Protein and Lipid Films at Equilibrium at Air–Water Interface

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ABSTRACT: The surface activity of β-casein, caseinate, and a whey protein isolate in aqueous solutions has been investigated over a range of protein concentrations ($1 \cdot 10^{-5}$ to 5% w/w) at pH 5 and 7. The surface pressure data were determined by the Wilhelmy plate method. Surface pressure data at low protein concentration indicate a low surface activity that rises to a plateau as the monolayer is saturated at higher protein concentrations. The protein concentration and the surface pressure at the plateau depend on the pH and the type of protein in the aqueous phase. Protein–monoglyceride interactions were investigated by spreading an insoluble monoglyceride (monopalmitin, monoolein, or monolaurin) on a film of protein previously adsorbed on the interface at equilibrium. The existence of protein–monoglyceride interactions depends on the interfacial composition and on the protein/monoglyceride ratio. The surface activity of mixed protein–monopalmitin and protein–monoolein films is determined by the lipid as the surface pressure of the mixed film is the same as the monoglyceride equilibrium spreading pressure, and the monolayer is not saturated by the protein. However, the protein determines the surface activity of mixed protein–monopalmitin and protein–monoolein films as the protein saturates the monolayer. For protein and monolaurin mixed films, protein determines the surface activity over the range of protein–monolaurin compositions due to monolaurin dissolution in the bulk aqueous phase.

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The stability and mechanical properties of dispersed food systems (emulsions and foams) depend on the way in which the constituent particles and macromolecules adsorb and interact at fluid–fluid interfaces. To stabilize food emulsions and foams, emulsifiers (lipids and proteins) must be placed at the interface, so they can form a film around droplets or bubbles, respectively. The optimal use of emulsifiers depends on knowledge of their interfacial physicochemical characteristics—such as surface activity, structure, stability, superficial viscosity, etc.—and the kinetics of the film formation at fluid–fluid interfaces (1,2).

Lipids and proteins at the interface reduce the interfacial tension between the phases and thus stabilize and improve the formation of food emulsions and foams (1,2). Proteins, in addition to lowering the interfacial tension, can form a continuous film at the interface *via* complex intermolecular interactions and thus impart structural rigidity. The small-molecule emulsifiers can cover the interface that proteins do not, resulting in an emulsion with smaller particles, leading to greater stability. But, competitive adsorption of proteins and lipids at fluid interfaces can affect the stability of food dispersions (3). However, more important in some products is the effect of the small molecules in destabilizing the emulsion (4). In the formulation of ice cream, the small-molecule emulsifier is added to break the adsorbed layer of protein and to allow the adsorption of fat to the surface of the air bubble. Thus, an important action of the small-molecule emulsifiers is to promote the displacement of caseins from the interface. In summary, knowledge of the lipids, proteins, and their mixtures at fluid–fluid interfaces is a key factor for the formation and stability of successful food dispersions (emulsions and foams).

There have been many studies of protein–lipid interactions in relation to the formation and stability of food emulsions and foams. Several groups have studied the interactions between proteins and soluble lipids, but much less is known about the details of protein–insoluble lipid interactions (5). We now report surface tension data of three milk proteins [β-casein, caseinate, and a whey protein isolate (WPI) with high content of β-lactoglobulin] and their mixtures with three food-permitted insoluble lipids (monopalmitin, monoolein, and monolaurin) at equilibrium. These experiments mimic the behavior of emulsifiers in food emulsions in which an oil-soluble lipid (monopalmitin, monoolein, or monolaurin) diffuses to the interface where a protein film is adsorbed from the aqueous bulk phase, followed by protein–lipid interactions in the interfacial region.

MATERIALS AND METHODS

Materials. β-Casein of 99% purity was supplied as purified from bulk milk by the Hannah Research Institute (Ayr, Scotland). WPI, a native protein with high content of β-lactoglobulin (protein 92 ± 2%, β-lactoglobulin >95%, α-lactalbumin <5%) obtained by fractionation, was supplied by Danisco Ingredients (Branbran, Denmark). Caseinate (a mixture of ≈38% β-casein, ≈39% α_{s1}-casein, ≈12% κ-casein, and ≈11% α_{s2}-casein) was purified from bulk milk and supplied by Unilever Research (Colworth, United Kingdom). Synthetic 1-monohexadecanoyl-*rac*-glycerol (monopalmitin), 1-mono-(*cis*-9-octadecanoyl)glycerol (monoolein), and 1-monododecanoyl-*rac*-glycerol (monolaurin) were supplied by Danisco

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Ingredients with over 95–98% purity. To form the surface film, monoglyceride was spread in the form of a solution with hexane/ethanol (9:1, vol/vol) as a spreading solvent. Analytical-grade hexane [Merck (Darmstadt, Germany), 99%] and ethanol (Merck, >99.8%) were used without further purification. The water used as subphase was purified by means of a Millipore (Milford, MA) filtration device (Milli-Q). To adjust subphase pH, buffer solutions were used. Acetic acid/sodium acetate aqueous solution (CH_3COOH/CH_3COONa) was used to achieve pH 5, and a commercial buffer solution called *trizma* was used for pH 7. All these products were supplied by Sigma (St. Louis, MO). Sodium azide (Sigma) was added (0.05 wt%) as an antimicrobial agent. Ionic strength was 0.05 M in all experiments.

Surface tension. Surface-tension measurements were used to determine protein and lipid spreading and protein adsorption at equilibrium and to explore the presence of protein–lipid interactions at the interface. Surface activity was expressed by the surface pressure, $\pi = \sigma_o - \sigma$, where σ_o and σ are the aqueous subphase surface tension and the surface tension of the aqueous solutions of protein and protein–lipid mixed films, respectively. Measurements were performed with a Krüss (Hamburg, Germany) K 10 digital tensiometer, based on the Wilhelmy method, with a roughened platinum plate, as described elsewhere (6–8). The temperature of the system was maintained constant within ±0.5ºC by a circulating Heto (Bikerod, Denmark) thermostat.

Measurements were also performed to study protein–lipid at the air–water interface. Fifty microliters of monopalmitin at $5.07 \cdot 10^{-4}$ M, monoolein at $3.97 \cdot 10^{-4}$ M, or monolaurin at $4.05 \cdot 10^{-4}$ M—dissolved in a mixture of hexane and ethanol, 9:1 (vol/vol)—was spread on a film of protein previously adsorbed from the subphase bulk. After the hexane–ethanol evaporated in 10–15 min, the solutions were placed in the dish and then in an enclosing box and were allowed to stand for 24 h to achieve the desired steady state protein–lipid interactions. Measurements were performed a minimum of five times. Surface-tension measurements were reproducible within ±0.5 mN/m.

Equilibrium surface pressure. The equilibrium spreading pressure (π_e) is the maximum surface pressure to which a spread monolayer may be compressed before monolayer collapse. Equilibrium surface pressures of protein in the range of 5 and 40ºC and of monoglycerides at 20ºC were measured by the Wilhelmy plate method as described elsewhere (6).

RESULTS AND DISCUSSION

Spreading of proteins at the air–water interface at the equilibrium. Equilibrium spreading pressures of β-casein, caseinate, and WPI at the air–water interface, at pH 5 and 7 and a temperature range between 5 and 40ºC, are shown in Figure 1. The magnitude of π_e was dependent on the protein, temperature, and aqueous phase pH. At pH 5, π_e was higher for caseinate and decreased for WPI and β-casein. However, at pH 7, π_e was lower for β-casein and was of the same order

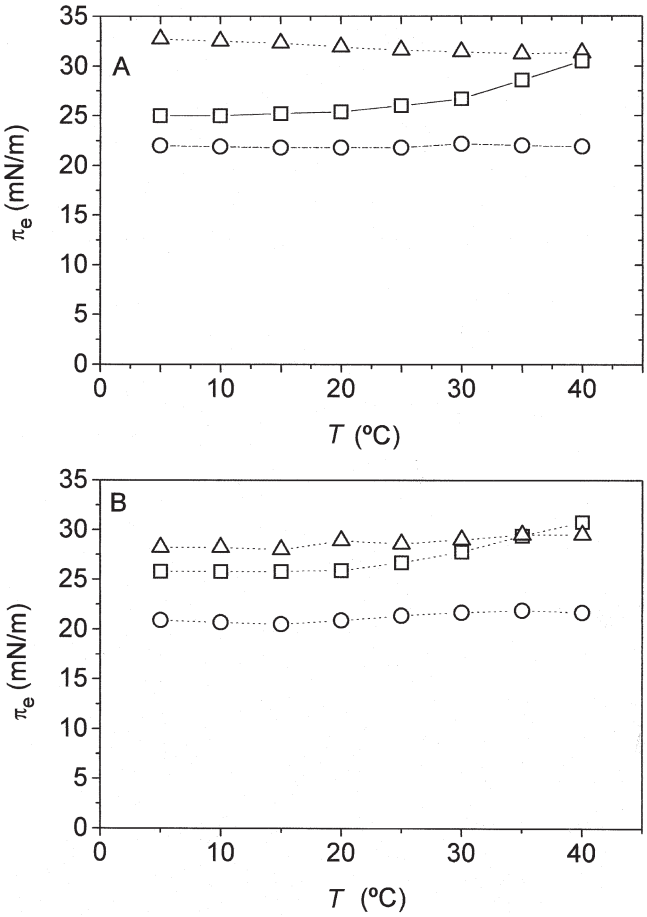

FIG. 1. Temperature dependence of equilibrium surface pressure (π_e) for spread monolayers of (○) β-casein, (△) caseinate, and (□) whey protein isolate (WPI), on air–water at (A) pH 5 and (B) pH 7.

of magnitude for caseinate and WPI, especially at temperatures higher than 25ºC. That is, at every pH and temperature, β-casein films show lower surface activity at the equilibrium than caseinate and WPI. The effect of temperature was also different for disordered proteins (β-casein and caseinate) than for an ordered protein (WPI). Figure 1 shows that π_e for β-casein and caseinate was not affected by temperature within the range of 5 and 40ºC. However, π_e for WPI increased with temperature, especially at temperatures above 25ºC. As is known (9), β-lactoglobulin in aqueous solutions at pH 5.5–7.5 is associated in the formation of dimers at room temperature. These dimers are dissociated at temperatures of 30–50ºC (9). However, we do not reject the possibility of interfacial gelation at higher temperatures, as was recently observed for WPI adsorbed films at the oil–water interface at 40ºC at a protein concentration in the bulk phase as low as $1 \cdot 10^{-5}$% w/w (10). Thus, these phenomena may be related to the temperature dependence of WPI films (Fig. 1).

Adsorption of proteins at the air–aqueous phase interface at equilibrium. Figures 2 to 4 show adsorption isotherms for β-casein (Fig. 2), caseinate (Fig. 3), and WPI (Fig. 4) on water at pH 5 and 7 at 20ºC. The protein concentration dependence on surface pressure showed classical sigmoidal

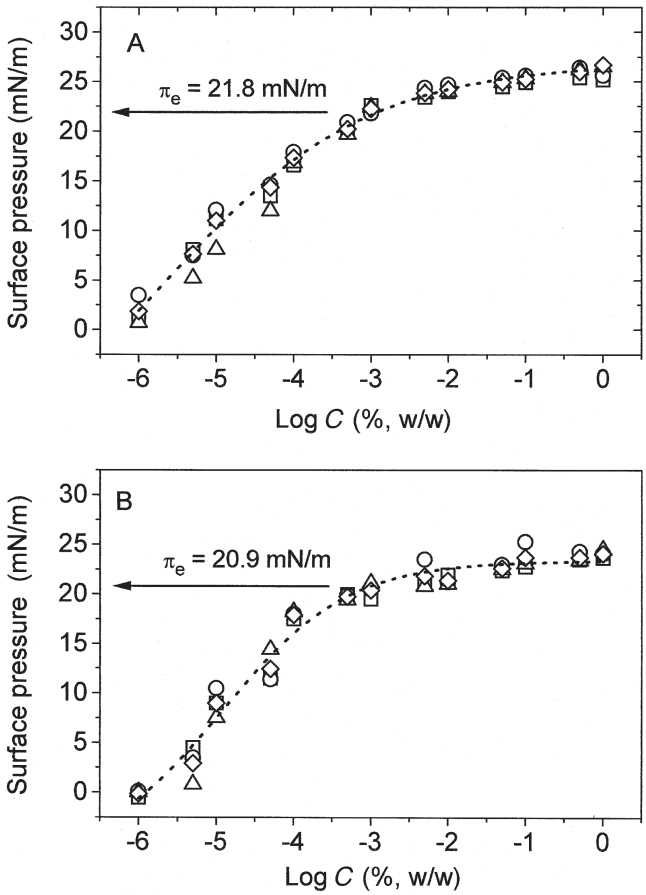

FIG. 2. Adsorption isotherm for β-casein on water at (A) pH 5 and (B) pH 7. Different symbols are for repetitive experiments. Temperature 20°C. The equilibrium spreading pressures for β-casein at pH 5 and 7 (from Fig. 1) are included by means of arrows.

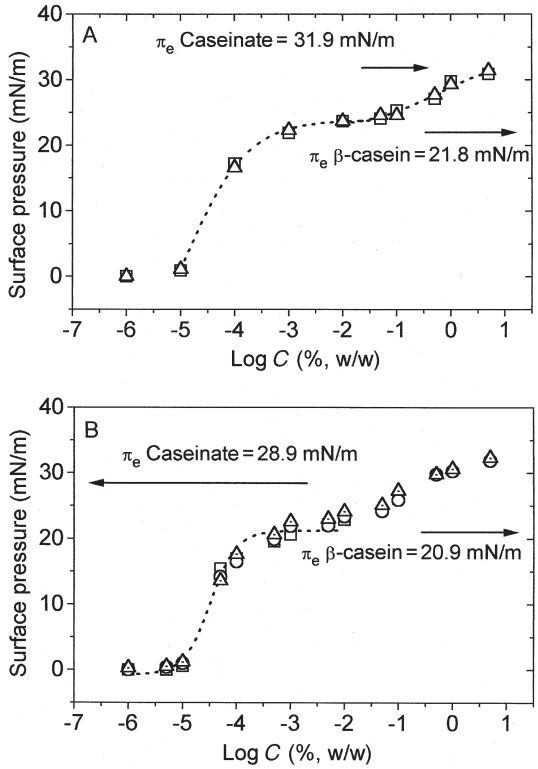

FIG. 3. Adsorption isotherm for caseinate on water at (A) pH 5 and (B) pH 7. Different symbols are for repetitive experiments. Temperature 20°C. The equilibrium spreading pressures for caseinate and β-casein at pH 5 and 7 (from Fig. 1) are included by means of arrows.

behavior. At low protein concentrations, the initial solutions caused only a small increase in the surface pressure. The surface pressure increased with protein concentration and tended to a plateau. This plateau commenced at the point where surface pressure reached its maximum value over the range of protein concentrations from $1 \cdot 10^{-3}$ to 1% w/w. However, some differences exist between proteins.

β-Casein (Fig. 2) showed significant surface activity at protein concentrations in the bulk phase of $1 \cdot 10^{-6}$% and $5 \cdot 10^{-6}$% for pH 5 and 7, respectively. The plateau commenced at $5 \cdot 10^{-3}$% at pH 7, but for pH 5 the surface pressure increased with protein concentration up to 1% w/w. The surface pressure values determined in this work were in good agreement with results reported for β-casein at the same temperature (11). On the other hand, the value of π_e for spread film was lower than that for the surface pressure at the plateau for adsorbed film.

Caseinate (Fig. 3) showed significant differences from β-casein:

(i) Caseinate showed significant surface activity at concentrations in the bulk phase of $1 \cdot 10^{-5}$%. This concentration was one order of magnitude higher than for β-casein. That is, at low concentrations, the surface activity of the proteins forming caseinate gave lower surface activity than β-casein alone.

(ii) The adsorption isotherms of caseinate showed a plateau at surface pressures close to that of pure β-casein, and then the surface pressure increased with protein concentration up to 5% w/w (the maximum studied protein concentration in the bulk phase). That is, at the plateau, the surface activity of the proteins forming caseinate gave higher surface activity than β-casein alone, behavior similar to that observed with spread films (Fig. 1). The value reported by Benjamins (12) of surface pressure at the plateau for β- and κ-caseins was in good agreement with that obtained in this work for β-casein, although the thickness and the equilibrium adsorption were higher for κ-casein than for β-casein. These results suggest that individual casein components in caseinate adsorb independently to the air–water interface, with few interactions between then.

(iii) As for pure β-casein, the value of π_e for spread caseinate film was lower than that of the surface pressure of adsorbed film at the maximum caseinate concentration in the bulk phase (5% w/w).

(iv) The pH had no significant effect on the adsorption isotherm for caseinate.

WPI showed (Fig. 4) significant surface activity at concentrations in the bulk phase of $5 \cdot 10^{-5}$% w/w, at pH 5 and 7. The surface pressure increased with protein concentration and tended to a plateau at the maximum protein concentration in the bulk phase (5% w/w). These data are in good agreement

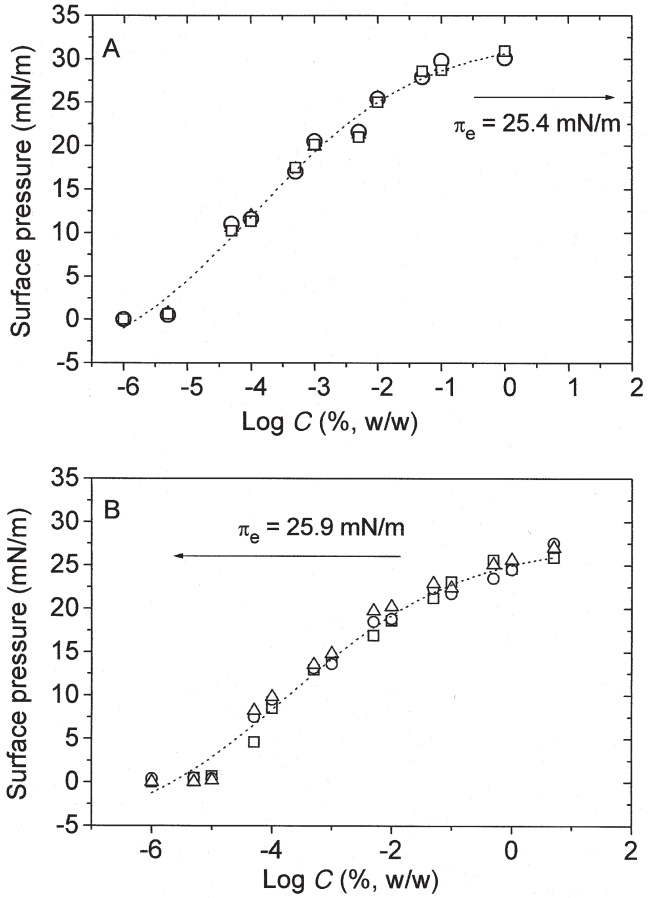

FIG. 4. Adsorption isotherms for WPI on water at (A) pH 5 and (B) pH 7. Different symbols are for repetitive experiments. Temperature 20ºC. The equilibrium spreading pressure for WPI at pH 5 and 7 (from Fig. 1) are included by means of arrows; see Figure 1 for abbreviation.

with the results of other authors for pure β-lactoglobulin (12,13). WPI-adsorbed films were sensitive to the pH. In fact, the surface activity was higher at pH 5 than at pH 7, especially at the region close to the plateau. The value of π_e for spread WPI film was lower than that of the surface pressure of adsorbed film at the maximum protein concentration in the bulk phase (5% w/w).

True equilibrium adsorption does not seem to be possible with proteins, even after 2 or 3 d (6,7,12). The origin of this slow aging effect is not known, but denaturation, evaporation/drying, or changes in intermolecular interactions have been proposed (12). Therefore, we considered the surface pressure measured after 24 h as the pseudo-equilibrium value.

The behavior of adsorbed protein films (Figs. 2–4) can be interpreted in terms of monolayer coverage by comparing the data of surface pressure determined here with that of surface concentration determined by ellipsometry (12) or surface radioactivity (14). At the lower protein concentrations, as the surface pressure is close to zero, the adsorbed protein residues may be considered as a two-dimensional ideal gas. Proteins at higher concentrations, but lower than that of the plateau, form a monolayer of irreversibly adsorbed molecules. As the plateau is attained, the monolayer is saturated by protein that is irreversibly adsorbed. At higher protein concentrations, the protein molecules form multilayers beneath the primary monolayer, but these structures do not contribute significantly to surface pressure (14). The presence of multilayers at the maximum protein concentration in the bulk phase (for β-casein, caseinate, or WPI) has been observed recently by Brewster angle microscopy (15).

The general characteristics of β-casein, caseinate, and WPI adsorption are practically the same at the two values of pH studied. However, the value of surface pressure at the plateau decreased as the pH increased. That is, the activity of β-casein and WPI at the plateau is higher at pH 5 than at pH 7. The adsorption characteristics of protein as a function of the aqueous phase pH could be interpreted in terms of the effect of pH on the structure of protein in the bulk phase and at the interface. The higher surface activity at pH 5, which is close to the isoelectric point (IEP) of the studied proteins, may be due to the reduction of electrostatic repulsion forces between charged adsorbing molecules. The maximum adsorption at the IEP has been explained by the absence of lateral repulsive forces between charged adsorbed molecules or by structural rearrangements in the adsorbing molecules (16). In fact, we have observed recently that spread monolayers of β-casein and caseinate at the air–water interface are more condensed at pH 5 than at pH 7 (15).

Finally, some differences were observed between surface pressure at the plateau for adsorbed protein and equilibrium spreading pressure for WPI (Fig. 4), caseinate (Fig. 3), and, especially for β-casein (Fig. 2), a phenomenon that should be associated with a different rearrangement of residues when the protein is either adsorbed or spread on the interface. Similar differences were observed between spread and adsorbed films for β-casein, bovine serum albumin, ovalbumin, and lysozyme from surface pressure-concentration curve (12). To further our knowledge of interfacial protein structure, studies on π-A isotherms for spread and adsorbed proteins at the air–water interface are under way at present.

Protein–lipid interactions at the air–water interface at the equilibrium. The effect of the protein/lipid ratio on the surface activity of mixed β-casein–monoglyceride, caseinate–monoglyceride, and WPI–monoglyceride systems at 20ºC is shown in Figures 5, 6, and 7, respectively. In these experiments, monoglyceride spread on a previously adsorbed protein film was maintained constant at surface densities of 12, 9.7, and 9.5 molecules.nm^{-2} for monopalmitin, monoolein, and monolaurin, respectively. So, the variation of the protein/lipid ratio is due to the protein added in the bulk phase over the range 5 to $1 \cdot 10^{-5}$% w/w. The monoglyceride density spread on the interface was higher than that required for the monolayer collapse, as was deduced from the π-A isotherm (17). The protein concentration dependence on surface pressure for protein–monoglyceride mixed systems showed a sigmoidal behavior. However, the surface activity of the mixed systems depended on the protein/monoglyceride ratio and the monoglyceride spread on the interface (Figs. 5–7).

For all protein–monoglyceride mixed films, the surface

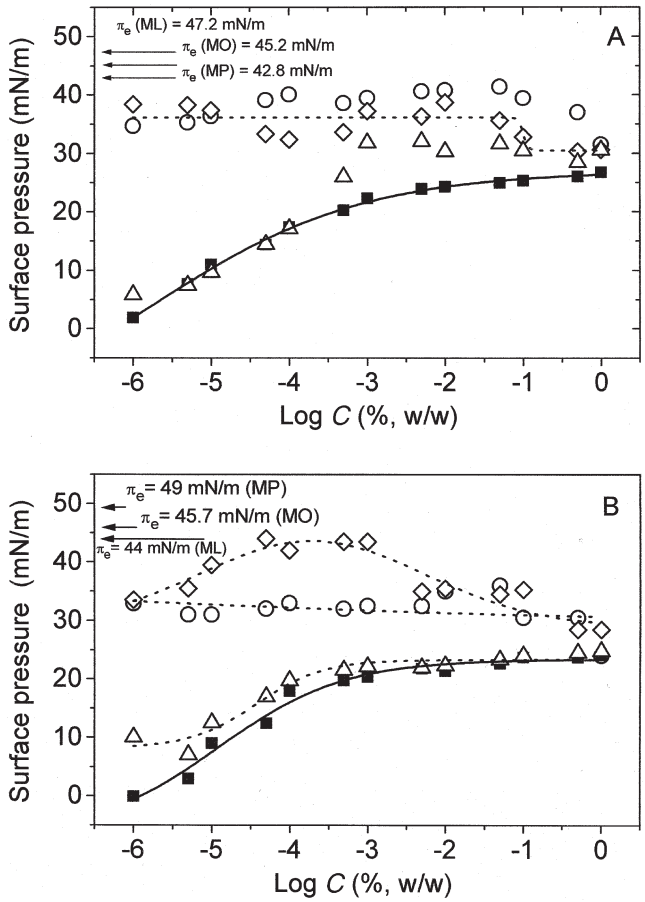

FIG. 5. Effect of the spreading of (○) monopalmitin (MP), (◇) monoolein (MO), and (△) monolaurin (ML) on (■) a film of β-casein previously adsorbed on the air–water interface. Temperature: 20°C. C = protein concentration. Lipid superficial density (molecule·nm^{-2}): MP (12), MO (9.7), and ML (9.5). The arrows indicate the equilibrium spreading pressure, π_e, for MP, MO, and ML. A = pH 5, B = pH 7.

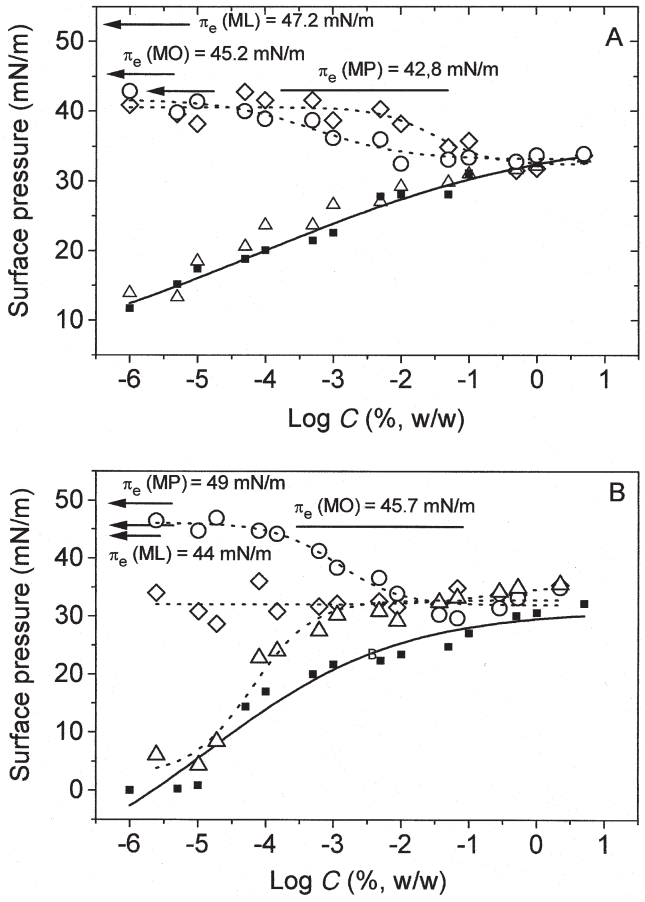

FIG. 6. Effect of the spreading of (○) MP, (◇) MO, and (△) ML on (■) a film of caseinate previously adsorbed on the air–water interface. Temperature: 20°C. Lipid superficial density (molecule·nm^{-2}): MP (12), MO (9.7), and ML (9.5). The arrows indicate the equilibrium spreading pressure, π_e, for MP, MO, and ML. See Figure 5 for other abbreviations.

pressure values approach those of pure protein films at higher relative protein concentrations in the mixed systems, as the protein saturated the monolayer. However, significant differences were observed at lower protein–monoglyceride ratios, especially for protein–monolaurin mixed films. In fact, for protein–monolaurin mixed films, the surface pressure followed the same dependence on protein concentration as pure protein, especially for pH 5. However, for protein–monopalmitin and protein–monoolein mixed films, the protein–lipid ratio dependence on surface pressure was more complex.

At lower relative protein concentrations, the surface pressure tended to the equilibrium surface pressure (π_e) of the pure monopalmitin or monoolein, which is indicated in Figures 5–7 by means of arrows (WPI–monoglyceride mixed film at pH 7 are an exception, Fig. 7B). However, the level of surface pressure at the minimum protein–monoglyceride ratio depended on the interfacial composition.

At the intermediate range of protein–monoglyceride concentrations, significant further reduction in the surface pressure was observed. The effect resulted in an inflection in the surface pressure curve in the intermediate region. Surprisingly, for β-casein–monoolein mixed films at pH 7, a maximum was observed in this region (Fig. 5B).

The general behavior for protein–monopalmitin and protein–monoolein mixed films at the air–water interface at pH 5 and 7 (Figs. 5–7) is practically the same as that observed with other monoglyceride–protein mixed films (7). As a consequence of these results, we can suggest that, at higher protein relative concentrations in protein–monoglyceride mixed films, the protein determines the surface activity. In contrast, monoglyceride (monopalmitin or monoolein) determines the surface activity of protein–monoglyceride mixed films at lower protein relative concentrations in the mixture. So, it can be suggested that, at the monoglyceride (monopalmitin or monoolein) surface densities spread here, the protein is removed, and the interface is saturated by a collapsed monopalmitin or monoolein film with liquid-condensed or liquid-expanded structure (6), respectively. In the intermediate region, the surface activity is determined by the existence of protein and monoglyceride at the interface. However, what is more difficult to establish is the degree of interactions between film-forming components in the mixed film. From π-A

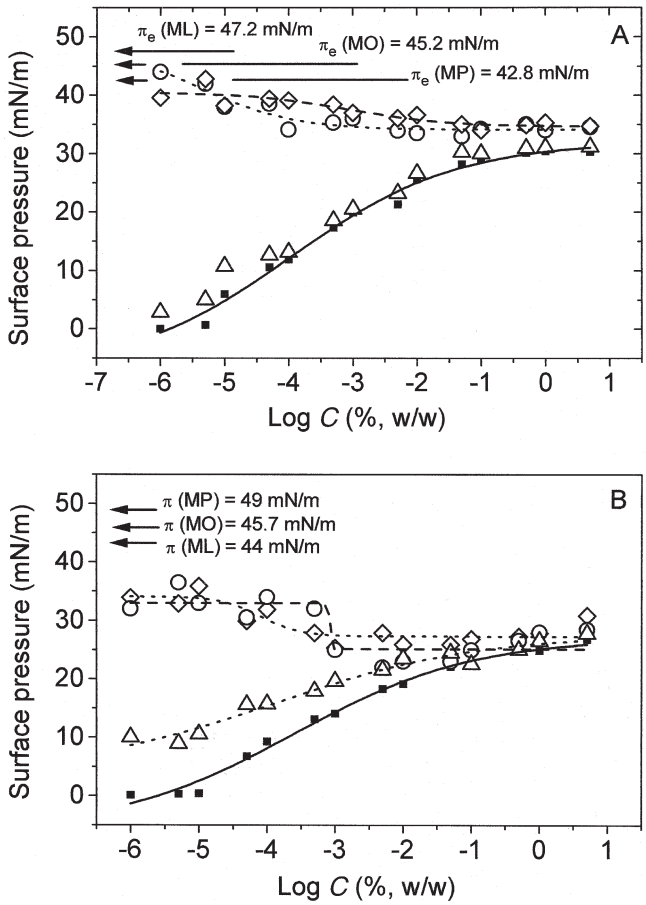

FIG. 7. Effect of the spreading of (○) MP, (◇) MO, and (△) ML (■) a film of WPI previously adsorbed on the air–water interface. Temperature: 20ºC. Lipid superficial density (molecule·nm^{-2}): MP (12), MO (9.7), and ML (9.5). The arrows indicate the equilibrium spreading pressure, π_e, for MP, MO, and ML. See Figures 1 and 5 for other abbreviations.

isotherms, it is clear that some degree of interaction exists between monoglycerides and proteins in spread mixed films, which are more pronounced as the monolayer is compressed at the highest surface pressures (18,19).

The removal of protein by surfactants is well documented in the literature (5). The degree of protein removal by a surfactant is affected by factors that are known to influence the binding strength of a protein to a surface. Thus, the removal of protein by surfactants has been found to decrease with conditions favoring conformational changes. However, removal of protein by a surfactant will be influenced not only by protein properties but also by the type of surfactant (5) and the aqueous phase composition (6,7).

Recent experiments (including π-A isotherm coupled with Brewster angle microscopy and relative thickness of spread monolayers) have shown (18,19), for the first time, that at higher surface pressures, after the protein collapse (at a surface pressure close to π_e), characteristic squeezing-out phenomena were observed with monoglyceride domains floating over a sublayer of collapsed residues of protein. The prevalence of monoglyceride increases with its concentration in the mixture and at higher surface pressures. In summary, on a microscopic level, the distribution of lipids and proteins in mixed spread films at the air–water interface is heterogeneous and depends on the surface pressure and the lipid–protein ratio in the mixed film. However, the distribution of monoglyceride and proteins at the air–water interface should be different when the monoglyceride is spread on a previously adsorbed protein, especially when protein concentration in the bulk phase is higher than that required for complete coverage, at the region plateau (Figs. 5–7). That is, the way in which proteins and monoglycerides are spread or adsorbed on the interface may have a role on the interfacial characteristics of the mixed film.

The general features described earlier for protein–monopalmitin and protein–monoolein mixed films are different for protein–monolaurin mixed films. In fact, the protein, at every protein–monolaurin ratio, determined the surface activity of protein–monolaurin mixed films. This phenomenon may be associated with the instability of monolaurin monolayers at the air–water interface. In fact, from relaxation experiments we have observed (20) a pronounced monolaurin monolayer molecular loss, which is more pronounced as the surface pressure increases, with a maximum at the surface density utilized in this study (at the collapse point). These results corroborate the theory that reduced interactions exist between monolaurin and protein at the air–water interface.

In this work, the existence of protein–monoglyceride interactions at the air–water interface has been proved by tensiometry. So, surface-tension measurement is an easier complementary experimental technique for providing information about the interfacial characteristics of pure protein and lipid films and about the existence of protein–lipid interactions at the interface. However, surface pressure–concentration experiments are not sufficient to allow a final picture of the nature of protein–monoglyceride interactions at the interface. In fact, from these experiments and from the discussion in the literature, it can be concluded that the interfacial characteristics of mixed emulsifiers at fluid interfaces depend at least on the nature of the interface (either air–water or oil–water) or on the way by which these emulsifiers are adsorbed to the interface (either by cooperative or competitive adsorption/spreading of the film-forming components) in a complicated manner.

On the other hand, lowering of the surface (interfacial) tension by emulsifiers (proteins and lipids) is only a first step in the production of stable food emulsions and foams. A low surface (interfacial) tension facilitates breaking up into smaller droplets. However, breaking requires rapid and substantial stretching of bubbles or drops, and consequently, the surface (interfacial) tension may be far from equilibrium. Thus, dynamic properties of adsorbed emulsifier layers are also important, due to their stabilizing function during emulsification.

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REFERENCES

1. Dickinson, E., *An Introduction to Food Colloids*, Oxford University Press, Oxford, 1992.
2. McClements, D.J., *Food Emulsions: Principles, Practice, and Techniques*, CRC Press, New York, 1999.
3. Dickinson, E., Structure, Stability, and Rheology of Flocculated Emulsions, *Current Opinion in Colloid & Interface 3*:633–638 (1998).
4. Berger, K.G., in *Food Emulsions,* edited by S.E. Friberg and K. Larsson, Marcel Dekker, New York, 1997, pp. 413–490.
5. Bos, M., T. Nylander, T. Arnebrandt, and D.C. Clark, in *Food Emulsifiers and Their Applications*, edited by G.L. Hasenhuette and R.W. Hartel, Chapman & Hall, New York, 1997, pp. 95–146.
6. Rodríguez Patino, J.M., and R. Martín, Spreading of Acylglycerols on Aqueous Surfaces at Equilibrium, *J. Colloid Interface Sci. 167*:150–158 (1994).
7. Rodríguez Niño, M.R., and J.M. Rodríguez Patino, Surface Tension of Protein and Insoluble Lipids at the Air–Aqueous Phase Interface, *J. Am Oil Chem. Soc. 75*:1233–1239 (1998).
8. Rodríguez Niño, M.R., and J.M. Rodríguez Patino, Surface Tension of Bovine Serum Albumin and Tween 20 at the Air–Aqueous Phase Interface, *Ibid. 75*:1241–1248 (1998).
9. Cayot, P., and D. Lorient, in *Food Proteins and Their Applications,* edited by S. Damodaran and A. Paraf, Marcel Dekker, New York, 1977, pp. 225–256.
10. Rodríguez Patino, J.M., M.R. Rodríguez Niño, and C. Carrera, Dynamic Interfacial Rheology as a Tool for the Characterization of Whey Protein Isolates Gelation at the Oil–Water Interface, *J. Agric. Food Chem. 47*:3640–3648 (1999).
11. Benjamins, J., J.A. de Feijter, M.T.A. Evans, D.E. Graham, and M.C. Phillips, Dynamic and Static Properties of Proteins Adsorbed at the Air/Water Interface, *Discuss. Faraday Soc. 59*: 218–229 (1975).
12. Benjamins, J., Static and Dynamic Properties of Proteins Adsorbed at Liquid Interfaces, Ph.D. Thesis, Wageningen University, 2000.
13. Makievski, A.V., R. Miller, V.B. Fainerman, J. Krägel, and R. Wüskneck, in *Food Emulsions and Foams: Interfaces, Interactions and Stability,* edited by E. Dickinson and J.M. Rodríguez Patino, Royal Society of Chemistry Cambridge, 1999, pp. 269–284.
14. Graham, D.E., and M.C. Phillips, Proteins at Liquid Interfaces. II. Adsorption Isotherms, *J. Colloid Interface Sci. 70*:415–426 (1979).
15. Rodríguez Patino, J.M., C. Carrera, and M.R. Rodríguez Niño, Structural Characteristics of β-Casein Monolayers at the Air–Water Interface, *Food Hydrocolloids 13*:401–408 (1999).
16. Norde, W., Adsorption of Proteins from Solution at the Solid–Liquid Interface, *Adv. Colloid Interface Sci. 25*:267–340 (1986).
17. Rodríguez Patino, J.M., C. Carrera, and M.R. Rodríguez Niño, Morphological and Structural Characteristics of Monoglyceride Monolayers at the Air–Water Interface Observed by Brewster Angle Microscopy, *Langmuir 15*:2484–2492 (1999).
18. Rodríguez Patino, J.M., C. Carrera, and M.R. Rodríguez Niño, Analysis of β-Casein–Monopalmitin Mixed Films at the Air–Water Interface, *J. Agric. Food Chem. 47*:4998–5008 (1999).
19. Rodríguez Patino, J.M., C. Carrera, and M.R. Rodríguez Niño, Is Brewster Angle Microscopy a Useful Technique to Distinguish Between Isotropic Domains in β-Casein–Monoolein Mixed Monolayers at the Air–Water Interface? *Langmuir 15*: 4777–4788 (1999).
20. Carrera, S., M.R. Rodríguez Niño, and J.M. Rodríguez Patino, Relaxation Phenomena in Monoglyceride Films at the Air–Water Interface, *Colloids Surfaces B 12*:175–192 (1999).

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