detailed molecular interpretation been put forward (Luck *et al.*, 1994). Other recent work has concerned itself with the influence of milk on the bioavailability of mineral ions when tea is consumed; the effect on iron absorption is controversial, whereas that on copper dialysability was not significant (Vaquero *et al.*, 1994).

The present paper deals with another phenomenon not previously investigated: the effect of milk on the surface films that appear when black tea is brewed in temporary hard water. The films formed in the absence of milk have been found to consist of oxidised tea polyphenols together with several per cent of calcium carbonate and other salts (Spiro & Jaganyi, 1994*a*). Addition of sugar increased the amount of this surface tea scum, whereas lemon tea was free of any film (Spiro *et al.*, 1996). In a preliminary account (Spiro & Jaganyi, 1993), we reported that added milk greatly increases the amount of surface film and a more detailed study is described below.

MATERIALS AND METHODS

Fresh pasteurised milk (Express), homogenised full cream, non-homogenised full cream, or skimmed, was bought in local shops. The single cream used was Eden Vale UHT.

The experiments were carried out in a similar way to those described previously (Spiro & Jaganyi, 1994*a*). In the standard procedure, one teabag of a popular black tea blend (Typhoo) was infused with stirring at 80°C for 5 min in 800 ml of hard London mains water whose composition was given by Spiro and Chong (1997). The teabag was then withdrawn and 40 ml of milk were added and mixed into the infusion. The mixture was quickly heated on a hotplate to 80°C, returned to the 80°C thermostat bath and any material on the surface was removed with an aluminium scoop. A surface film was then allowed to form at 80°C for 60 min before being quantitatively collected with a scoop and the pH of the solution measured. The film itself was dried and weighed. In other experiments, some of these parameters (number of teabags, type of water, volume and type of milk, temperature) were varied. Duplicate or triplicate experiments were always carried out and the mean values are reported.

Chemical microanalyses, Fourier transform infrared (FTIR) spectra, and electron micrographs of the surface films were obtained as described in the previous paper (Spiro & Chong, 1997).

RESULTS AND DISCUSSION

Nature of the water used

The results obtained on adding 40 ml of homogenised full cream milk to various black tea infusions are summarised in Table 1. All the milky tea films were medium brown in colour. With London mains water, 47 mg of film developed compared with only 9·7 mg in the absence of milk. A simple calculation shows that this marked increase cannot be accounted for by the calcium and magnesium in the milk. According to the literature (Holland *et al.*, 1991), 40 ml of milk at 80°C contain 46 mg of calcium which will have increased the calcium content in the mixed infusion from 105 mg $litre^{-1}$ to 155 mg $litre^{-1}$, a rise of 48%. However, even doubling the calcium content of hard water had been found to produce only 22% more tea scum (Spiro & Jaganyi, 1994*b*). The small amount of magnesium in the milk (5·6 mg) could have been responsible for another 5% of scum. In total, therefore, only about a 2 mg increase in the tea scum weight can be explained by the extra alkaline earth ions introduced with the milk. Indeed, as the last column in Table 1 shows, most of the large mass of the milky tea scum can be attributed to the milk itself. However, the fact that the film over milky tea weighed more than 9·7 (tea) + 47·6 (milk) = 57·3 mg is the first indication that the two types of film are not simply additive.

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

Water used	w (mg)	pH	w (mg) Milk only[a]	w (mg) Tea only[b]
London mains water	47·6[c]	6·65	47·6	9·7
London mains water + 1 mM EDTA	0·0			0·0
Distilled water	0·0	6·17	0·0	0·0
Distilled water + 2·72 mM $CaCl_2$	4·0	5·87	32·3	0·0
Distilled water + 2·72 mM $CaCl_2$ + 0·1 M NaOH	8·7[d]	6·53		
Distilled water + 2·72 mM $CaCl_2$ + 5·44 mM $NaHCO_3$	39·3	6·66	34·1	8·9

[a]Milk only data from Spiro and Chong (1997).
[b]Tea only data from Spiro and Jaganyi (1994*b*).
[c]When the tea infusion was added to the milk, 46·5 mg of film were formed.
[d]The addition of NaOH after the teabag had been removed was designed to raise the pH. In two such sets of experiments the average mass of film ranged from 5·9 mg with pH 6·46 to 11·4 mg with pH 6·59.

The presence of calcium (and magnesium) ions had been found to be crucial for the formation of both tea scum (Spiro & Jaganyi, 1993) and milk film (Spiro & Chong, 1997). Table 1 demonstrates that the same was true for the film formed over milky tea, since none was found either with distilled water or with London mains water in which these ions had been complexed by adding EDTA (ethylenediaminetetraacetic acid disodium salt; BDH AnalaR). Nevertheless, calcium ions on their own were not quite sufficient either, even when the pH had been adjusted to be close to that of mains water. As Table 1 shows, only relatively small amounts of milky tea film were produced in $CaCl_2$ solutions, whereas milk on its own had formed a substantial film under these conditions. Only when both calcium and bicarbonate ions were present did large amounts of milky tea film appear. It follows that milky tea films will form only when tea is brewed in temporary hard water. No such film will appear when tea has been brewed in soft water (which contains no calcium) and only small amounts when permanent hard water (containing no bicarbonate) has been used.

Variation with concentration of milk and temperature

Figure 1 shows that the mass of milky tea film collected after 1 h at 80°C or 70°C increased steadily with increasing concentration of milk, although the amount

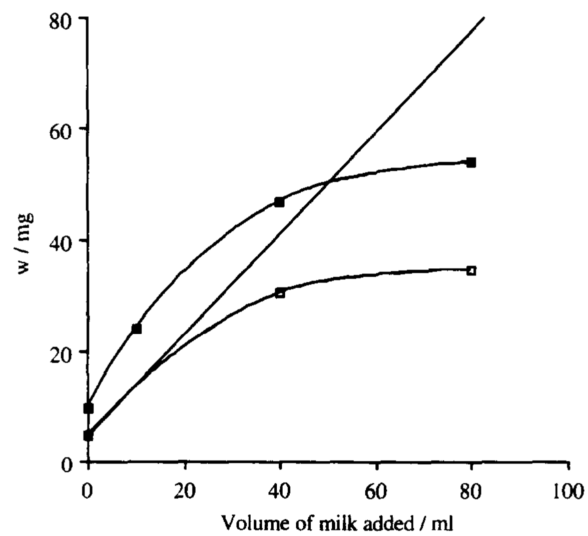

Fig. 1. Variation of the mass of milky tea film formed after 1 h when increasing volumes of homogenised full cream milk were added to 800 ml of tea infusion in London mains water kept at 80°C (■) or at 70°C (□). The line represents the amount of milk film obtained at 80°C without any tea present (Spiro & Chong, 1997).

formed levels off gradually. The 80°C curve can be compared with the line representing the mass of milk film obtained at the same temperature in the absence of tea (Spiro & Chong, 1997). The intercept, with no milk present, is clearly greater with a tea infusion (which forms tea scum) than with hard water alone (which forms a layer of $CaCO_3$; Spiro *et al.*, 1996). At low milk concentrations, the mass of milky tea film continues to be greater than that of the milk film, but the curves cross over at higher concentrations of milk. Here the presence of tea reduces the mass of film formed.

When 40 ml of homogenised milk were added to the hard water tea infusion, the activation energy for milky tea film formation between 70°C and 80°C was 44 kJ mol^{-1}. Although smaller than the 67 kJ mol^{-1} found for the formation of milky films under the same conditions, both point to chemically-controlled rather than diffusion-controlled processes.

Type of milk used

The amount of milky tea film depended greatly on the kind of milk employed. As Table 2 shows, the mass collected ranged from 24 mg with skimmed milk to 182 mg with UHT single cream and, in general, the mass increased the greater the percentage of fat in the milk. However, the structure of the milk was at least as important, since non-homogenised full cream milk produced three times as much film as homogenised milk with the same fat content. The last column in Table 2 shows that there was a very similar trend in the absence of tea with milk and hard water systems alone (Spiro & Chong, 1997).

To a first approximation, therefore, one might consider milky tea films to be essentially milk films even though the phenomenon itself is mainly apparent to tea drinkers. However, comparison of the last columns in Table 2 brings out two major differences. In the case of skimmed milk, the presence of tea greatly increased the mass of film, to a value larger than that obtained by naively adding the masses of tea alone (10 mg) and of milk film alone (6 mg). Moreover, the activation energy for the formation of the film over tea containing skimmed milk was only 22 kJ mol^{-1} between 70°C and 80°C. With UHT single cream, on the other hand, the amount of milky tea film was significantly less than that of milk (i.e. cream) film alone. The effect brought about by tea solubles is also apparent when the chemical microanalytical data in Table 3 are compared with the compositions of the corresponding milk films (Spiro & Chong, 1997). With skimmed milk the tea films contained much more carbon, more hydrogen and more nitrogen but less phosphorus and less calcium. In contrast, with both the full cream milks and with the single cream, the tea films contained similar percentages of carbon and hydrogen, less nitrogen, more phosphorus and more calcium.

Table 2. Properties of the different types of milk used and the masses (*w*) of the films formed when 40 ml of these were added to 800 ml of tea infused in London mains water and allowed to stand at 80°C for 1 h

Milk used	Milk composition[a] (g per 100 ml)			*w* (mg)	
	Carbohydrate	Protein	Fat	Tea + milk	Water + milk[b]
Skimmed	5·0	3·4	0·1	24[c]	6
Homogenised full cream	4·8	3·4	3·9	47	48
Non-homogenised full cream	4·7	3·3	4·0	146[d]	146
UHT single cream	3·9	2·6	19·3	182	238

[a]Figures taken from the milk cartons used.
[b]Milk only data from Spiro and Chong (1997).
[c]This film was a lighter brown than the other milky tea films.
[d]When the tea infusion was added to the milk, 188 mg of film were formed.

Effect of increased tea concentration

The above experiments were carried out with only one teabag in 800 ml of water in order to produce a large amount of film for study. In everyday tea drinking, however, the tea leaf:water ratio is much larger. To simulate the effect of a stronger cup of tea, five teabags instead of one were infused for 5 min to prepare the 80°C infusion in London mains water before 40 ml of homogenised full cream milk were added to it. This produced only 0·6 mg of film after 1 h. To check that this low result had not been caused by the reduced pH of 5·76, the experiment was repeated with the addition to the mixture of a small amount of 0·1 M NaOH solution to raise the pH to 6·56. This time 1·8 mg of film were collected, still far below the value of 47 mg found when only one teabag was used. It follows that the mass of milky tea film formed on a normal cup of tea, which stands for only a few minutes and gradually cools, is quite small.

Fourier transform infrared spectra

Figure 2 shows the FTIR spectrum of the film formed from a tea infusion containing skimmed milk. It is closely similar in shape to the spectrum of a skimmed milk film (Spiro & Chong, 1997). The superimposed dashed spectrum of a black tea scum is quite different. It does not contain the large peaks at 1079 and 558 cm^{-1} which were ascribed to ionic phosphate or the peak at 1541 cm^{-1} attributed to N–H bend in –CONH (Spiro & Chong, 1997). These three peaks are likely to have arisen from casein micelles. On the other hand, the pronounced tea scum peak at 1618 cm^{-1}, probably due to carboxylate ion although previously ascribed to carbonate ion (Spiro & Jaganyi, 1994*a*), does not appear when skimmed milk is present. One can therefore conclude that there was no significant amount of tea scum in the skimmed milk film and that the polyphenols were incorporated inside the casein micelles as suggested by Luck *et al.* (1994).

The FTIR spectra for films above tea infusions containing homogenised or non-homogenised full cream milk were substantially similar to those of the milk films themselves. The main differences were that the two milky tea films exhibited more pronounced peaks at 3308 and 3311 cm^{-1} respectively (attributed to O–H stretch) and stronger peaks at 1652 and 1654 cm^{-1}, respectively (attributed to C=O stretch in amide). The homogenised milky tea film also showed a larger peak than did the homogenised milk film at 1096 cm^{-1}, a wavenumber ascribed to ionic phosphate. The FTIR spectra of the films formed above the single cream and the single cream + tea solutions displayed no significant differences.

Table 3. Analysis (in wt%) of milky tea films formed in London mains water by the standard procedure

Milk used	C (±0·3)	H (±0·3)	N (±0·1)	P	Ca[a] (±0·1)	Mg	Mn
None[b]	40·4	4·04	0·53	0·27	6·06	0·36	0·92
Homogenised full cream	57·9	8·54	3·11	2·77	7·43	0·13	0·30
Non-homogenised full cream	60·5	8·45	2·81	1·22	4·68	0·05	0·14
Skimmed milk	33·9	4·07	6·33	5·23	13·3	0·23	0·53
UHT single cream	61·3	9·65	2·13	1·27	3·10		

[a]The sodium concentrations were between 0·04 and 0·11 wt%.
[b]Taken from Spiro and Jaganyi (1994*a*). The calcium and magnesium concentrations should probably be higher (Spiro *et al.*, 1996).

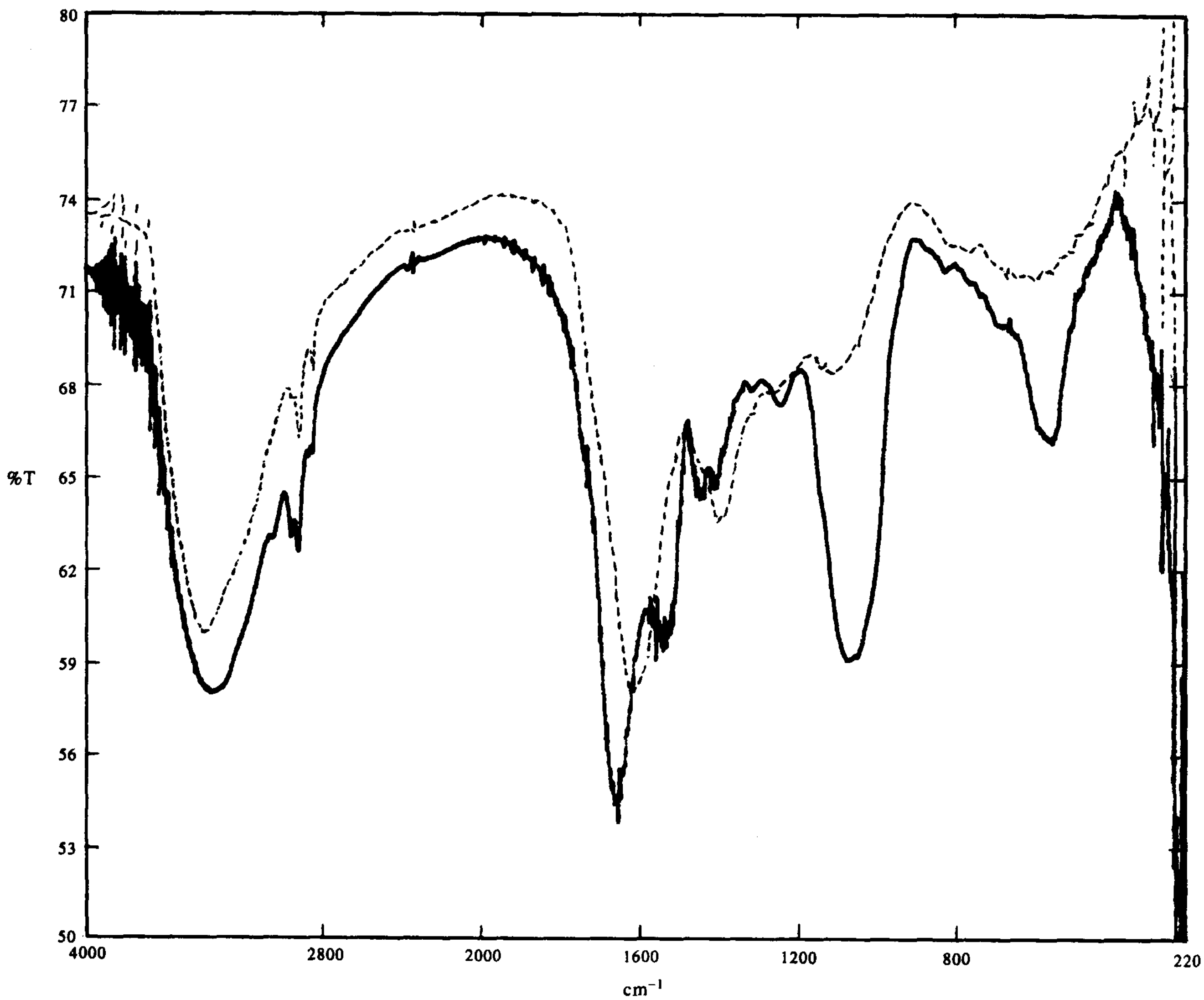

Fig. 2. FTIR spectrum of the film formed from a mixture of 40 ml of skimmed milk in 800 ml of tea infusion prepared in London mains water and kept at 80°C for 1 h. The superimposed FTIR spectrum (dashed line) is that of the film formed above the same tea solution but without added milk.

Scanning electron microscopy

The electron micrograph of milky tea film in Fig. 3 shows flaky material containing dark spots which are even larger and more diffuse in some other micrographs. These may indicate incorporated black tea polyphenols. The scattered white particles are likely to be calcium carbonate or phosphate, similar to those found on tea scum (Spiro & Jaganyi, 1994*a*). The milky tea film micrographs show none of the bubbles seen on milky films (Spiro & Chong, 1997).

General discussion

Both tea infusions and milk are chemically and structurally complex and their combination is even more so. All three types of system have been shown to form surface films in hot temporary hard water but not in hot distilled water. There the overt similarity ends. The surface layer formed on hard water infusions of black tea is largely the result of the oxidation of tea polyphenolic constituents at the surface (Spiro & Jaganyi, 1993, 1994*a,b*), while the film produced on dilute solutions of milk arises mainly from the calcium-induced aggregation of fat globules in the bulk solution which then rise to the top (Spiro & Chong, 1997). The surface films found on hot milky tea are, however, not simply the sum of the two. In particular, the results in Fig. 1 and the FTIR data indicate that normal tea scum does not form in the presence of a moderate amount of milk. One reason for this may be that the surface layer of milk fat limits the access of oxygen, while another and probably more important cause is the well-documented interaction between tea polyphenols and milk proteins in the solution (Brown & Wright, 1963; Luck *et al.*, 1994).

Our findings point to milk constituents as the main components of milky tea films. The subject is therefore best approached by considering the effect on hot dilute milk solutions of adding tea solubles, principally tea polyphenolics such as catechins, theaflavins and thearubigins (Willson & Clifford, 1992). These play at least four different roles:

1. They partly chelate calcium ions. This will decrease the free calcium ion concentration and should therefore reduce the amount of all milky tea films. It is likely to be a major reason for the almost complete absence of film over concentrated tea infusions.
2. The acidic tea polyphenols will reduce the pH. This will increase the solubility of calcium phosphate and release some calcium ions, so partly counteracting

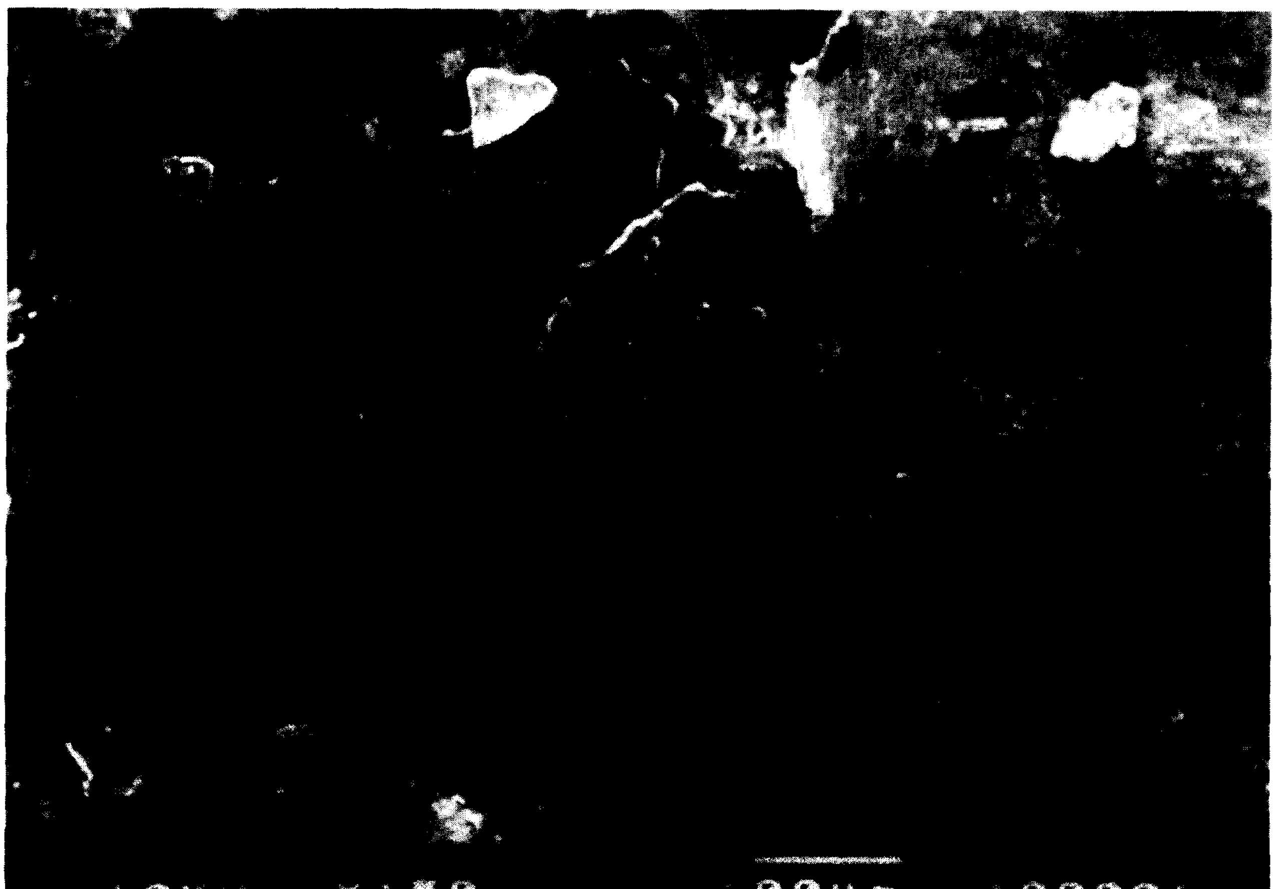

Fig. 3. Scanning electron micrograph of the film formed from a mixture of 40 ml of homogenised full cream milk and 800 ml of hard water tea infusion kept at 80°C for 1 h. The white bar at the bottom represents 100 μm.

effect (1) above. Some of the bicarbonate ions in the hard water will be converted to carbon dioxide which will reduce the amount of film. The function of the bicarbonate ion itself is unclear. Although its presence is not important in forming milk film (Spiro & Chong, 1997), it appears to play a significant role in the formation of milky tea film. The results in Table 1 suggest that its effect on pH is not the whole reason, and its association with calcium ions at 80°C in dilute solution is slight (extrapolation of the data of Jacobson and Langmuir (1974) to 80°C gives a stability constant for $CaHCO_3^+$ of 56 litre mol^{-1}).

3. The polyphenols will associate with milk α- and β-caseins by hydrogen bonding (Brown & Wright, 1963). Haslam's group has identified the hydrophobic and proline-rich aspects of caseins as the ones which facilitate the strongest complexation with polyphenols (Luck *et al.*, 1994). These workers have proposed that the tea polyphenols will then be removed from the free solution by sequestration within the casein micelles, being located inside many of the submicelles. This accords with our FTIR spectra which showed little difference between the films formed from solutions of skimmed milk and from solutions of skimmed milk + tea. Our experiments with these two systems suggest that the polyphenol-containing casein micelles are more prone to aggregation by calcium ions and produce significantly more surface film.
4. Tea polyphenols will also be attracted to the protein-containing membranes which stabilise fat globules. They will thereby tend to prevent calcium ions binding to the phosphoseryl groups of the membrane caseins and thus hinder the clustering of the globules. This would account for the smaller amount of film in the presence of tea at higher milk concentrations (Fig. 1), the reduction in the mass of film in tea infusions containing single cream, and contribute to the lack of film at high tea concentration. However, the concentration of polyphenols in our standard experiments will not have been large enough to bind to all possible protein sites on the membranes of the fat globules in homogenised milk, whose surface area will have been an order of magnitude larger than for the globules in non-homogenised milk.

The present research has therefore shed light on the films formed on tea brews to which various kinds of milk have been added. The presence of the milk prevented the formation of most of the black tea scum because the tea polyphenols were sequestered inside casein micelles or incorporated within aggregates of fat globules. This explains why milky tea does not leave deposits on cups and mugs which are as dark and difficult to remove as those left by tea without milk.

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