Enzymatic Hydrolysis of Milk Proteins Used for Emulsion Formation. 2. Effects of Calcium, pH, and Ethanol on the Stability of the Emulsions

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Oil-in-water emulsions were prepared with native milk proteins (caseinate and β-lactoglobulin) or the same proteins after different extents of breakdown by treatment with trypsin. The effect of trypsin treatment of the emulsions after formation was also investigated. The stability of emulsions was measured by determining the particle sizes with light scattering. Different destabilization conditions were used; namely, decreasing pH, adding calcium ions, or incorporation of ethanol. Comparisons were made between emulsions containing modified and unmodified proteins. Trypsinolysis rendered caseinate emulsions less stable to decreasing pH and increasing concentrations of ethanol, but the stability to calcium increased and correlated with the surface composition of individual types of casein molecules and their calcium sensitivity. Emulsions containing β-lactoglobulin, on the other hand, showed improved stabilities with hydrolysis under all conditions compared with emulsions containing unhydrolyzed protein.

Keywords: *Oil-in-water emulsions; proteins; peptides; adsorption; aggregation; instability*

INTRODUCTION

Formation and stability of oil-in-water emulsions containing milk proteins that have been hydrolyzed by trypsin was described in the first part of this study (Agboola and Dalgleish, 1996c), which demonstrated that emulsions made from hydrolyzed caseinate were generally stable after formation, although they tended to be somewhat less stable than emulsions made with untreated protein. Emulsions made from hydrolyzed β-lactoglobulin (β-lg) were less stable during storage, and the stability appeared to depend on the amount of unhdrolyzed protein present in the emulsion. On the other hand, emulsions prepared with untreated protein that were subsequently treated with trypsin were particularly stable, both for caseinate and β-lg. These stability properties were measured in a simple aqueous buffer at pH 7.0. Of course, in practice, food emulsions need to be stable under a wider range of conditions, such as the presence of destabilizing ions, low pH, or changing solvent quality. It was therefore necessary to study the stability of our emulsions in conditions that would be appropriate to some foods.

In this study, we describe the stability properties of emulsions containing hydrolyzed proteins with respect to calcium addition, reduction in pH from neutral, and addition of ethanol solution. These properties are compared with those of the emulsions made with native proteins, as described in earlier publications (Agboola and Dalgleish, 1996a,b).

MATERIALS AND METHODS

Preparation of protein solutions, protein hydrolysis, formation of 20% soy oil-in-water emulsions, separation of the cream phase from the serum phase, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and particle size distributions have been described in detail in the preceding paper (Agboola and Dalgleish, 1996c). Solutions of protein (caseinate or β-lg) were treated with trypsin for different times before being used to form emulsions. In addition, emulsions were made with unmodified proteins and were then subjected to hydrolysis by trypsin for different times; these are referred to in this paper as surface-hydrolyzed emulsions.

Destabilization of Emulsions. Emulsions formed with hydrolyzed protein solutions or those containing surface-hydrolyzed proteins were treated with calcium or ethanol, or subjected to decreased pH as described earlier for destabilization reactions carried out under shearing conditions (Agboola and Dalgleish, 1996a,b). About 200-μL aliquots of emulsion sample were added to 110 mL of 20 mM imidazole/HCl buffer containing the appropriate concentration of calcium chloride or ethanol, or at the appropriate pH (acetate/acetic acid buffer), in the small volume presentation unit of a Mastersizer X (Malvern Instruments Inc., Southboro, MA). This unit contains a rapidly rotating paddle that drives the solution through the measuring cell and coincidentally provides a shearing action on the suspension, which affects the destabilization reaction between emulsion droplets. It is impossible, however, to calculate the shear rate because of the complex geometry of the instrument. However, all emulsions were measured under the same conditions of dilution and shear. The particle size distributions were measured (without removing the diluted emulsion) at intervals of ~1 min, while stirring continued (stirrer speed of 300 rpm), until the aggregation was too extensive to be measured accurately or for 1 h. Results are presented in terms of the weight-average particle size (d_{43}), as the best reflection of the changes in the masses of the particles, although it must be understood that this assumes that the aggregated emulsion droplets are spherical, which will only be true if coalescence occurs.

RESULTS

Effects of Calcium on Surface-Hydrolyzed Emulsions. The extent of hydrolysis of 0.5 or 1% caseinate on the surface of the oil droplets markedly affected the calcium sensitivity of the emulsions, as shown in Figure 1A. Although the unhydrolyzed emulsions were unstable in the presence of 13 mM Ca^{2+}, hydrolysis for <20 min rendered the emulsions more stable, and no aggregation occurred over the time scale of the experiment. When the unhydrolyzed emulsions were diluted

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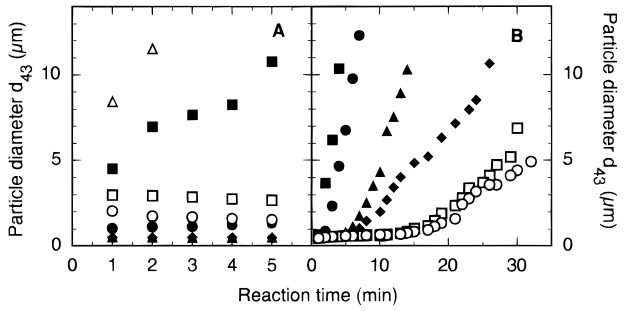

Figure 1. Time courses of destabilization of surface-hydrolyzed emulsions by solutions of 13 mM Ca^{2+}: (A) emulsions containing 1% caseinate, and hydrolyzed for (■) 0 min, (●) 1 min, (▲) 5 min, (◆) 15 min, (○) 25 min, (□) 30 min, and (△) 45 min; (B) emulsions containing 1% β-lg and hydrolyzed for (■) 0 min, (●) 15 min, (▲) 25 min, (◆) 40 min, (○) 60 min, and (□) 90 min.

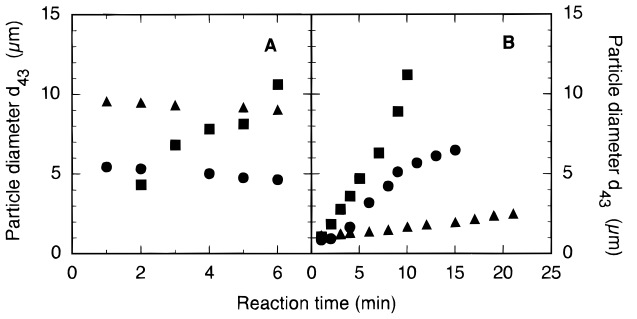

Figure 2. Calcium-induced destabilization of emulsions formed with proteins hydrolyzed in solution: (A) 1% caseinate hydrolyzed for 5 min in concentrations of Ca^{2+} of (●) 13 mM, (▲) 15 mM, and (■) 13 mM (nonhydrolyzed protein); (B) 1% β-lg solutions hydrolyzed for (■) zero time, (●) 4 h, and (▲) 24 h.

into solution containing 13 mM Ca^{2+}, there was an immediate increase in the particle size, followed by growth of the particle size with time. After trypsinolysis of the emulsion for times as little as 1 min, there was much less initial aggregation and no subsequent aggregation could be detected. The most stable particles were formed after ~5 min of treatment with trypsin. Then, stability decreased until, after hydrolysis for 45 min, the trypsinolyzed emulsion containing 1% caseinate was less stable than the unhydrolyzed emulsion. After hydrolysis for 25 and 30 min, the particles showed aggregation when they were diluted in to the buffer containing calcium, but remained stable thereafter. Emulsions containing 0.5% caseinate behaved similarly to those containing 1% caseinate, although instability to Ca^{2+} developed after 60, rather than 45 min. However, this was the initial instability that occurred immediately after dilution, and stirring thereafter produced a slight decrease, rather than increase, of the average particle size.

The time courses of calcium destabilization of hydrolyzed β-lg emulsions are shown in Figure 1B. The same pattern of aggregation as described earlier for emulsions formed with native β-lg (Agboola and Dalgleish, 1996a) was followed by the hydrolyzed emulsions because two distinct phases ("lag" and "growth") appeared in the reaction. Hydrolysis reduced the sensitivity of the emulsions to calcium, and the extent of this reduction appeared to be directly related to the extent of protein hydrolysis; however, at hydrolysis times >1 h, a limiting behavior pattern was reached and there was very little difference in the behavior of the emulsions. As hydrolysis increased, the apparent lag time before aggregation increased and the slopes of the growth phase of the reaction decreased. This result was in contrast to the caseinate system where the longest hydrolysis resulted in increased sensitivity to calcium. In emulsions with β-lg, the stabilization appeared not to be reversed by extended proteolysis.

Effect of Calcium on Emulsions Made with Hydrolyzed Protein. The calcium-induced behavior of emulsions made with hydrolyzed protein solutions is shown in Figure 2. Emulsions formed with hydrolyzed caseinate were instantly destabilized to form aggregates that were comparable in size to the immediately formed calcium-induced aggregates of the emulsions made with nonhydrolyzed emulsions. However, there was no further aggregation; instead, the average size of the destabilized emulsions decreased very slowly with the duration of shearing (Figure 2A). This time course is somewhat similar to that obtained from surface hydrolyzed emulsions (30 min hydrolysis or less) aggregating under the influence of calcium (Figure 1A), although in surface-hydrolyzed emulsions, the initial aggregate size was much smaller. Generally, as shown in Table 1, the average size of the initial aggregates was proportional to the calcium concentration and there seemed to be no significant effect of the extent of hydrolysis. Emulsions made with caseins that had been hydrolyzed for 5 and 30 min behaved very similarly.

Table 1. Initial Particle Size Averages (d_{43}) of Emulsions Formed with Hydrolyzed Caseinate and Treated with Different Concentrations of Calcium

Ca (mM)	0.5% 5 min	0.5% 30 min	1% 5 min	1% 30 min	0.5% native	1% native
13	0.63[a]	0.90[a]	5.43[a]	6.66[a]	0.50[a]	4.76
15	1.03[b]	0.89[a]	9.56[b]	11.66[b]	0.51[a]	NA
18	1.56[c]	2.45[b]	NA	12.34[c]	1.12[b]	NA
20	2.35[d]	5.04[c]	NA	NA	1.52[b]	NA

[a] Two different times (5 and 30 min) of hydrolysis were used, and two concentrations (0.5 and 1%) of caseinate; NA = not available; values with different superscripts within the same column are significantly different ($P < 0.05$); see text for full details.

Emulsions formed with hydrolyzed β-lg solution were not as sensitive to calcium ions as the ones formed from native protein (Figure 2B), and greater extent of hydrolysis of the protein solution produced emulsions that were less sensitive to calcium. However, none of the emulsions was completely stable because time-dependent aggregation continued and no limiting level was reached. In these emulsions prepared from hydrolyzed β-lg, there did not appear to be any dependence of the properties on the concentration of protein. Thus, the same trend of reduced calcium sensitivity with the extent of hydrolysis was observed for surface-hydrolyzed emulsions and emulsions formed after the hydrolysis of the protein.

Effects of pH on Surface-Hydrolyzed Emulsions. Although caseinate-stabilized emulsions were not destabilized at pH values >5.1, the hydrolyzed emulsions were less stable at low pH; that is, emulsions formed containing either 0.5 or 1% caseinate and then hydrolyzed were destabilized at pH 5.3 and below. As shown in Figure 3A, this increased susceptibility to low pH was noticeable after only 1 min of surface hydrolysis, and, after 5 min of surface hydrolysis, immediate destabilization to give particles of average size >10 μm was observed when the emulsion was diluted into buffer at pH 5.1. Thus, pH-induced destabilization is increased with the extent of surface hydrolysis of caseinate. In

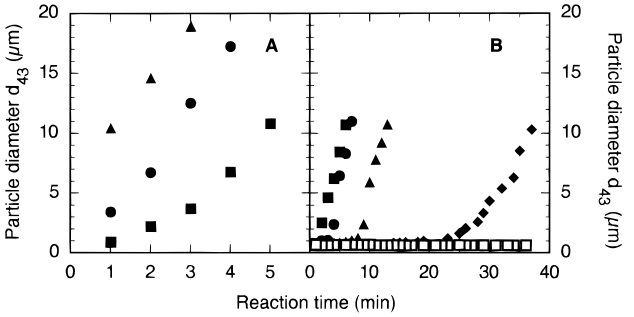

Figure 3. Effect of pH on the stability of emulsions formed and then surface-hydrolyzed: (A) 1% caseinate emulsion hydrolyzed for (■) 0 min, (●) 1 min, and (▲) 5 min; (B) 0.5% β-lg emulsion hydrolyzed for (■) 0 min, (●) 5 min, (▲) 10 min, (◆) 15 min, and (□) 30 min. The results on the emulsion formed with caseinate were obtained at pH 5.1 and those on the β-lg-stabilized emulsion were measured at pH 5.3.

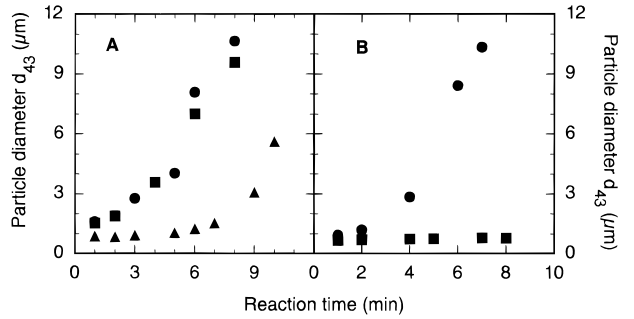

Figure 4. Effect of pH on the stability of emulsions formed with hyrolyzed caseinate: (A) emulsions containing 0.5% protein diluted into buffer at pH 5.1, and (B) emulsions containing 1% protein diluted into buffer at pH 5.3. Hydrolysis times were (▲) 0 min, (■) 5 min, and (●) 30 min. Emulsions formed with 1% nonhydrolyzed caseinate did not destabilize at pH 5.3.

contrast to the increase in stability to calcium-induced association found for intermediate extents of hydrolysis, there were no indications of any positive effect of hydrolysis on the stability of the emulsions to low pH.

However, the stability of surface-hydrolyzed emulsions containing β-lg to low pH was increased with the time of surface hydrolysis, as shown typically for pH 5.3 in Figure 3B. The increase in stability was not significant until after 10 min of hydrolysis, but it was clear that considerable increases in stability could be produced, especially when the hydrolysis was extensive. This seemed to be similar to the trend observed for the effect of Ca^{2+} on surface-hydrolyzed emulsion containing β-lg (Figure 1B). In contrast to calcium-induced association, however, the slope of the latter stage of the aggregation appeared to be relatively independent of the extent of hydrolysis.

Effect of pH on Emulsions Made with Proteins Hydrolyzed in Solution. Emulsions formed with hydrolyzed caseinate solution were also less stable to reduction in pH than emulsions formed with untreated caseinate. For emulsions formed with 0.5% protein and tested at pH 5.1, hydrolysis times of 5 or 30 min gave virtually no difference between the effects; both emulsions destabilized to the same extent compared with the untreated material (Figure 4A). Of the emulsions formed with 1% caseinate, however, the one formed with casein hydrolyzed for 5 min was not destabilized at pH 5.3 (Figure 4B), whereas that formed with casein hydrolyzed for 30 min was destabilized at that pH (compared with destabilization at pH 5.1 only for

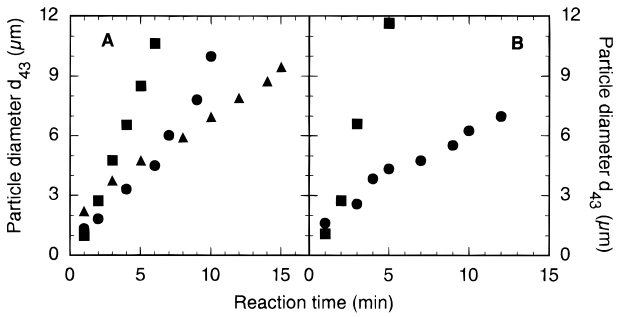

Figure 5. Effect of pH 5.3 on the stability of emulsions formed with solutions of β-lg that were hydrolyzed for different times: (A) 0.5% β-lg hydrolyzed for (■) 0 min, (●) 4 h, and (▲) 24 h, or (B) 1% β-lg solution hydrolyzed for (■) 4 h, and (●) 24 h. Destabilization of emulsions formed with nonhydrolyzed 1% β-lg at pH 5.3 was too rapid to be followed by our techniques.

untreated caseinate). Thus, the stability of the emulsions to pH was reduced with the extent of hydrolysis only in the emulsions formed with 1% caseinate. Experiments at pH 5.2 showed that the emulsion formed with 1% caseinate solution that had been hydrolyzed for only 5 min reacted very extensively, too rapidly to be followed with the Mastersizer, within the first minute of reaction, compared with the emulsion formed with the native caseinate solution that aggregated very much more slowly with time of shearing. We have pointed out in an earlier paper that emulsions formed with 1% caseinate are less stable to reduced pH than those formed with 0.5% protein (Agboola and Dalgleish, 1996b).

In contrast to the caseinate system, emulsions formed with β-lg solution were generally more stable to low pH as shown in Figure 5. Increasing the hydrolysis time beyond 4 h did not improve the stabilization of emulsions formed with 0.5% β-lg. However, the emulsion formed from protein hydrolyzed for a longer time was more stable, if the rate of aggregation was expressed relative to the original particle size. The emulsion containing 0.5% β-lg hydrolyzed for 24 h had a particle size of $>2\ \mu$m, and the increase in size with time is small relative to that size. Relative to its initial size (1.3 μm), the emulsion made from a solution hydrolyzed for 4 h, however, has a faster increase with time, showing that it is more sensitive to pH 5.3. The influence of time of solution hydrolysis was much clearer in 1% protein systems where emulsions formed after 24 h hydrolysis were much more stable than those prepared after 4 h of hydrolysis of the protein. Overall, it was clear that hydrolysis improved the stability of emulsions formed with β-lg solution to pH because the reactions could be followed at pH 5.3; a pH value that resulted in large visible aggregates ($d_{43} = 20.57 \pm 2.1$ mm) in emulsions made with unmodified β-lg. It should, be emphasized, however, that only improvements in the stability were seen and that complete stabilization at low pH values was not achieved.

Effects of Ethanol on Surface-Hydrolyzed Emulsions. Hydrolysis of caseinate emulsions reduced their stability to ethanol (Figure 6A). Emulsions hydrolyzed for only 5 min showed extensive aggregation in 35% ethanol, where unmodified emulsions remained stable. Even 30% ethanol caused significant aggregation of fully hydrolyzed (>45 min) 0.5 or 1% caseinate-stabilized emulsions. The pattern of aggregation was similar to the timecourses obtained from nonhydrolyzed emulsions, with an initial high rate of aggregation followed by a leveling off of the particle size. The only difference

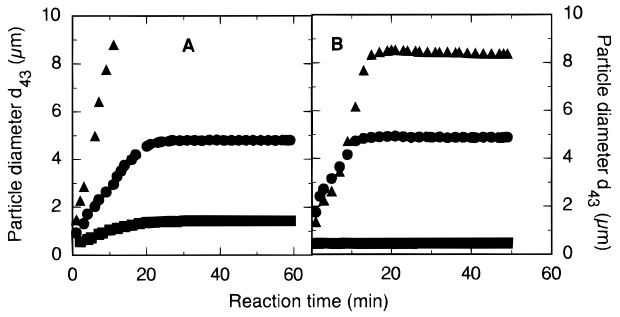

Figure 6. (A) Destabilization by 35% ethanol of surface-hydrolyzed emulsions containing 1% caseinate, with hydrolysis times of (■) 0 min, (●) 5 min and (▲) 30 min. (B) Destabilization by 45% ethanol of emulsions prepared from 1% caseinate solution that had been hydrolyzed for (■) 0 min, (●) 5 min, (▲) 30 min.

was the increased level of aggregation because average sizes as large as 9 μm were recorded at the plateau level.

Surface hydrolysis of emulsions containing β-lg improved their stability to ethanol. When the emulsions were introduced into ethanol, an immediate increase in size was found, but no further change occurred during the rest of the experiment. This result was similar to the behavior of the aggregates formed from ethanol-induced associations of the native β-lg-stabilized emulsions (Agboola and Dalgleish, 1996b). The effects of hydrolysis time and ethanol concentration on the stability of the emulsions are shown in Table 2. The effect of hydrolysis was to progressively increase the stability of the surface-hydrolyzed emulsions to ethanol, as gauged by the values of the particle sizes. The emulsions hydrolyzed for longer periods of time were stable at higher ethanol concentrations. Indeed, after 1.5 h of hydrolysis, ethanol had little effect on the stability of the emulsions.

Effects of Ethanol on Emulsions Formed from Proteins Hydrolyzed in Solution. Hydrolysis of caseinate before making the emulsions also reduced the stability progressively with the time of hydrolysis (Figure 6B). The same trend of high initial rate of aggregation followed by a leveling out of the particle size was observed for both native emulsions and emulsions formed with 5-min-hydrolyzed solution. In the emulsion formed with 30-min-hydrolyzed solution and treated with 45% ethanol, the particle size had not leveled off before the reaction was stopped because of the formation of significant amounts of very large particles (>80 μm). In 40% ethanol solution, however, the slow phase was reached at an average size of ~5 μm (not shown). There were no significant differences ($p \geq 0.05$) between the ethanol-induced associations of emulsions formed with either 0.5 or 1% hydrolyzed caseinate solutions (with respect to the slope of the initial phase and the final average size). It was clear from these results that although emulsions formed with hydrolyzed caseinate solutions were more susceptible to aggregation by ethanol compared with those formed with nonhydrolyzed caseinate, they were not as sensitive as surface-hydrolyzed emulsions. From Figure 5 it can be seen that 35% ethanol was able to destabilize surface-hydrolyzed emulsions in a way that was comparable to the effect of 45% ethanol on emulsions formed with hydrolyzed caseinate solution.

Compared with the ethanol-induced aggregation of caseinate-stabilized emulsions, emulsions formed with hydrolyzed β-lg solutions were stable in the presence of 45% ethanol despite the fact that native β-lg-stabilized emulsions can be destabilized at ethanol concentrations as low as 30%, when they have been stored for some hours after the emulsions are formed (Agboola and Dalgleish, 1996b). When compared with the surface-hydrolyzed β-lg systems, emulsions formed from solution were less sensitive to ethanol even when the hydrolysis was just for 4 h. In contrast to the emulsions formed with native β-lg, where aging of the emulsions decreased the ethanol stability, no such effect was found for emulsions formed from hydrolyzed β-lg solution or for surface-hydrolyzed emulsions.

DISCUSSION

Calcium sensitivities of surface-hydrolyzed caseinate-stabilized emulsions were affected by the protein composition at different extents of hydrolysis. From our knowledge of the breakdown of the individual caseins (Agboola and Dalgleish, 1996c), we know that the calcium-sensitive proteins (α_s- and β-caseins) are broken down at an earlier stage of the hydrolysis with trypsin than is the calcium-stable κ-casein. This trend continued during hydrolysis for ~30 min, and is paralleled by the increased stability of the emulsions to calcium ions. It seems reasonable to assume that this increased stability results from the protective role of intact κ-casein, somewhat in the manner of its stabilizing action in casein micelles (Swaisgood, 1992). As the extent of hydrolysis increased and the κ-casein was destroyed in its turn, the emulsions became less stable. Destabilization by Ca^{2+} continued even after most of the κ-casein was lost so it is probable that some of the peptides that remained adsorbed to the interface could bind Ca^{2+} and so encourage particle associations, especially when the κ-casein fraction has been fully hydrolyzed (i.e., after 45 min of hydrolysis). Of course, the extensive hydrolysis also removes the possibility of steric stabilization of the emulsions, which will also contribute to rapid aggregation.

The initial sizes of the calcium-induced aggregates of emulsions formed with hydrolyzed caseinate solutions were larger than those in the surface-hydrolyzed systems, probably because hydrolysis of κ-casein in solution

Table 2. Average Particle Sizes (d_{43}) Obtained for Ethanol (EtOH)-Destabilized Native or Surface-Hydrolyzed Emulsions Made with 1% β-lg[a]

	d_{43} (μm)					
EtOH (% v/v)	no hydrolysis	10 min of hydrolysis	15 min of hydrolysis	30 min of hydrolysis	1 h of hydrolysis	1.5 h of hydrolysis
25[a]	0.46	0.45	0.45	0.44	0.46	0.45
30[a]	0.72	0.68	0.65	0.61	0.64	0.59
35	3.04	2.06	0.85	0.67	0.66	0.62
40	8.89	4.48	2.31	0.91	0.70	0.68

[a] Hydrolysis with trypsin was carried out for different times, and the values reported are the steady values measured immediately on dilution of the emulsion into ethanol solution; the nonhydrolyzed emulsion was allowed to age for 24 h before the measurement was made.

occurs more in parallel with the other casein molecules (Agboola and Dalgleish, 1996c); thus, the protective function of the protein may be reduced. After the initial increase in size on introducing Ca^{2+}, there was no further association during shearing of emulsions formed with hydrolyzed caseinate. Different and possibly shorter peptides will now serve as surfactants in the different emulsion systems in place of the longer flexible casein molecules. According to Van Hekken and Strange (1993), enzymatic hydrolysis of caseins results in peptides that are not predisposed to associate; this tendency could also be carried forward to the emulsion droplets to which the peptides are adsorbed, giving a rather weak tendency towards aggregation. The limiting particle size may arise from mechanical breakdown of aggregated emulsion droplets under shear (Dickinson and Williams, 1994), so the limiting size may reflect the strengths of interparticle bonds. Thus, such bonds in emulsions made from hydrolyzed casein are weak; calcium-induced aggregates of emulsions formed with unmodified caseinate do not fall apart under similar shearing conditions. These results suggest that hydrolysis in caseinate emulsion systems leads to the loss of calcium-sensitive sites so that fewer sites are available for association, compared with emulsions formed with nonhydrolyzed caseinate. Possible losses are peptides containing the serine phosphate (Leaver and Dalgleish, 1990) or the carboxylic acid groups. It is possible that peptides in solution could cause destabilization, but we consider the possibility to be small because the emulsion droplets can be collected by centrifugation and resuspended in new buffer with very little change in their properties. This result is in agreement with earlier studies that showed reduction in the calcium sensitivity of enzyme-hydrolyzed caseinopeptides in solution (Bingham *et al*., 1972; Aoki *et al*., 1987). We did not test the reversibility of these calcium-induced reactions, although studies using intact proteins suggested that even the chelation of the Ca^{2+} with complexing agents did not cause the aggregates to dissociate (Agboola and Dalgleish, 1995).

The behavior of emulsions containing hydrolyzed β-lg was similar, whether or not the protein was hydrolyzed on the surface or in solution. This similarity may be because there was little difference in the products of hydrolysis despite the difference in the overall rate of hydrolysis, as shown in part 1 of this study, (Agboola and Dalgleish, 1966c) and as reported by Persaud (1995). The reduction in calcium-sensitivity could be related to the loss of some carboxylic acid residues from the protein at the interface into the serum phase during surface hydrolysis. It is also possible that the peptides containing these calcium-sensitive residues were not very surface active. The presence of these peptides in the serum phase was not likely to contribute to the destabilization of the emulsions droplets, as confirmed in separate experiments, in which no significant difference was seen between the particle size averages of the Ca^{2+}-induced aggregates of emulsions containing the complete hydrolyzed proteins on the one hand and of the same emulsions from which the nonadsorbed peptides had been removed by centrifugation.

The influence of hydrolysis on the pH-sensitivity of the hydrolyzed emulsions agreed with research findings that the isoelectric point (p*I*) of caseinate hydrolysates is increased toward the basic range, whereas that of β-lg hydrolysates is lowered toward the acidic range (Chobert *et al*., 1988; Turgeon *et al*., 1992; Van Hekken and Strange, 1993). This isoelectric shift is an indication of the properties of the peptides formed from the hydrolyses. The results may have relevance to the utilization of protein hydrolyzates in food products or improvements in existing products, because low pH is important in many foods. The results support the earlier results on the importance of isoelectric pH in the destabilization of emulsions formed from intact proteins (Agboola and Dalgleish, 1996b).

The observation that the same pattern of ethanol-induced aggregation was obtained in the surface-hydrolyzed caseinate emulsions as well as emulsions formed from hydrolyzed caseinate solutions indicates a similar mechanism of association, which has been suggested to be Ostwald ripening (Agboola and Dalgleish, 1996b). Reduction in ethanol stability of hydrolyzed emulsions could be explained by the decreased amount of material in the interfacial layer, which may reduce the surface viscoelasticity and increase the possibility of diffusion of oil molecules through the adsorbed layer. The extent of hydrolysis was important because it directly reduced the ability of the interfacial layers to retard Ostwald ripening. It is not clear why surface-hydrolyzed emulsions and emulsions made from hydrolyzed casein show different sensitivities to ethanol. Differential adsorption of peptides is a possibility, which may lead to different surface coverage or to different surface structures. For example, interactions on the interface between peptides obtained from α_s-caseins at the interface have been reported by Lee *et al*. (1987).

The loss of some secondary structures in β-lg on hydrolysis presumably reduced the sensitivity of the hydrolyzed emulsions to ethanol. This improved stability may be a result of an improvement in the steric stabilization due to increased flexibility of the interfacial proteins. It is also probable that hydrolysis caused changes in the electrostatic properties of β-lg (e.g., exposure of charged residues), which conferred stability to the emulsions by an alteration in the distribution of charge away from the interface.

CONCLUSIONS

The relative sensitivity of the individual casein molecules to Ca^{2+} had profound effects on the extent and mechanism of calcium-induced destabilization of emulsions containing hydrolyzed caseinate. This sensitivity agreed with changes in the composition of the casein molecules with time of hydrolysis. In contrast, emulsions containing hydrolyzed β-lg behaved similarly to those containing nonhydrolyzed β-lg, although with less Ca^{2+} sensitivity. Under the influence of pH and ethanol, the stability of emulsions containing hydrolyzed caseinate was reduced whereas the stability of emulsions containing hydrolyzed β-lg was improved relative to that of emulsions made with nonhydrolyzed proteins. These results may have important uses in the development or improvement of dairy-based food commodities.

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