

Effect of pH on the Stability and Surface Composition of Emulsions Made with Whey Protein Isolate

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Emulsions (20 wt % soy oil) made with various concentrations (0.5–2.5 wt %) of whey protein isolate (WPI) were most stable at pH 7 and least stable at pH 5.5. Emulsions made with imidazole buffer at pH 6 were stable, but those made with citrate buffer at the same pH were unstable. Emulsions prepared at pH 3, using citrate buffer, were stable. At pH 7 β -lactoglobulin and α -lactalbumin adsorbed in proportion to their concentration, but at lower pH values α -lactalbumin was found to adsorb preferentially, depending on the protein concentration, pH, and buffer. When emulsions (2 wt % WPI) were acidified from pH 7 to 3 more α -lactalbumin became adsorbed and the emulsion was stable, but reducing the pH from 7 to 6 did not alter the interfacial composition of protein and the emulsion became unstable. The behavior of the whey proteins depends on variations of tertiary and quaternary structure with pH.

Keywords: *Whey proteins; emulsions; emulsion stability; adsorption; surface composition*

INTRODUCTION

Most investigations into the basic behavior of food proteins in emulsions have used just one concentration of a purified protein, or a mixture of two such proteins, at pH 7 and in solutions of low ionic strength. This is quite far removed from the situation in real food emulsions, in which more crude protein preparations and protein mixtures are used with a wide range of solution conditions (protein concentration, pH, and ionic strength). Recent investigations have begun to involve more complicated systems by using caseinate as opposed to individual caseins (Fang and Dalgleish, 1993), using whey protein isolate (WPI) instead of individual whey proteins (Hunt and Dalgleish, 1994), and varying the concentration of protein. Indeed, the concentration of protein has been found to be a very important factor in determining whether or not competitive adsorption occurs in emulsions made with mixtures of caseinate and WPI, and therefore protein concentration is an important consideration in both model and more complex mixtures (Hunt and Dalgleish, 1994).

In this paper we define the effect of pH on the stability and composition of emulsions made with different concentrations of WPI. Previous studies have commented on the ability of whey proteins to stabilize emulsions at different pH values (Lee *et al.*, 1992; Shimizu *et al.*, 1981; 1985; Yamauchi *et al.*, 1980) and even examined the composition of adsorbed protein as a function of pH (Shimizu *et al.*, 1981; Yamauchi *et al.*, 1980), but these studies did not relate the stability of the emulsion to interfacial protein composition and did not consider the effect of protein concentration. Furthermore, no studies have been reported on the effect of reducing the pH after homogenization on the stability of the emulsion or the protein composition at the surface of the emulsion droplet. We report on the effect of adjusting the pH, both before and after homogenization, on the stability of the emulsions made with WPI and relate this to the interfacial composition of protein for a range of protein concentrations.

MATERIALS AND METHODS

Soy oil, sodium dodecyl sulfate (SDS), and buffer salts were purchased from Sigma Chemicals Co., St. Louis, MO. 2-Mercaptoethanol was obtained from Fisher Chemical Co., Mississauga, ON. WPI was provided by Protose Separations Inc., Teeswater, ON.

Emulsion Preparation. Oil-in-water emulsions were prepared using a Microfluidizer M110S (Microfluidics Corp., Newton, MA) at an input pressure of 0.3 MPa (corresponding to a homogenization pressure of 42 MPa or 6200 psi), using soy oil (20 wt %) and buffered WPI solution. Protein concentrations ranged from 0.5 to 2.5 wt % in the aqueous phase, and solutions were filtered (0.22 μ m pore) prior to homogenization. For the particular whey protein isolate and filters used, very little protein was removed by this filtration step, showing that the proteins had dispersed completely in the solution. For emulsions made at pH 7 and 6, imidazole/HCl was used as the buffering agent. Sodium citrate/citric acid buffer was used for lower pH values and also for additional experiments at pH 6. Each sample was circulated through the homogenizer for 10 strokes of the pump, collected, and then subjected to a further 10 passes before finally being collected. When necessary, emulsions were acidified by the addition of HCl until the required pH was reached and were stored at 4 °C. To monitor changes in the composition of adsorbed protein in acidified samples with time, emulsions were left stirring at room temperature for up to 48 h, during which time aliquots were removed and analyzed. Sodium azide (0.01%) was used to prevent bacterial growth.

Determination of Particle Sizes and Particle Size Distribution. The size distribution of the emulsion droplets was determined using a Mastersizer X (Malvern Instruments Ltd., Malvern, U.K.), with optical parameters defined by the manufacturer's presentation code 0303. Milli-Q water was used as the dispersant, and the dilution factor was approximately 1 in 1000.

In some cases the average droplet diameter of undiluted emulsions was determined from dynamic light scattering measurements using an Autosizer Hi-C (Malvern Instruments). This instrument comprises a single optic fiber which transmits light of wavelength 780 nm and collects backscattered light (180°) from the sample, which is held in a cell at 25 °C. This represents a recent development in light scattering technology because measurements can be made directly on turbid samples, and therefore any effects from dilution can be eliminated. Diffusion coefficients were calculated by the method of cumulants, and from these the average apparent diameter was obtained by applying the Stokes–Einstein

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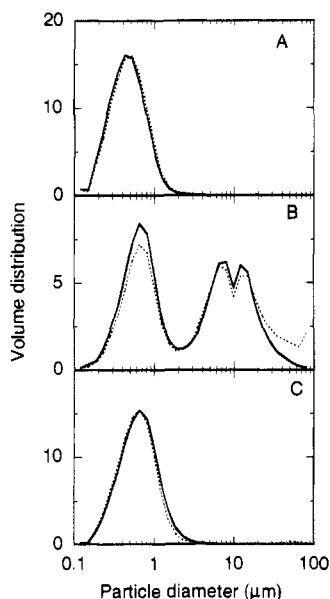


Figure 1. Particle size distribution (vol % against size), measured by the Mastersizer, of emulsions (20 wt % soy oil) made with 1 wt % WPI: A, prepared at pH 7 (imidazole buffer), showing the fresh emulsion (solid line) and a 3-week-old emulsion (broken line); B, prepared at pH 5.5 (citrate buffer), showing the fresh emulsion (solid line) and 2-day-old emulsion (broken line); C, prepared at pH 3 (citrate buffer), showing the fresh emulsion (solid line) and 3-week-old emulsion (broken line).

equation. The details of the theory (van der Meeren *et al.*, 1993; Horne, 1991; Pine *et al.*, 1988) and operation of the instrument (McNeil-Watson, 1991) are described more fully elsewhere. A sample time of 300 μ s and an experimental time of 500 s were used in all cases.

Determination of Surface Composition of Protein.

The composition of protein adsorbed at the surface of the emulsion droplets was determined directly by analyzing the cream phase using electrophoresis and photometric scanning of the stained protein bands as described in a previous paper [see Hunt and Dalgleish (1994) for details].

RESULTS AND DISCUSSION

Emulsions Prepared at Different pH Values.

Figure 1 contrasts the size distribution of fresh and aged emulsions made with 1.0 wt % WPI at various pH values. At pH 7 the size distribution of the fresh emulsion was monomodal and changed very little over a period of 3 weeks (Figure 1A). In contrast, at pH 5.5 the size distribution of the fresh emulsion appeared to be bimodal, and over a period of 2 days large flocs and/or coalesced droplets were produced which exceeded the detection limit of the reverse Fourier optics configuration ($>80 \mu\text{m}$) (Figure 1B). At lower protein concentration (0.5 wt %) the emulsion was so unstable that a clear serum layer was visible in a matter of hours. When emulsion was prepared at pH 3, a monomodal size distribution was obtained for the freshly prepared emulsion. This emulsion was stable for up to 2 weeks, but after 3 weeks small quantities of large flocs and/or coalesced droplets formed as evidenced by the appearance of a second peak in the size distribution (Figure 1C).

The stability of emulsions made at pH 6 depended on the buffer system. Freshly prepared emulsions (1 wt % WPI) made with imidazole were quite stable. The fresh emulsion had a monomodal size distribution which was superimposable on the size distribution of the same emulsion aged over 2 weeks (Figure 2A). In contrast,

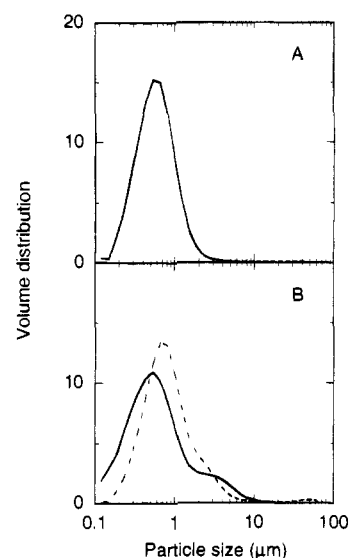


Figure 2. Particle size distribution (vol % against size), measured by the Mastersizer, of emulsions (20 wt % soy oil) made with 1 wt % WPI at pH 6: A, imidazole buffer, showing the fresh emulsion (solid line) and 2-week-old emulsion (broken line); B, made with citrate buffer, showing the fresh emulsion (solid line) and 2-day-old emulsion (broken line).

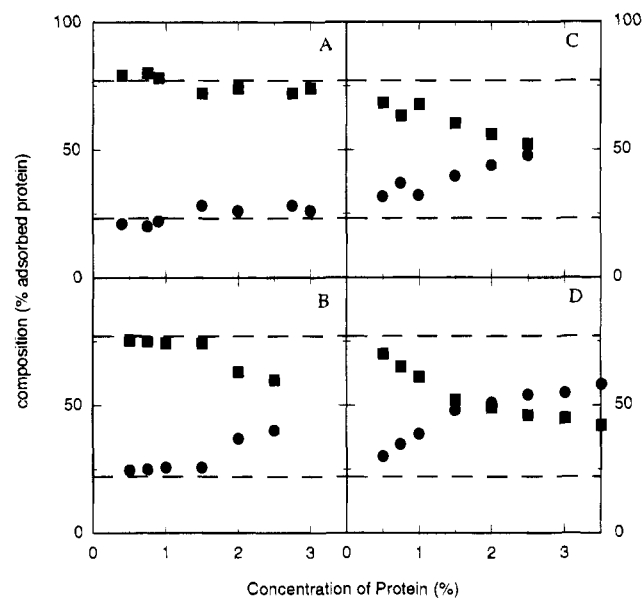


Figure 3. Relative proportions of β -lactoglobulin (■) and α -lactalbumin (●) found in the cream phase of emulsions (20 wt % soy oil) at different concentrations of WPI: A, imidazole buffer at pH 7; B, imidazole buffer at pH 6; C, citrate buffer at pH 6; D, citrate buffer at pH 3. The broken lines refer to the proportion of the two proteins in the whole emulsion.

when the emulsion was prepared with citrate buffer, the size distribution of the fresh emulsion was skewed and deteriorated rapidly (Figure 2B). This was evidenced by large increases in the $d_{4,3}$ after just 2 days. Increasing the protein concentration to 2.0 wt % produced a narrower size distribution, but after 6 days $d_{4,3}$ increased sharply, so even at high concentrations of protein stability was not achieved.

To understand further the effect of pH on emulsion stability and to try to elucidate the effect of the buffer on emulsion stability at pH 6, the composition of protein adsorbed to the surfaces of the emulsion droplets was determined. The results are shown in Figure 3, in which the relative proportion of α -lactalbumin and β -lactoglobulin in the cream phase is plotted against the

protein concentration. At pH 7 the proportion of α -lactalbumin and β -lactoglobulin in the cream phase was the same as their overall composition in the emulsion (Figure 3A); i.e., there was no preferential adsorption of one protein over the other. This was true for the range of protein concentrations studied and agrees with earlier findings (Dickinson *et al.*, 1988; Hunt and Dalgleish, 1994). However, for emulsions made at pH 6 with imidazole buffer there was preferential adsorption of α -lactalbumin over β -lactoglobulin when the protein concentration was greater than 1.5 wt % (Figure 3B). Despite the difference in the composition of the adsorbed protein at higher protein concentrations, the total amount of adsorbed material was the same at pH 6 as at pH 7 [see Hunt and Dalgleish (1994) for the effect of protein concentration on surface concentration at pH 7.0].

When citrate buffer was used at pH 6, as opposed to imidazole, preferential adsorption of α -lactalbumin over β -lactoglobulin was observed for all protein concentrations studied (Figure 3C), and at pH 3, the behavior was even more pronounced (see Figure 3D). Indeed, at protein concentrations of 2 wt % and greater, more α -lactalbumin than β -lactoglobulin was found in the cream phase of emulsions made at pH 3. The surface concentration of protein in emulsions made using citrate buffer was higher than those prepared with imidazole buffer at pH 7 because, for a given concentration, the specific surface area of the emulsion was always lower at pH 3. For emulsions prepared at pH 6 with citrate buffer, values of the surface concentration are unreliable because of the poor stability of the emulsions, making them susceptible to coalescence during centrifugation. It should be emphasized that at no stage in the experiments did we use different ratios of α -lactalbumin to β -lactoglobulin; i.e., the same batch of WPI was used throughout.

It is significant that this behavior depended on the concentration of protein. For emulsions made at pH 6 with imidazole buffer, preferential adsorption was observed only at concentrations greater than 1.5 wt %, and it is above this concentration that protein is no longer the limiting factor in determining the size of emulsion droplets. This is in agreement with our recent study on the adsorption behavior of proteins in emulsions stabilized by mixtures of caseinate and WPI in which competitive adsorption was observed only when there was more than enough protein to cover the nascent interface (Hunt and Dalgleish, 1994). For emulsions made at pH 6 and pH 3 with citrate buffer, protein concentration (0.5–2.5 wt %) had much less effect on the size of emulsion droplets and some degree of preferential adsorption was observed at each concentration. Increasing the protein concentration had the effect of increasing the amount of α -lactalbumin, and since this was adsorbed preferentially at these lower pH values, we observed an increase in adsorbed α -lactalbumin as protein concentration was increased.

Yamauchi *et al.* (1980) and Shimizu *et al.* (1981) conducted similar experiments on emulsions (20 wt % coconut oil) made with whey concentrate. They also found increased adsorption of α -lactalbumin at the expense of β -lactoglobulin at pH 5 and 3 compared to emulsions made at pH 7. Shimizu *et al.* (1981) quantified the behavior by densitometry and found much greater preferential adsorption of α -lactalbumin over β -lactoglobulin than was observed in our experiments, but since Shimizu *et al.* (1981) used 4 wt % protein and

the average diameter of the emulsion droplets was 4.0–4.5 μm (giving a very large excess of protein in solution), their findings may be consistent with those reported here. Furthermore, the whey concentrate sample used in these earlier experiments contained small amounts of casein which are known to affect the adsorption behavior of whey proteins, especially in conditions of excess protein (Hunt and Dalgleish, 1994).

The behavior described above is likely to be partly a consequence of how close the pH of the sample is to the *pI* values of the main proteins, namely β -lactoglobulin (*pI* = 5.2) and α -lactalbumin (*pI* = 4.1–4.8), but other factors must also be considered, especially if we are to explain the effect of buffer on the stability of emulsions made at pH 6. For example, the conformation and quaternary structure of the whey proteins are very sensitive to environmental conditions, and it is likely that conformational and aggregation changes contribute to the observed behavior. At pH 7 β -lactoglobulin exists primarily as a dimer and α -lactalbumin is in the N (native) conformation, but at pH 3 β -lactoglobulin monomers are prevalent and α -lactalbumin has changed to the A (acidic) conformer (Brew and Grobler, 1992; Hambling *et al.*, 1992). The A-conformer of α -lactalbumin is referred to as a molten globule since, although much of the tertiary structure is lost, there are only small changes in the secondary structure. The dissociation of calcium from α -lactalbumin, resulting from the protonation of the coordinating β -carboxyl groups, is thought to induce the change from the N- to the A-conformer as pH is reduced (Brew and Grobler, 1992). Ostensibly, these changes relate to the preferential adsorption of α -lactalbumin at pH 3, especially since the change of α -lactalbumin to the A-conformer exposes more hydrophobic domains of the protein (Timasheff, 1964; Hanssens and van Cauwelaert, 1978) and increases molecular flexibility. The loss of calcium bound to α -lactalbumin may explain the difference in emulsion stability for emulsions made at pH 6 with either imidazole or citrate buffer. The change from the N- to the A-conformer usually occurs at pH values less than 5, but if the citrate buffer chelates the calcium this change may be effected at pH 6; therefore, the greater preferential adsorption of α -lactalbumin in citrate buffer (Figure 3C) may be partly because it is present as the A-conformer. The reason for the instability of the emulsions made at pH 6 with citrate buffer is not clear, but perhaps changes in protein structure, in both solution and the adsorbed layer, render them less effective emulsifiers and stabilizers under these solution conditions. Emulsions made at pH 5.5 were very unstable, and it was not possible to determine the composition of the adsorbed layer of protein. This pH is close to the *pI* of β -lactoglobulin (*pI* = 5.2) and α -lactalbumin (*pI* = 4.1–4.8) and, therefore, solubility is likely to be reduced. In addition, octamers of β -lactoglobulin exist in equilibrium with monomers and dimers (Hambling *et al.*, 1992), and these may limit the availability of β -lactoglobulin molecules for adsorption (in effect, reducing the concentration of protein) and contribute to the poor emulsifying properties of WPI at pH 5.5.

Acidified Emulsions. Figure 4A shows the size distribution of particles in emulsions (2 wt % WPI) prepared at pH 7 and measured immediately after acidification with HCl to the desired pH. Reducing the pH to 5.5 produced a bimodal distribution of particles and rapid aggregation which produced a clear serum

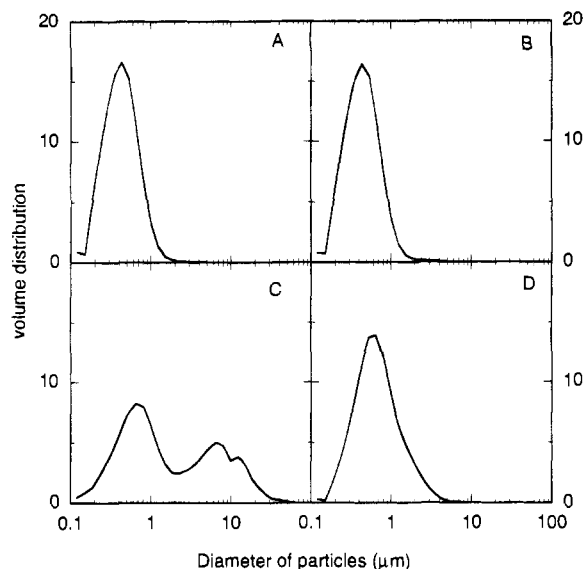


Figure 4. Effect of acidification on the size distribution (vol % against size) of emulsions (20 wt % soy oil; 2.0 wt % WPI) made at pH 7. The distributions were measured immediately after the pH adjustment: A, control emulsion at pH 7; B, pH 6; C, pH 5.5; D, pH 3.

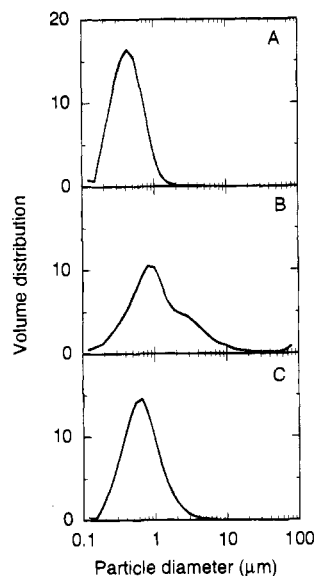


Figure 5. Size distribution (vol % against size) of emulsions (20 wt % soy oil; 2.0 wt % WPI) made at pH 7 and measured 9 days after acidification: A, control emulsion at pH 7; B, pH 6; C, pH 3.

layer after a few hours (Figure 4C). There was no immediate effect on the size distribution of emulsion droplets when the pH was reduced to 6, but reducing the pH to 3 significantly broadened the size distribution (Figure 4B,D, respectively). However, over a period of days the size distribution of the emulsion which had been acidified to pH 6 deteriorated while that for the emulsion at pH 3 approached that of the control emulsion (Figure 5); i.e., the average size of the emulsion droplets at pH 6 increased steadily over a period of up to 13 days, while over the first 2 days the average droplet size decreased for the emulsion at pH 3, after which time an equilibrium was reached. This behavior may be a result of flocs forming as the pH passed through the *pI* and/or a mixing phenomenon leading to transient regions of high acidity.

The effect of acidification on the average size of emulsion droplets in the undiluted emulsions was

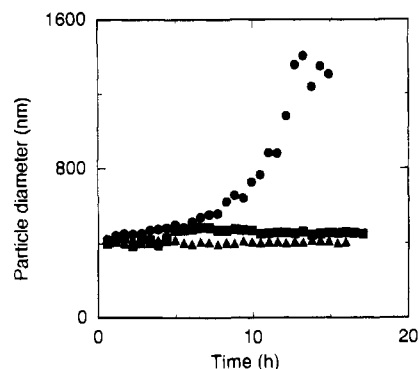


Figure 6. Apparent average droplet diameter of acidified emulsions (20 wt % soy oil; 2.0 wt % WPI) measured by the Hi-C (25 °C) as a function of time: ■, control emulsion at pH 7; ●, emulsion acidified from pH 7 to 6; ▲, emulsion acidified from pH 7 to 3.

monitored also with the Hi-C. Figure 6 shows that there was very little change in the apparent droplet diameter of the control emulsion (pH 7) and the emulsion which had been acidified to pH 3. In contrast, the average diameter of emulsion droplets where the pH had been reduced to pH 6 increased at an increasing rate from the time of acidification, and after 15 h, there was insufficient light backscattered to produce meaningful results (an emulsion made at pH 6 with imidazole buffer was shown to be stable when the droplet size was measured with the Hi-C). It is evident that the destabilization of the acidified emulsion at pH 6 was observed more clearly by measurement of the droplet size in the concentrated emulsion. Presumably, dilution broke up weak flocs during the early stages of destabilization, whereas these were detected by the Hi-C since the emulsion remains at its original concentration.

It is important to note that the effect of pH on emulsion stability is path dependent: when an emulsion is made at pH 6 (imidazole buffer), it is stable, but when the pH is reduced from pH 7 (imidazole) to pH 6, the emulsion becomes unstable. To understand further this behavior the composition of protein adsorbed at the surface of the emulsion droplet after acidification was monitored as a function of time. Figure 7 shows that when the pH of an emulsion made with 2 wt % protein at pH 7 was reduced to pH 6, there was no change in the composition of the adsorbed protein; i.e., β -lactoglobulin and α -lactalbumin were adsorbed in proportion to their concentration in the emulsion. When the emulsion was acidified from pH 7 to 3, the composition of the adsorbed protein changed: more α -lactalbumin adsorbed and β -lactoglobulin was displaced. Since we observed no changes in interfacial composition upon reducing the pH from 7 to 6, it is not possible to explain the instability of the emulsion on the basis of changes in the interfacial composition. We must therefore conclude that the structures of the adsorbed layers must be different for emulsions made at pH 7 and 6 and that when the emulsion is acidified from pH 7 to 6, both the composition and the structure of the adsorbed layer remain unchanged (i.e., in their "pH 7 state") and the emulsion becomes unstable. We have, however, no evidence of what the structures of the adsorbed layers are at the different pH values. This result is consistent with earlier results which showed that the extent and rate of exchange between globular proteins at the oil-water interface are very limited at pH 7 and were related to the rigid nature of the proteins (Dalgleish *et al.*, 1991; Dickinson *et al.*, 1988, 1991). At pH 3,

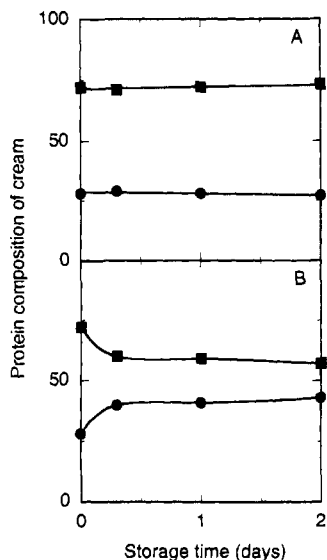


Figure 7. Effect of reducing the pH of emulsions (20 wt % soy oil; 2 wt % WPI) from pH 7 to 6 (A) and 3 (B) on the proportion of β -lactoglobulin (■) and α -lactalbumin (●) in the cream phase as a function of time after acidification.

however, α -lactalbumin exists in the form of a molten globule (the A-conformer) and, therefore, has increased flexibility because of the loss of tertiary structure. This will facilitate the compositional changes in the adsorbed layer when the emulsion is acidified from pH 7 to 3. Furthermore, at pH 3 β -lactoglobulin is dissociated into monomers, and monomers of β -lactoglobulin are more likely to facilitate compositional and structural changes at the interface compared with dimers and octamers. Indeed, this is consistent with a recent study which showed that β -lactoglobulin was displaced more readily from the surface of emulsion droplets by Tween 20 at pH 3 than at pH 7 (Chen and Dickinson, 1993).

Conclusions. WPI can make stable emulsions under acidic conditions (pH 3) as well as at pH 7, but the composition of the adsorbed layer at pH 3 contains much higher levels of α -lactalbumin than at pH 7. The preferential adsorption of α -lactalbumin is likely to be related to changes in the conformation and quaternary structure of the whey proteins as the pH is reduced. Furthermore, buffer had a large influence on the stability of emulsions made at pH 6: with citrate buffer, emulsions were unstable, but those made with imidazole were stable. The reason for this is not clear at this stage but is likely to be related to conformational changes in the proteins and interactions between them.

When the pH of an emulsion (2 wt % WPI) made at pH 7 with imidazole buffer was reduced to pH 6, no change in the interfacial composition of protein was observed and, rather surprisingly, the emulsion became highly unstable, showing that the effect of pH on emulsion stability is path dependent. Furthermore, we found that the composition of adsorbed proteins in emulsion made at pH 7 and 6 (imidazole buffer) was very similar at protein concentrations <2 wt %. Therefore, we must conclude that the structures of the adsorbed layer are different at pH 7 and 6, and we cannot change from one structure to the other when the pH of the emulsion is altered because of the rigid conformation of the proteins. In comparison, when the pH was reduced from pH 7 to 3, more α -lactalbumin became adsorbed. This is likely to be facilitated by conformational and quaternary structure changes, namely the transition of α -lactalbumin to the molten

globule A-conformer and the dissociation of β -lactoglobulin to monomers.

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