Stability of Emulsions Formed Using Whey Protein Hydrolysate: Effects of Lecithin Addition and Retorting

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Oil-in-water emulsion (4 wt % soy oil) containing 4 wt % whey protein hydrolysate (WPH) (27% degree of hydrolysis) was prepared in a two-stage homogenizer. Other emulsions containing 4 wt % WPH but including two levels (0.1, 0.25 wt %) of either unmodified commercial soy lecithin or hydroxylated lecithin were similarly prepared. The effect of retorting at 121 °C for 16 min on creaming stability, droplet size distribution, microstructure, surface concentration, and surface composition was then studied. The effect of retorting was an immediate destabilization of the lecithin-free emulsions. Addition of unmodified lecithin slightly improved the stability of the retorted emulsions, but did not prevent creaming and coalescence. However, addition of hydroxylated lecithin markedly improved the creaming stability after retorting and retarded coalescence. It appears that the most important factor affecting creaming stability was the particle diameter, in accordance with Stoke's law. Incorporation of unmodified lecithin encouraged coalescence and increased average droplet diameter, but the mechanism by which creaming stability is preserved in systems containing hydroxylated lecithin is unclear.

Keywords: *Lecithin; phospholipid; hydrolysate; creaming; surface composition*

INTRODUCTION

Agboola et al. (1998) reported the destabilization of oil-in-water emulsions containing highly hydrolyzed whey protein hydrolysate (WPH) as the sole emulsifier/stabilizer. The best conditions for producing emulsions that were fairly stable to creaming and coalescence were low homogenization pressure and high WPH concentration. Under these conditions, emulsions could be held at 20 °C for >1 week without destabilization. This stability was attributed to an increasing proportion of higher molecular mass peptides (>5000 Da) in the emulsion with the higher concentration of added WPH, as well as higher surface peptide load of droplets obtained using low-pressure homogenization. Because WPH-containing emulsions are usually sterilized to obtain a long shelf life, the retort stability is an important requirement.

Lecithins are important ingredients in the commercial manufacture of emulsions. Commercial lecithins are mixtures of several phospholipids and fats. According to Prosise (1985), the major phospholipids in crude soybean are phosphatidylcholine (23%), phosphatidylethanolamine (20%), and phosphatidylinositol (14%). Many studies have been carried out on lecithins reporting their surface active properties (Rhydag and Wilton, 1981; Davis and Hasrani, 1985; Weete et al., 1994; Van der Meeren et al., 1995), their competition with proteins at oil/water interfaces (Courthaudon et al., 1991; Dickinson and Iveson, 1993; Fang and Dalgleish, 1993, 1996), and their interactions with proteins (Nakamura et al., 1988; Fang and Dalgleish, 1995).

Commercial lecithins increased the heat stability of recombined milk systems especially in the neutral pH region and lecithin composition had a very significant effect on heat stability (Singh et al., 1992; McCrae and Muir, 1992). Caseinate-stabilized emulsions containing crude soy lecithin were more heat stable than those containing refined lecithin, and lecithin concentration affected the heat stability of emulsions (Cruijsen, 1996). These previous studies have not considered surface coverage and compositions of the lecithins, and the mechanism by which lecithins improve the heat stability of emulsion is still not very clear.

Hydroxylated lecithin, a commercially available modified lecithin, is produced by the introduction of hydroxyl groups to the fatty acid double bonds of concentrated and purified soya bean lecithin (Schmidt and Orthoefer, 1985). This reaction leads to increased water dispersibility and enhanced oil-in-water emulsifying properties. The objectives of this study were 2-fold: first, to study the effect of unmodified lecithin and hydroxylated lecithin on the stability of unheated and retorted emulsions containing highly hydrolyzed whey protein; and second to investigate the mechanism of retort (in)stability in the presence of these lecithins by determining the peptide and phospholipid composition of the interface.

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MATERIALS AND METHODS

Materials. Whey protein hydrolysate (WPH), 27% degree of hydrolysis (ALATAL 821), was supplied by the New Zealand Dairy Board, Wellington, New Zealand. The dried product contained 90.5% protein nitrogen, 4.5% moisture, 2.8% ash, 0.1% fat, and 0.2% lactose. Soy oil was purchased from Davis Trading Co., Palmerston North, New Zealand. Soy lecithin (CENTROLEX F) and hydroxylated soy lecithin (PRECEPT 8120) were supplied by Central Soya Co. Inc., IN. For simplicity, these preparations are subsequently referred to as lecithin and hydroxylated lecithin, respectively. The phospholipid content of both lecithins was 95% (as acetone insolubles). All other reagents were of analytical grade and were supplied by BDH Chemicals Ltd., Poole, England.

Preparation of Emulsions. Emulsions were formed by first preparing a 960 g solution containing 40 g of WPH in MilliQ-water at room temperature (20 ± 2 °C). The pH of these solutions was in the range 6.8−7.0. Lecithin or hydroxylated lecithin (1 or 2.5 g) was dispersed in 40 g of oil at 60 °C before mixing with the WPH solution. This mixture was then passed through a two-stage Rannie homogenizer (Roholmsvej 8 DK2620, Albertslund, Denmark) at no input pressure and then homogenized at a first-stage pressure of 20.6 MPa, and a second-stage pressure of 3.4 MPa. The emulsions were homogenized twice for more effective mixing of the oil phase. At least three emulsions were prepared for each treatment.

Retorting. Emulsions were double-seamed under vacuum in 120 mL cans. The cans were then sterilized at 121 °C in a retort for 16 min, and cooled with cold water immediately to room temperature. Temperatures in the retort and can center were continuously monitored with thermocouples.

Measurement of Particle Sizes. The droplet size distribution was determined by light scattering using a Mastersizer E (Malvern Instruments Ltd., Worcs., England). The presentation factor was 2NAD (i.e., refractive index and absorption of emulsion particles of 1.456 and 0, respectively), and a polydisperse model was chosen for the size distribution. Emulsion droplets were sized using distilled water as the dispersant. All measurements were carried out at 20 °C.

Determination of Creaming Stability. Creaming stability was determined as described by Agboola et al. (1998). About 15 mL of freshly prepared emulsion was poured into specially constructed "stability tubes" (300 mm long, 8 mm internal diameter). The tubes were graduated with 0.1 mL divisions. The separated cream layer was read after 24 h storage at 20 °C.

Measurement of Surface Peptide Concentration. The emulsion was centrifuged at 20000*g* for 30 min in a Sorvall RC5C temperature-controlled centrifuge (Dupont Co., Wilmington, DE). The cream was dried on a Whatman No. 1 filter paper and analyzed for total nitrogen (in milligrams) using the Kjeldahl method with a Tecator Kjeltec System (Tecator AB, Hoganas, Sweden). A mercury catalyst gave much more reproducible results than a selenium catalyst. A factor of 6.38 was used to convert milligrams of nitrogen to miligrams of peptides. From the Mastersizer data, specific surface area (SSA) in meter squared per gram of oil was obtained for each emulsion and was used to calculate the surface concentration (Γ) as follows:

$$\Gamma = \frac{\text{mg of peptides/g of oil}}{\text{SSA (m}^2\text{/g of oil)}} \quad (1)$$

Measurement of Surface Phospholipid Concentration. Total lipids were extracted from the dried cream using the Rose Gottlieb procedure, with 0.15 g of NaCl/g of sample, to achieve a better recovery of phospholipids (Walstra and de Graaf, 1962). To the extracted lipid were added 16 mL of 20% sulfuric acid, 4 mL of perchloric acid, and 2 mL of nitric acid. The whole mixture was then digested using the Buchi automatic digester (Buchi Laboratory Technik, Ag. Switzerland). Total phosphorus oxidized to phosphate in the digest was measured at 820 nm using the colorimetric phosphomolybdate reaction (Watanabe and Olsen, 1965). The phosphorus was converted to total phospholids by using a factor of 31.7 as recommended for soy lecithin (List et al., 1977). Surface phospholipid concentration was calculated from SSA as in eq 1.

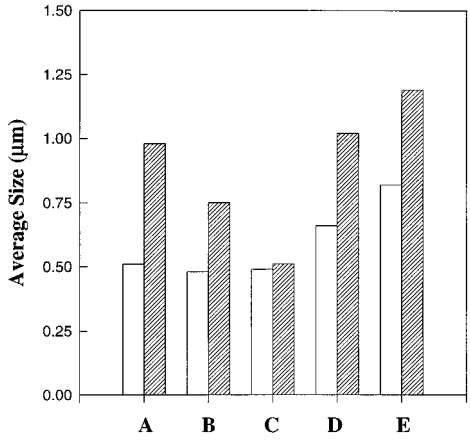

Figure 1. Average particle sizes (d_{32}) of unheated (white bars) and heated (hatched bars) WPH-stabilized emulsions containing (A) no lecithin; (B) 0.1% hydroxylated lecithin; (C) 0.25% hydroxylated lecithin; (D) 0.1% lecithin; (E) 0.25% lecithin.

In each case, at least three separate emulsions were analyzed, and typical variations in surface peptide and phospholipid concentrations were between 3 and 6%. The results shown are the means of three sets of emulsions.

Microscopic Examination of Emulsions. Nile blue (fluorescent dye) at 0.1 wt % was added to the samples which were then mounted on a Leica TCS 4D confocal scanning laser microscope (Leica Laser Technik, GmbH, Heidelberg, Germany). The laser source was Ar/Kr, and the excitation wavelength was 488 nm. Samples were viewed under oil immersion using ×100 objective (numerical aperture = 1.4).

RESULTS

Effect of Phospholipids in Unheated Emulsions. The volume-surface average droplet diameters (d_{32}) of the emulsions containing hydroxylated lecithin were not significantly different ($P > 0.05$) from those of the emulsions without lecithin (Figure 1), and the size distributions were also similar (Figure 2A). Emulsions containing lecithin, on the other hand, showed a bimodal distribution with many droplets between 1 and 10 μm (Figure 2C) and a significant increase ($P \leq 0.05$) in d_{32} (Figure 1). Furthermore, emulsions containing 0.25% lecithin had a higher d_{32} and higher frequencies of droplets between 1 and 10 μm (second major peak). These results were in good agreement with those obtained using confocal laser microscopy (Figure 3A,C,E).

Emulsions formed with or without hydroxylated lecithin did not cream after 24 h storage at 20 °C (Table 1). However, in emulsions containing lecithin, creaming occurred within 24 h.

Effect of Retorting. The heating profile (Figure 4) indicates very good heat transfer between the retort and the can centers. After retorting the control (no lecithin), the d_{32} doubled (Figure 1A), and the size distribution of the emulsion became bimodal (Figure 2B). A new peak was observed with a size range of 6−30 μm. Such a distinct population of larger droplets was also apparent from the confocal laser micrographs (Figure 3B). These large droplets appeared to have developed from coalescence of the smaller ones.

Both light scattering and microstructure analysis indicated that emulsions containing 0.25% hydroxylated lecithin were unchanged after retorting (Figures 2B and

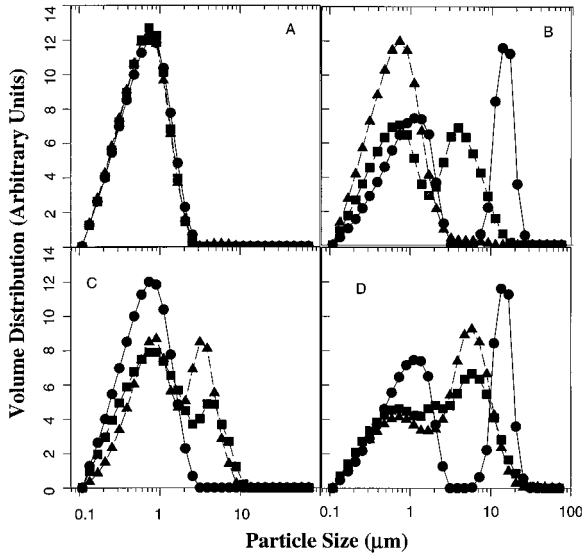

Figure 2. Particle size distributions of unheated (A, C) and heated (B, D) WPH-stabilized emulsions containing either hydroxylated lecithin (A, B) or lecithin (C,D) at various levels (●, no lecithin; ■, 0.1%; ▲, 0.25%).

Table 1. Creaming Stability (24 h) of Retorted and Unheated Emulsion Samples

	(mL cream layer)	
sample	unheated	retorted
no lecithin	0	0.7
0.1% Lec·OH[a]	0	0.1
0.25% Lec·OH	0	0
0.1% lecithin	0.1	0.5
0.25% lecithin	0.2	0.4

[a] Lec·OH, hydroxylated lecithin.

3C,D). However, when emulsions containing 0.1% hydroxylated lecithin were retorted, the d_{32} increased significantly ($P \leq 0.05$), and there were many droplets between 2 and 10 μm (Figure 2B). The micrographs also indicated many fairly large droplets (not shown).

Effects of heating on the d_{32} and size distribution of emulsions containing lecithin were similar at both levels of addition. The d_{32} increased, and the size distribution shifted toward higher particle size ranges. Emulsions containing 0.1% lecithin formed smaller droplets than those at 0.25% lecithin both before and after retorting (Figures 1D,E and 2D). After retorting, the average change in diameter of emulsions containing lecithin was smaller than the change observed in the control (no lecithin).

Retorted lecithin-free emulsions (Table 1) showed extensive creaming after 24 h storage at 20 °C. Emulsions containing hydroxylated lecithin were the most stable after retorting, and the stability increased with the level of added lecithin. Emulsions containing 0.25% hydroxylated lecithin did not cream even after 48 h storage at 20 °C. Emulsions containing lecithin creamed, although less than the control, and there was no significant difference ($P > 0.05$) in the extent of creaming with the two different lecithin levels.

Surface Composition and Concentration. For unheated emulsions, the control emulsion had the highest surface peptide, while the emulsions containing hydroxylated lecithin did not contain any surface phospholipid (Table 2). Addition of lecithin or hydroxylated lecithin resulted in a decrease in surface peptide concentrations, the decrease being greater in the case of lecithin. In emulsions containing lecithin, the amount of phospholipid at the interface increased with the level of added lecithin. The total surface load also increased as a result.

After retorting the lecithin-free emulsion, there was a dramatic reduction in the milligrams of peptides per grams of fat (Table 3), but the surface load actually increased because of the larger droplets and reduced surface area. In emulsions containing lecithin, retorting increased the adsorption of phospholipids which led to a reduction in the milligrams of peptides per grams of fat. However, the decreased surface area on retorting resulted in an increase in peptide surface loads, and the total surface loads roughly doubled in emulsions containing 0.1% lecithin and more than doubled in emulsions containing 0.25% lecithin. The phospholipid surface loads were higher than the peptide surface loads. Retorting emulsions containing hydroxylated lecithin caused some adsorption of phospholipid, but the amounts were lower than in lecithin-containing emulsions. The adsorbed phospholipids led to increased total surface loads.

DISCUSSION

The results obtained from both laser light scattering and confocal microscopy showed that retorting at 121 °C for 16 min of WPH-stabilized emulsions containing no lecithin resulted in destabilization of emulsions which appears to occur mainly via a coalescence mechanism. The adsorbed peptide layers in these emulsions lack the cohesiveness of the parent proteins and have poor ability to provide steric or charge stabilization (Agboola et al., 1998). Consequently, retorting of these emulsions causes droplet aggregation, leading to coalescence. It appears that desorption of some loosely adsorbed peptides occurs during heating, as indicated by the decrease in the amounts of peptides associated with the oil surface (Table 3) after retorting, which is likely to enhance droplet aggregation and coalescence. Retorted WPH-stabilized emulsions appeared to contain two distinct sets of particles in the bimodal size distributions (Figures 2 and 3). This showed that larger droplets aggregated faster than the smaller ones, in accordance with the orthokinetic aggregation mechanisms (Darling, 1987; Agboola and Dalgleish, 1996a,b).

The effect of lecithin on the size distribution of WPH-stabilized emulsions indicates a weakening of the interface, since coalescence occurred even without heating. This could be brought about by the adsorption of lecithin in preference to WPH peptides at the interface (Table 2), because of competition between the two. Alternatively, nonadsorbed lecithin may complex with those peptides with better surfactant properties and hence reduce their adsorption during emulsification. It has been shown that lecithin, compared to proteins, can promote coalescence via a reduction in both electrostatic and steric repulsion potentials (Rhydag and Wilton, 1981; Van der Meeren et al., 1995; Cruijsen, 1996). Furthermore, the decrease in the interfacial tension (Van der Meeren et al., 1995) on adsorption of lecithin could also have contributed to the destabilization of the emulsions. This is essentially because low interfacial tension leads to low internal (Laplace) pressures within the droplets, making them unable to withstand the external pressures (e.g., van der Waals, buoyancy, and shear). This leads to an increased probabilty of droplet rupture.

There was apparently no adsorption of hydroxylated lecithin at the oil surface in unheated emulsions. This

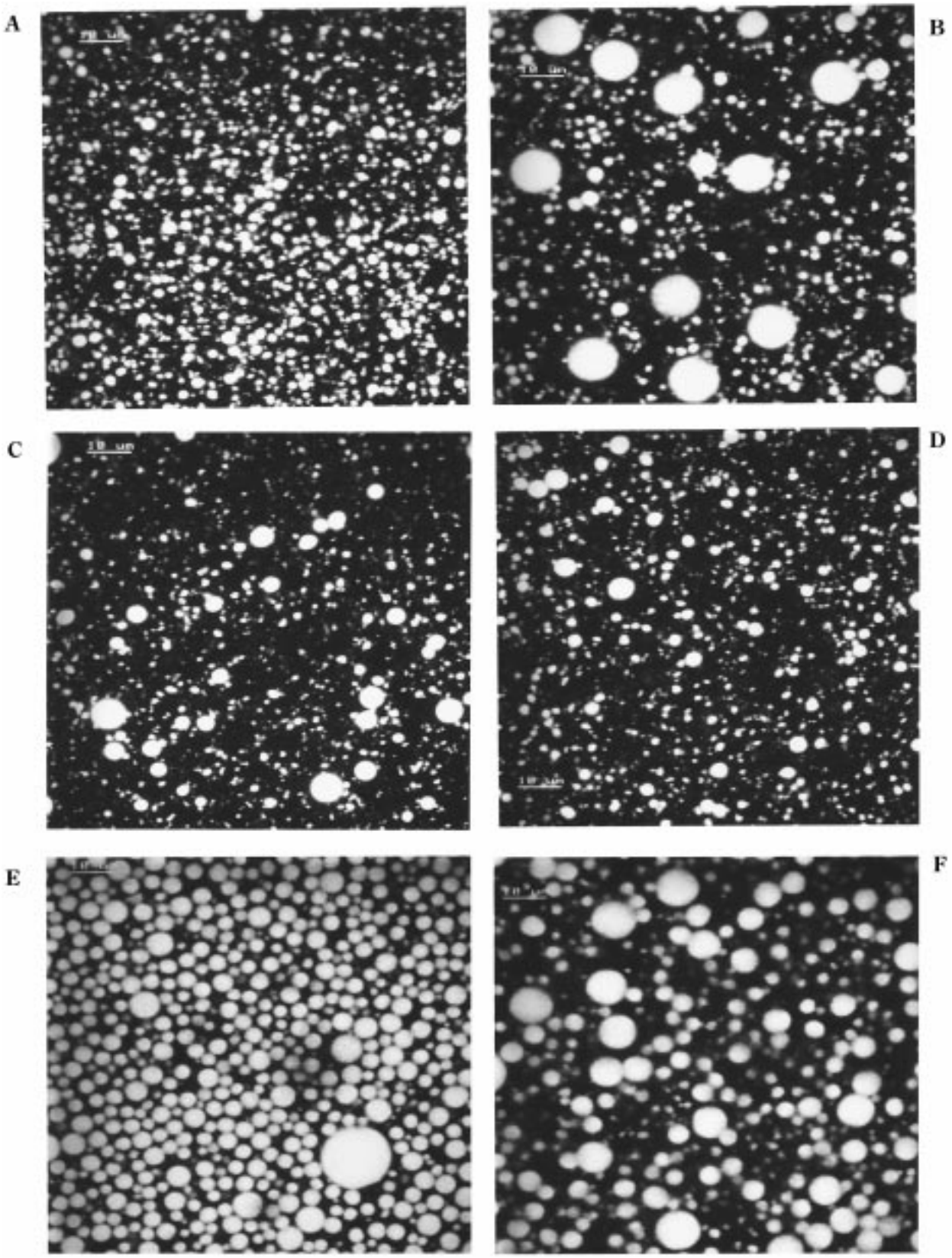

Figure 3. Typical confocal micrographs of unheated (A, C, E) and heated (B, D, F) WPH-stabilized emulsions containing no lecithin (A, B); 0.25% hydroxylated lecithin (C, D); 0.25% lecithin (E, F).

is possibly related to a reduction in the hydrophobic properties of the surfactant by the addition of the hydrophilic hydroxyl groups. Hydroxylation of lecithin to give the desired modification in functionality is usually achieved by 10−25% reduction in the number of fatty acid double bonds (Schmidt and Orthoefer, 1985). A large proportion of unmodified fatty acid chains should still be available for interaction with the oil phase. However, it is possible that steric hindrance by the hydroxylated fatty acid chains perhaps enhanced by hydration of these chains may reduce adsorption via nonhydroxylated fatty acid chains. The decrease in

Table 2. Surface Compositions of Unheated Emulsions

emulsion sample	mg of peptides/g of fat	mg of peptides/m^2	mg of phosolipid/g fat	mg phosolipid/m^2	total surface load (mg/m^2)
no lecithin	20.40	2.02	0	0	2.02
0.1% Lec·OH[a]	15.84	1.62	0	0	1.62
0.25% Lec·OH	12.94	1.32	0	0	1.32
0.1% lecithin	12.59	1.54	4.8	0.50	2.04
0.25% lecithin	8.29	1.37	8.83	1.24	2.61

[a] Lec·OH, hydroxylated lecithin.

Table 3. Surface Compositions of Retorted Emulsions

emulsion sample	mg of peptides/g of fat	mg of peptides/m^2	mg of phosolipid/g of fat	mg of phosolipid/m^2	total surface load (mg/m^2)
no lecithin	12.2	2.38	–	–	2.38
0.1% Lec·OH	12.92	1.90	4.95	0.62	2.52
0.25% Lec·OH	10.74	1.19	11.8	1.04	2.23
0.1% lecithin	9.56	1.92	12.10	2.07	3.99
0.25% lecithin	7.76	1.94	21.02	4.47	6.41

[a] Lec·OH, hydroxylated lecithin.

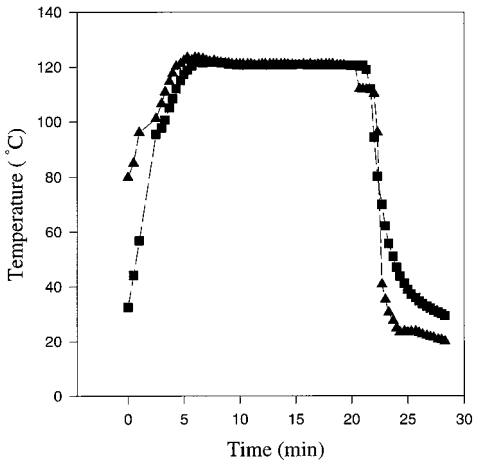

Figure 4. Typical heating profiles obtained during sterilization of emulsions. Symbols: ■, can center; ▲, retort.

interfacial peptide loading in emulsions containing hydroxylated lecithin (Table 2) could be due to formation of complexes between peptides and hydroxylated lecithin in solution. We ruled out the possibility of desorption of interfacial peptides during the analysis since there was no indication of droplet coalescence even after centrifugation used for determination of surface load.

Retorting increased the amount of either unmodified lecithin or hydroxylated lecithin associated with the oil phase (Tables 2 and 3). This may be due to a reorganization of the interface due to the heating process, leading to adsorption of lecithin on top or between the adsorbed peptides. Furthermore, the adsorption of peptide/lecithin complexes from the aqueous phase may be enhanced at high temperatures at the expense of noncomplexed peptides.

The reasons for the continued stability of the emulsions containing hydroxylated lecithin but not unmodified lecithin after retorting are not very clear. The surface concentration results (Tables 2 and 3) showed some adsorption of hydroxylated lecithin at the droplet surface after retorting; this adsorption may increase the overall charge and hydration at the oil droplet surface, resulting in an increased stability during heating. Alternatively, stabilization could be caused by some kind of interactions by the nonadsorbing components, including the possibility that hydoxylated lecithin selectively binds to certain peptides in the aqueous phase, leaving the better surfactant peptides to adsorb at the oil surface. Another possibility is that the rheology of the aqueous phase was affected by addition of hydroxylated lecithin via the formation of phospholipid vesicles. However, preliminary experiments showed that all the emulsions after retorting had similar apparent viscosities and yield values (S. O. Agboola, unpublished results). Clearly, this aspect of the work needs to be further investigated.

The droplet size distribution appeared to be the dominant factor controlling the gravity creaming. The results are thus in qualitative agreement with Stoke's law (Darling, 1987). Also, unlike emulsions containing just proteins or peptides (Leman and Kinsella, 1989; Agboola et al., 1998), the surface concentration data did not appear to be an important factor in determining the stability in these emulsions. This is presumably due to changes after the emulsions were formed, including competition at the interface and the retort-induced destabilization.

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LITERATURE CITED

Agboola, S. O.; Dalgleish, D. G. Kinetics of calcium-induced instability of oil-in-water emulsions: studies under quiescent and shearing conditions. *Lebensm. Wiss. Technol.* **1996a**, *29*, 425−432.

Agboola, S. O.; Dalgleish, D. G. Effects of pH and ethanol on the kinetics of destabilisation of oil-in-water emulsions containing milk proteins. *J. Sci. Food Agric.* **1996b**, *72* 448−454.

Agboola, S. O.; Singh, H.; Munro, P. A.; Dalgleish, D. G.; Singh, A. M. Destabilization of oil-in-water emulsions formed using highly hydrolyzed whey proteins. *J. Agric. Food Chem.* **1998**, *46*, 84−90.

Courthaudon, J. L.; Dickinson, E.; Christie, W. W. Competitive adsorption of lecithin and β-casein in oil-in-water emulsions. *J. Agric. Food Chem.* **1991**, *39*, 1385−1368.

Cruijsen, H. Physical stability of caseinate-stabilized emulsions during heating. Ph.D. Thesis, Agricultural University, Wageningen, The Netherlands, 1996.

Darling, D. F. Kinetic aspects of emulsion behaviour. In *Food Structure & Behaviour*; Blansard, J., Lillford, P. J., Eds.; Academic Press Ltd.: London, 1987; pp 107−147.

Davis, S. S.; Hasrani, P. The coalescence of oil droplets stabilized by phospholipid emulsifiers. *J. Colloid Interface Sci*. **1985**, *108*, 285−287.

Dickinson, E.; Iveson, G. Adsorbed films of β-lactoglobulin + lecithin at the hydrocarbon−water and triglyceride−water interfaces. *Food Hydrocolloids* **1993**, *6*, 533−541.

Fang, Y.; Dalgleish, D. G. Casein adsorption on the surfaces of oil-in-water emulsions modified by lecithin. *Colloids Surf. B* **1993**, *1*, 357−364.

Fang, Y.; Dalgleish, D. G. Studies on interactions between phosphatidylcholine and casein. *Langmuir* **1995**, *11*, 75−79.

Fang, Y.; Dalgleish, D. G. Competitive adsorption between dioleylphosphatidylcholine and sodium caseinate on oil−water interfaces. *J. Agric. Food Chem*. **1996**, *44*, 59−64.

Leman, J.; Kinsella, J. E. Surface activity, film formation and emulsifying properties of milk proteins. *CRC Crit. Rev. Food Sci. Nutr*. **1989**, *28*, 115−138.

List, G. R.; Heakin, A. J.; Evans, C. D.; Black, L. T.; Mounts, T. L. Factor for converting elemental phosphorus to acetone insolubles in crude soybean oil. *J. Am. Oil Chem. Soc*. **1977**, *55*, 521−522.

McCrae, C. H.; Muir, D. D. Heat stability of recombined milk: influence of lecithins on the heat coagulation time−pH profile. *J. Dairy Res*. **1992**, *59*, 177−185.

Nakamura, R.; Mizutani, R.; Yano, M.; Hayakawa, S. Enhancement of emulsifying properties of protein by sonicating with egg yolk lecithin. *J. Agric. Food Chem*. **1988**, *36*, 729−732.

Prosise, W. E. Commercial lecithin products: food use of soybean lecithin. In *Lecithins*; Szuhaj, E., List, G. R., Eds.; American Oil Chemists Society: Champaigne, IL, 1985; pp 163−182.

Rhydag, L.; Wilton, I. The function of phospholipids of soybean lecithin in emulsions. *J. Am. Oil Chem. Soc*. **1981**, *58*, 830−837.

Schmidt, J. E.; Orthoefer, F. T. Modified Lecithins. In *Lecithins*; Szuhaj, E., List, G. R., Eds.; American Oil Chemists Society: Champaigne, IL, 1985; pp 203−211.

Singh, H.; Sharma, R.; Tokley, R. P. Influence of incorporation of soya lecithin into skim milk powder on the heat stability of recombined evaporated milk. *Aust. J. Dairy Technol*. **1992**, *47*, 33−37.

Turgeon, S. L.; Gauthier, S. F.; Paquin, P. Interfacial and emulsifying properties of whey peptide fractions obtained with a two-step ultrafiltration process. *J. Agric. Food Chem*. **1991**, *39*, 673−676.

Van der meeren, P.; Van der deelen, J.; Baert, L. On the relationship between dynamic interfacial tension and o/w emulsion stabilizing properties of soybean lecithins. *Prog. Colloid Polym. Sci*. **1995**, *98*, 136−139.

Walstra, P.; deGraaf, J. J. Note on the determination of the phospholipid content of milk products. *Neth. Milk Dairy J*. **1962**, *16*, 283−287.

Watanabe, F. S.; Olsen, S. R. Test of an ascorbic acid method for determining phosphorus and $NaHCO_3$ extracts from soil. *Soil Sci. Soc. Proc*. **1965**, *29*, 677−678.

Weete, J. D.; Betageri, S.; Griffith, G. L. Improvement of lecithin as an emulsifier for water-in-oil emulsions by thermalization. *J. Am. Oil Chem. Soc*. **1994**, *71*, 731−737.

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