Destabilization of Oil-in-Water Emulsions Formed Using Highly Hydrolyzed Whey Proteins

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Oil-in-water emulsions (4 wt % soy oil) were prepared with 0.5−5 wt % whey protein hydrolysate (WPH) (27% degree of hydrolysis), in a two-stage homogenizer using various first-stage pressures of 10.3, 20.6, and 34.3 MPa and a constant second-stage pressure of 3.4 MPa. Destabilization studies on the emulsions were carried out for up to 24 h, using both laser light scattering and confocal laser microscopy. It was found that emulsions formed with <2% WPH showed oiling off and coalescence at all homogenization pressures. Emulsions formed with 2, 3, and 4% WPH showed coalescence and creaming only, while slight flocculation but no creaming occurred in emulsions formed with 5% WPH. Furthermore, the apparent rate of coalescence increased with homogenization pressure but decreased with WPH concentration. In contrast, the surface concentration of WPH increased with the WPH concentration in the emulsions but decreased with homogenization pressure. Analysis of WPH by high-performance liquid chromatography showed an increase in the concentration of high molecular weight peptides at the droplet surface compared to the WPH solution. This was considered very important for the stability of these oil-in-water emulsions.

Keywords: *Emulsions; homogenization; destabilization; mechanisms; hydrolysate*

INTRODUCTION

It is well-known that for proteins to behave as good emulsifiers/stabilizers, they must possess both hydrophobic and hydrophilic segments within the polypeptide chain for adsorption onto the oil and aqueous phases, respectively (Kinsella, 1984; Parker, 1988; Dalgleish, 1989). While most proteins satisfy this requirement, only in a very few proteins with flexible structure (e.g. caseins) are these segments available for adsorption and interaction with the phases. Various workers (Jost and Monti, 1982; Turgeon et al., 1991, 1992; Mahmoud, 1994; Agboola and Dalgleish, 1996a) have shown that flexibility and thus availability of the different segments is facilitated by moderate enzymatic hydrolysis of proteins containing a high proportion of secondary structures (e.g. whey proteins). However, extensive hydrolysis, due to the production of many short peptides, has been found to be detrimental to the emulsifying and stabilizing properties of proteins (Chobert et al., 1988; Agboola and Dalgleish, 1996a,b).

Many nutritional and health formulations such as hypoallergenic infant formulas and enteral formulations are essentially oil-in-water emulsions containing a large proportion of short peptides obtained from extensive hydrolysis of milk proteins (Schmidl et al., 1994; Mahmoud, 1994). However, presumably due to difficulties in emulsion formation, there are very few studies on the properties and destabilization of emulsion systems in which these types of hydrolysates were the only emulsifiers/stabilizers. Related studies have focused on the emulsifying properties (or lack thereof) of the peptides, while very little attention was paid to the mechanism(s) of destabilization (Chobert et al., 1988; Turgeon et al., 1992; Agboola and Dalgleish, 1996a,b). A knowledge of these mechanisms is very important to improve the formation and stability of these emulsion systems. In this study, we report the formation, characterization, and destabilization of oil-in-water emulsions containing highly hydrolyzed whey proteins.

MATERIALS AND METHODS

Materials. Whey protein product that has been hydrolyzed to a 27% degree of hydrolysis (WPH 931) was supplied by the New Zealand Dairy Board, Wellington, New Zealand. The dried product contained 90.5% protein, 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. All other reagents were of analytical grade and were supplied by BDH Chemicals Ltd. (Poole, England).

Preparation of Emulsions. A typical oil-in-water emulsion (4 wt % soy oil, deionized water, pH 6.8−7.0) was prepared at 50 °C, using a two-stage Rannie homogenizer (Albertslund, Denmark) operating at various first-stage pressures (10.3, 20.6, or 34.3 MPa) and a constant second-stage pressure of 3.4 MPa. Appropriate quantities of whey protein hydrolysate (WPH) were dissolved in Milli-Q water at room temperature (20 ± 2 °C) to give WPH concentrations in the range 0.5−5 wt % of the final emulsion. Soy oil (50 °C) and WPH solutions were combined and mixed by a single pass through the homogenizer at atmospheric pressure prior to homogenization. The emulsions were homogenized twice for more effective mixing of the oil phase. At least three emulsions were prepared for each treatment.

Measurement of Particle Sizes. The droplet size distribution and hence the volume−surface average particle diam-

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eter (d_{32}) was measured by light scattering using a Mastersizer E (Malvern Instruments Ltd., Worcestershire, 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. However, to determine the incidence of flocculation, the droplets were suspended in 1% sodium dodecyl sulfate (SDS) solution for 30 min before sizing (Tomas et al., 1994). All measurements were carried out at 20 °C.

Determination of Creaming Stability. About 15 mL of a freshly prepared emulsion was poured into specially constructed "stability tubes" (30 cm in length, 8 mm i.d. and 10 mm o.d.). The tubes were graduated with each division equal to 0.1 mL. Various levels, corresponding to oil, cream, and serum layers, were read after 6, 12, and 24 h storage at 20 °C. Results after 24 h are reported.

Measurement of Surface Concentration. Surface concentration was determined according to a modification of the depletion method (Dickinson, 1987) using two different centrifugal force/time regimes. The emulsion was first centrifuged at 5090*g* for 30 min in a Sorvall RC5C temperature-controlled centrifuge (DuPont Co., Wilmington, DE), after which time most of the cream was removed. This mild centrifugation was necessary to prevent breakup of emulsions and desorption of loosely bound peptides from the interface. The subnatant was then centrifuged at 30000*g* for 35 min to separate the remaining cream from the serum phase. The serum (subnatant) was filtered through a 0.22 μm Millipore membrane filter (Millipore Corp., Bedford, MA); ~4.8 g of this serum was then analyzed for total nitrogen (TN) using the Kjeldahl method with a Tecator Kjeltec System (Tecator AB, Hoganas, Sweden). The nitrogen content of 5 g of equivalent (percent WPH in the emulsion) solution of hydrolysate was similarly determined; the difference between this and the serum TN was the surface nitrogen. A factor of 6.38 was used to convert the milligrams of nitrogen to milligrams peptides. This value was then related to the amount of oil associated with the serum sample (~200 mg) to calculate the milligrams of peptides per gram of oil. From the Mastersizer data, specific surface area (SSA) in square meters per gram of oil was obtained for each freshly prepared emulsion and was used to calculate the surface concentration (Γ) in milligrams of peptides per square meter as follows:

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

Microscopic Examination of Emulsions. Emulsions formed with 0.5, 2, and 4% WPH using first-stage homogenization pressures of 10.3 and 34.3 MPa were analyzed for their microstructure. Nile blue (fluorescent dye) at 0.1 wt % concentration was added to the samples, which were mounted on a Leica TCS 4D confocal scanning laser microscope (Leica Laser Technik, GmbH, Heidelberg, Germany). The laser source was Ar/Kr and was used at an excitation wavelength of 488 nm. Samples were viewed either under oil immersion using the ×100 objective lens (all samples) or in air using the ×10 objective lens (0.5% WPH-stabilized emulsions only). Typical micrographs are presented.

Removal and Collection of Interfacial Peptides. The method employed by Persaud (1995) was slightly modified to obtain the interfacial peptides. Emulsions were centrifuged at 20000*g* for 30 min, and the subnatant was discarded. The cream was resuspended in Milli-Q water and recentrifuged at 20000*g* for a further 30 min to wash off adhering peptides. The resulting cream was then carefully removed and again suspended briefly in Milli-Q water to further remove serum peptides. Emulsions containing 4% WPH were used for this experiment; emulsions formed using <4% WPH gave very weak creams that were difficult to remove from the centrifuge tubes. The washed cream was then spread thinly on a Whatman No. 1 filter paper and allowed to dry. To 1 g of this cream were added 0.2 g of Tween 20 and 2.3 mL of Milli-Q water, making the Tween 20 content ~6%. This mixture was stirred for 1 h, after which time it was mixed with half its volume of a 2:1 mixture of chloroform/methanol and then centrifuged at 19500*g* for 15 min. The supernatant (containing interfacial peptides) was carefully collected and freeze-dried for HPLC analysis.

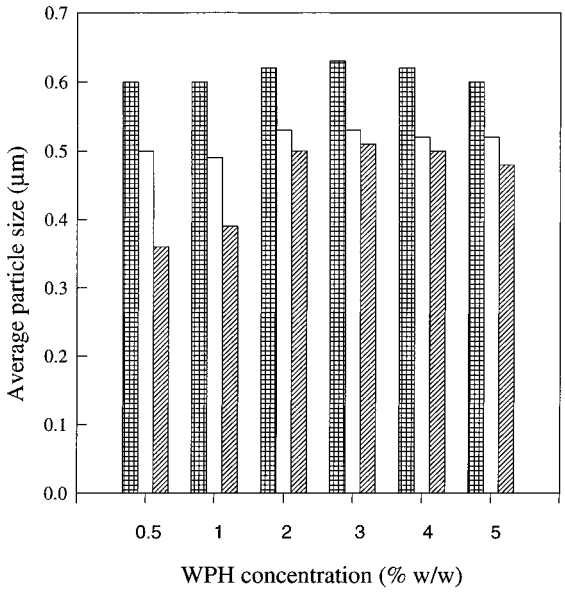

Figure 1. Average particle sizes (d_{32}) of emulsions stabilized by various concentrations of WPH. Emulsions were formed at various first-stage homogenization pressures of 10.3 (crossed bars), 20.6 (clear bars), or 34.3 MPa (slashed bars).

Peptide Analysis by HPLC. Solutions (0.5% w/v) of the WPH product (total peptides), defatted serum, and interfacial peptides were passed through a 0.45 μm Millipore filter and analyzed using both reversed phase (RP-) and gel permeation (GP-) HPLC. The serum was defatted prior to the HPLC analysis by adding half its volume of a 2:1 chloroform/methanol mixture, centrifuging the mixture at 19500*g* for 15 min, and then freeze-drying the supernatant. RP-HPLC was conducted using a Waters 600 multisolvent delivery system (Millipore Corp., Milford, MA) and a C_{18} Haisil 300 analytical column (Higgins Analytical Inc., Mountain View, CA). The mobile phases were Milli-Q water (solvent A) and 90% (v/v) ethanol (solvent B) (Lee and Warthesen, 1996). Both mobile phases contained 0.1% (v/v) hydrochloric acid and were passed through a 0.45 μm Millipore membrane filter. After 25 mL of sample was loaded, a linear gradient elution from 0 to 100% solvent B in 50 min at a flow rate of 0.5 mL/min was employed at room temperature (20 °C) for the separation of peptides. The peaks were detected at an absorbance of 220 nm.

The molecular weight profiles of the peptides were analyzed by gel permeation chromatography (Waters 600E delivery system attached to a Waters 717+ autosampler) using a Supelco Progel TM/TSK G2000SW column (7.5 mm i.d. × 60 cm, 10 μm pore size) (Supelco Inc.). An aliquot (50 μL) of each filtered sample was loaded onto the column. The mobile phase used for the elution consisted of 36% acetonitrile, 0.1% trifluoroacetic acid, and 64% Milli-Q water (v/v). Elution was carried out at 0.5 mL/min under isocratic conditions for 65 min, and absorbance was monitored at 205 nm.

RESULTS AND DISCUSSION

Formation of Emulsions. The combined effects of both the homogenization pressure and the WPH concentration on d_{32} and size distribution of the emulsion droplets are shown in Figures 1 and 2, respectively. At any given WPH concentration, the d_{32} decreased with increasing homogenization pressure, as expected from the increasing power density with pressure (Walstra,

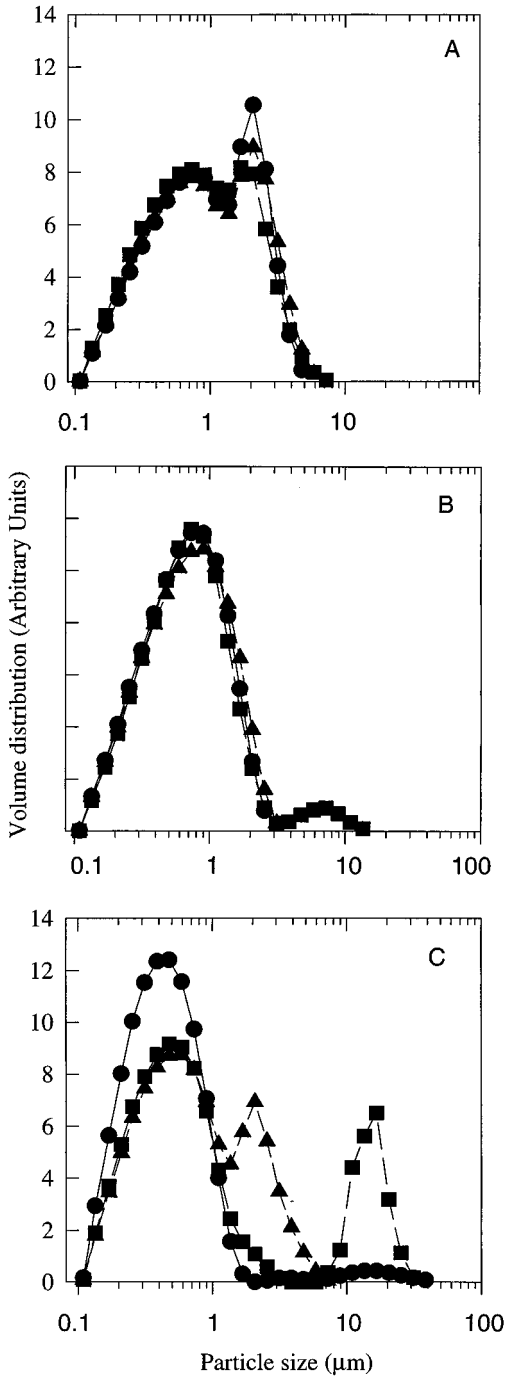

Figure 2. Size distributions of 0.5 (●), 2 (■), and 4% (▲) WPH-stabilized emulsions formed at first-stage homogenization pressures of (A) 10.3, (B) 20.6, or (C) 34.3 MPa.

1993). In this regard, the emulsions behaved essentially like those stabilized with intact proteins (Tornberg and Hermansson, 1977; Tornberg, 1978a; Walstra, 1987). It was also clear from Figure 2 that higher proportions of small particles were formed with increasing homogenization pressure and hence lower average sizes. Emulsions formed using a first-stage homogenization pressure of 10.3 MPa (Figure 2A) showed comparable size distribution profiles at all WPH concentrations used, with all of the particles being below 10 μm. Increasing the homogenization pressure to 20.6 MPa increased the proportion of particles between 0.1 and 3 μm (Figure 2B). Some particles above 10 μm were also formed in emulsions stabilized using 2% WPH. When the pressure was increased to 34.3 MPa, the proportion of

Table 1. Creaming Stability (Milliliters Separated) of WPH-Stabilized Emulsions after 24 h at 20 °C

	first-stage homogenization pressure					
	10.3 MPa		20.6 MPa		34.3 MPa	
WPH (wt %)	oiling off	creaming	oiling off	creaming	oiling off	creaming
0.5	0.1	0	0.1	0	0.2	0
1	0.1	0	0.1	0	0.1	0.3
2	0	0.3	0	0.5	0	0.7
3	0	0.2	0	0.6	0	0.65
4	0	0	0	0.2	0	0.2
5	0	0	0	0	0	0

droplets in the size range between 0.1 and 1 μm was reduced (Figure 2C). However, formation of large emulsion droplets (>10 μm) was also observed. The proportion of these large droplets was apparently not enough to increase the d_{32} beyond those formed at lower pressures (see Figure 1). The formation of these large droplets was likely related to the inability of the predominantly short peptides in the WPH product to adequately stabilize the greater surface area produced by homogenizing at very high pressures. During homogenization, it is known that the size of the droplets depends to a very large extent on the power input from the equipment (Darling and Birkett, 1987; Walstra, 1983, 1993). As fresh surfaces are being created, the available emulsifiers are adsorbed from the solution phase. It is expected that while many of the peptide species would be adsorbed instantaneously, the majority of the short peptides would be desorbed because of competition from more suitable emulsifiers (Turgeon et al., 1991) or would simply never adsorb at all. The short peptides, because of the lack of secondary and tertiary structures, would have poor ability to provide steric stabilization and strong interfacial films. In addition, peptide−peptide interactions in solution may diminish their affinity for the interface. Since the concentration of "suitable" peptides is assumed to be limited in the WPH, the end result is an immediate recoalescence of bare oil droplets. This situation, as expected, worsens with increasing homogenization pressure and the attendant increase in surface areas being created.

The direct corollary of this inadequacy of WPH to emulsify the oil droplets is that increasing its concentration would produce more stable emulsions. This was found to be true as the WPH concentration was increased from 2 to 5%. Emulsions formed at 2 and 3% WPH showed the highest d_{32} (Figure 1), and as the WPH concentration increased, d_{32} reduced significantly at all homogenization pressures ($P \leq 0.05$) except at 20.6 MPa. The d_{32} of emulsions produced with 0.5% and 1% WPH and especially at 34.3 MPa were, however, lower than at higher concentrations, reflecting the fact that in those systems, small particles had the highest frequencies. This apparent inconsistency was clarified by two alternative methods to light scattering. First, a great deal of oiling off was visually observed at these low concentrations, suggesting that a considerable proportion of the oil (15−30%) remained unemulsified or coalesced extensively immediately after homogenization (see Table 1). It is therefore reasonable to conclude that there was much better oil emulsification at WPH concentrations of 2% and above (no oiling off). Also, the confocal micrograph (Figure 3B) of a typical emulsion formed at low WPH concentration showed some huge oil droplets, which were apparently low in proportion (number concentration) compared to the small droplets.

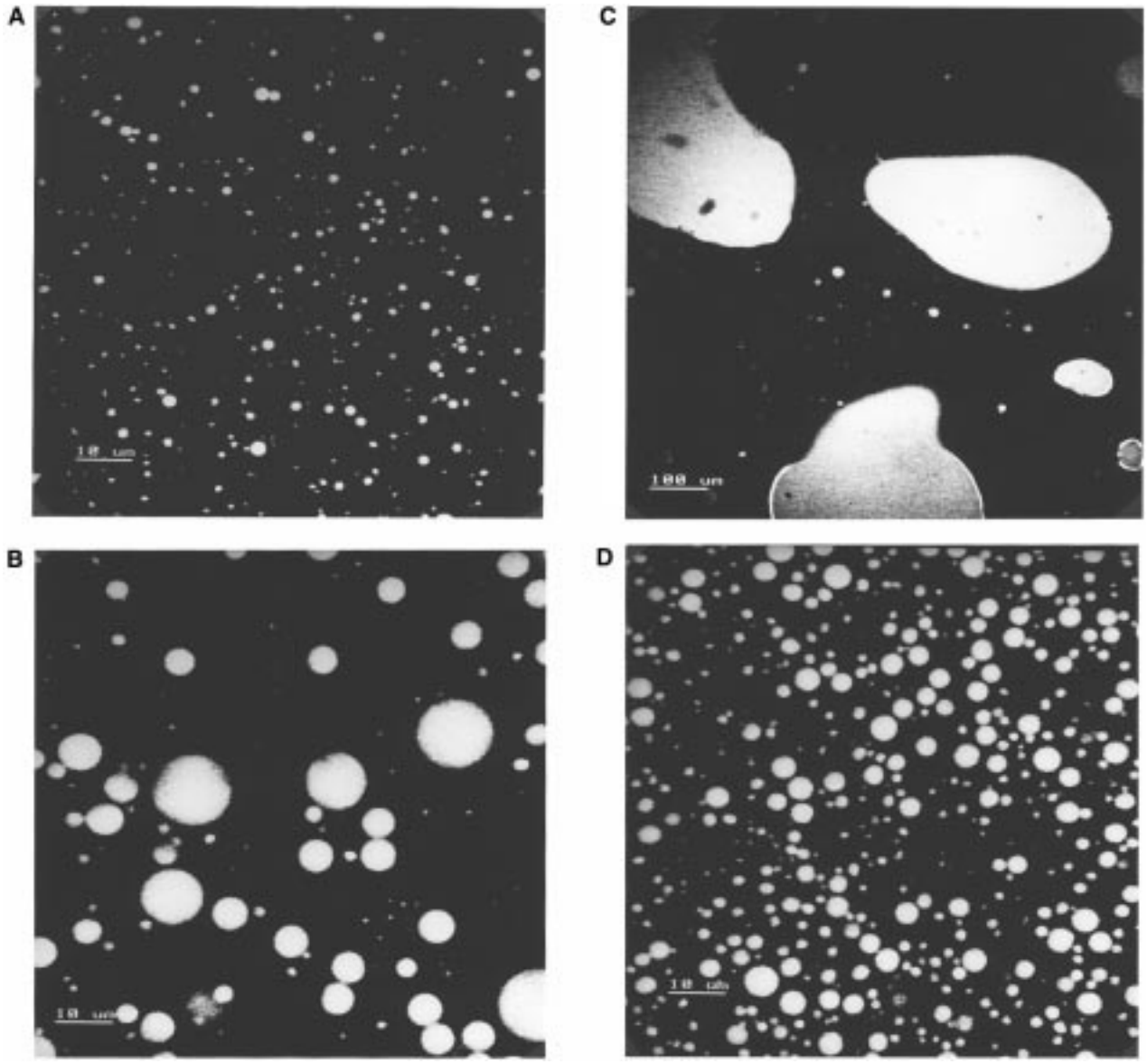

Figure 3. Confocal micrographs of emulsions formed at 34.3 MPa first-stage homogenization pressure. The emulsions contained 0.5 (A, C), 2 (B), or 4% WPH (D).

It therefore appears that small-angle laser light scattering was inadequate to accurately size these emulsions since size distribution data did not show such particles (Figure 2). This may be due to either or a combination of two factors. In the first instance, these large droplets are outside the range of the 45 mm (focal length) lens used in the Mastersizer. Second, it is possible that these huge oil droplets were broken down in the turbulent shear field of the Mastersizer before reaching the measuring cell. Microscopy thus proved to be a very valuable tool in obtaining a clearer picture of the emulsion behavior. Similar clarifications have been reported for protein-stabilized emulsions (Agboola and Dalgleish, 1995). In comparison to the emulsions formed at low WPH concentration, emulsions formed with 2 and 4% WPH (Figure 3B,D) showed a corresponding decrease in d_{32} with WPH concentration, in agreement with the general trend already obtained using light scattering (Figures 1 and 2).

Destabilization of Emulsions. Oil-in-water emulsions stabilized with proteins can destabilize in many ways, although the major destabilizing mechanisms are flocculation and coalescence (Kinsella, 1984; Darling, 1987; Walstra, 1987; Parker, 1988). These mechanisms could also contribute to creaming, sedimentation, oiling off, and ultimately the emulsion breakup into its separate oil and water phases. Emulsions formed with WPH at 4% and below showed no difference in their d_{32} values or size distribution patterns whether suspended in either distilled water or 1% SDS solution before sizing. This indicates little or no flocculation between the droplets (Tomas et al., 1994). Comparatively, emulsions containing 5% WPH showed a consistent, significant ($P \leq 0.05$), although slight, reduction in d_{32} ($\sim$0.02 μm) when suspended in SDS solution. This indicates slight flocculation between droplets. This observation was independent of either the extent of destabilization (hours of storage) or the homogenization pressure. Flocculation normally occurs before droplets can coalesce. After droplet flocculation, coalescence depends on the probability of the interfacial film between the droplets rupturing in finite time. This probability has been found to decrease with the thickness of the interfacial layer (Phillips, 1981; Leman et al., 1988; Leman and Kinsella, 1989). Presumably, increasing concentration of the peptides caused an improvement

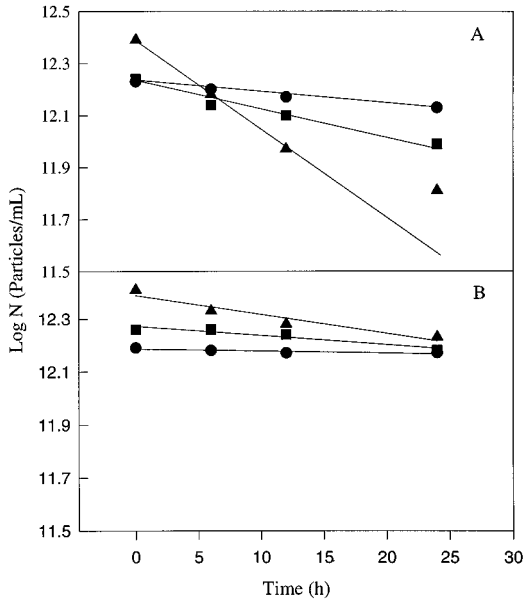

Figure 4. Relationships between particle number concentrations (N_c) and storage time of the emulsions formed with (A) 2 or (B) 4% WPH at 20 °C. Slope of each line represents the apparent rate of coalescence (R_c). Symbols represent the first-stage homogenization pressures: (●) 10.3, (■) 20.6, and (▲) 34.3 MPa.

in the integrity of the interfacial layer, which was able to resist coalescence.

Coalescence of oil droplets in emulsions is usually represented by a first-order rate equation as follows (Darling, 1987; Walstra, 1987):

$$N_t = N_0 e^{-kt}$$

where N_t is the number concentration of emulsion droplets at time t, N_0 is the number concentration of freshly formed emulsion droplets (time zero), and k is the rate constant, which is related to the probability of the interdroplet film (interfacial layer) rupturing in time t. The slope of the logarithmic plot of N_c with time $[-d(\log N_c)/dt]$ gives an indication of the rate of coalescence; this calculated value depends on the initial number concentration, as well as the influence of other destabilization mechanisms, e.g., flocculation (Walstra, 1987). Also, this equation is only applicable at the initial stages of destabilization as the rate of coalescence is always changing with time as a result of changes in size distribution, particle interactions, and surface properties of the emulsions (Darling, 1987).

In this study, the apparent rate of coalescence (R_c) was estimated for emulsions containing 2−5% WPH (no oiling off occurred in these emulsions) by plotting log N_c versus time (Figure 4). N_c, i.e. the number of particles per unit volume of emulsion, was calculated from volume−mean diameter (d_{30}) and the volume fraction of oil in the emusion (Foust et al., 1980). The calculated value of R_c decreased with the WPH concentration and increased with the homogenization pressure (Table 2). Although the initial number concentrations of the emulsions were lowest at 10.3 MPa, the emulsions underwent the lowest changes with time, translating into the most stable (i.e. lowest extent of creaming) at each WPH concentration. Also, increasing the WPH concentration to 5% showed a stable system (neither creaming nor oiling off occurred in 24 h) irrespective of the homogenization pressure.

Table 2. Apparent Rate of Coalescence (R_c in mL^{-1} h^{-1}) of Emulsions Formed with WPH at Various First-Stage Homogenization Pressures

	homogenization pressure		
WPH (wt %)	10.3 MPa	20.6 MPa	34.3 MPa
2	1.45×10^{10}	3.21×10^{10}	7.46×10^{10}
3	7.10×10^{9}	4.34×10^{10}	4.93×10^{10}
4	4.59×10^{9}	1.36×10^{10}	3.70×10^{10}
5	2.25×10^{9}	4.58×10^{9}	1.79×10^{10}

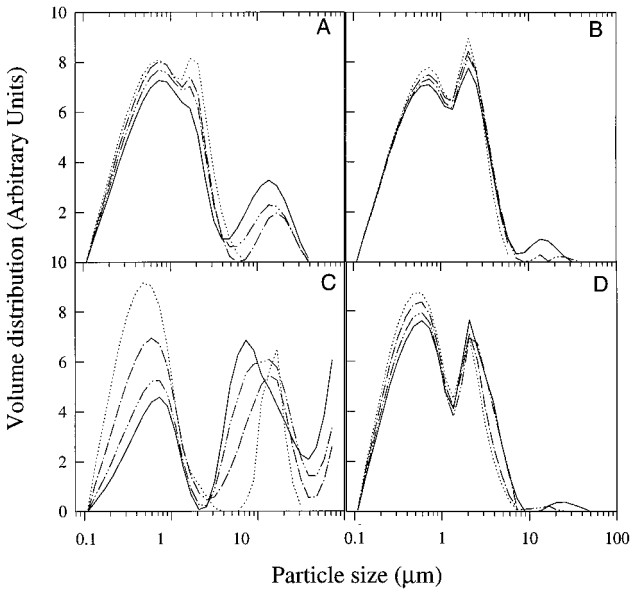

Figure 5. Effect of storage time [(···) 1 h; (−·−) 6 h; (−··−) 12 h; (—) 24 h] on the size distribution of emulsions formed with 2% WPH (A, C) or 4% WPH (B, D). Emulsions were formed using homogenization pressures of 10.3 (A, B) or 34.3 MPa (C, D). The curves in panel C show a large proportion of particles above 80 μm, which could not be measured using the 45 mm lens in the Mastersizer.

As shown in Table 1, however, the properties of emulsions formed at ≤1% WPH were clearly different from the others in terms of oiling off. This indicates low emulsifying ability by the WPH at such low concentrations, leading to spontaneous formation of "free oil" in the system. While this may be a major defect in some emulsions (Kinsella, 1984; Parker, 1988), it is seldom observed in emulsions stabilized by intact milk proteins even at low protein concentrations (Fang and Dalgleish, 1993; Agboola and Dalgleish, 1995). This represents a major difference in properties of emulsions formed using peptides compared to those formed using intact milk proteins.

The successful application of stability tubes toward quantifying the changes in stability of these emulsions under storage is considered very significant. Not only did it directly agree with the rate of destabilization as measured by the apparent rate of coalescence, it was also not necessary to alter the nature of these weak emulsions by employing various accelerated tests (Dickinson, 1987; Tornberg, 1978b).

Figure 5 shows typical size distribution patterns of the emulsions during storage at 20 °C. From Figure 5A (2% WPH, 13.6 MPa), we noticed a monomodal distribution in the emulsion measured within 1 h of homogenization. After 6 h, however, there was a second peak indicating the formation of large droplets >10 μm. Furthermore, the size of this peak increased as the storage continued for 12 and 24 h. Interestingly, the

Table 3. Molecular Weight Profiles of Total, Serum, and Interfacial Peptides Obtained Using GP-HPLC

MW range	total peptides (%)	defatted serum peptides (%)	interfacial peptides (%)
>20000	0.0	0.0	1.7
10000−20000	0.2	0.2	2.0
5000−10000	1.9	1.9	6.8
2500−5000	9.1	9.4	15.9
1000−2500	24.1	24.1	26.3
500−1000	21.3	21.4	14.9
250−500	21.2	21.2	18.3
<250	22.3	21.8	14.1

emergence and growth of this second peak occurred in parallel with the decrease in the size of the first peak. At 34.3 MPa (Figure 5C), the same trend was observed, although at a much larger scale corresponding to the reduced stability as the homogenization pressure was increased. Increasing the concentration of WPH to 4 wt % resulted in much smaller changes in particle size distributions (Figure 5B,D).

The results outlined above serve as further evidence of the inability of these highly hydrolyzed peptides to adequately stabilize the more numerous oil droplets formed at high homogenization pressures. It also highlights the improvement conferred on these systems by increasing the WPH concentration and hence the amount of more suitable peptides. It appears that the critical factor in the destabilization of these emulsions is the influence of homogenization pressure and WPH concentration on surface concentration and composition.

Surface Concentration and Composition. Although the method of directly estimating the surface protein from the cream layer (Hunt and Dalgleish, 1994) has some advantages over the depletion method, this was not applicable to the emulsions stabilized by WPH. First, the molecular weight profile of the peptides (Table 3) shows a large proportion of small molecular weight species (<1000), which will be difficult to quantify using SDS−polyacrylamide gel electrophoresis. Also, the cream layers obtained after centrifugation were very weak, presumably owing to low adsorption of surfactants.

Figure 6 shows the surface concentration of the emulsions at various WPH concentrations and homogenization pressures. The values of surface concentration at 0.5 and 1% WPH were calculated by assuming 4% oil in the system. The proportion of surface peptides was calculated to be between 2.7 and 3.2% of the initial WPH. Since this proportion was fairly constant over the entire concentration and homogenization pressure range, it was evident that the surface concentration will increase with both the d_{32} (reducing specific surface area) and added WPH. This was indeed found to be so, with the curves tending toward plateaus as the WPH concentration increased beyond 3%. This was presumably because we were approaching saturated monolayer surface coverage. The surface concentration range obtained for the WPH-stabilized emulsions in this study was similar to the values reported for caseinate or whey protein-stabilized emulsions containing comparable concentrations of surfactants (Fang and Dalgleish, 1993; Hunt and Dalgleish, 1994; Srinivasan et al., 1996). Although the oil volume fraction was much higher in those studies (20−30%), the unusually low level of surface activity of the WPH (~3% adsorption) compensated for the low oil volume fraction used in our experiments. The surface concentration curves also

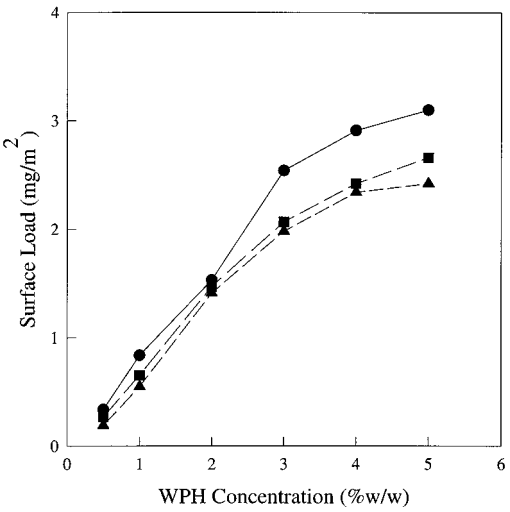

Figure 6. Effects of WPH concentration on surface load of emulsions formed at various first-stage homogenization pressures: (●) 10.3, (■) 20.6, and (▲) 34.3 MPa.

indicated higher surface loads for emulsion droplets formed at 10.3 MPa. This may be due to the formation of a higher proportion of larger oil droplets compared to the emulsions formed at higher homogenization pressures, at which very small particles predominate at any given WPH concentration (Figure 2C). This increased surface concentration may have contributed to the observed stability of the emulsions formed at low homogenization pressure (Table 2; Figure 4). The importance of high surface concentration conferred by milk proteins in improving emulsion stability has been reported by Phillips (1981), Parker (1988), Leman and Kinsella (1989), and Singh et al. (1993).

Table 3 shows the molecular weight profiles of the total, serum, and interfacial peptides. It indicates that there was very little difference between the molecular weight profile of total and serum peptides, presumably because of the low level of adsorbed peptides. There was, however, a shift in the proportion of higher molecular weight species on the interface; the proportion of peptides above 5000 increased 5-fold from only 2.1% in total peptides to 10.5% at the interface, and those in the range 2500−5000 almost doubled at the interface. The results indicated clearly the importance of high molecular weight species in their ability to remain adsorbed to the interface to stabilize oil-in-water emulsions. Similar suggestions have been made by Turgeon et al. (1991, 1992) and Agboola and Dalgleish (1996a).

Although the proportions of low molecular weight species were reduced at the interface, they were still present in high amounts. It was also confirmed by RP-HPLC (profile not shown) that most of the peptides were still at the interface, albeit in much smaller concentrations. Since several washing steps were included in the isolation of the peptides, we discounted contamination from the serum. The RP-HPLC results also showed that a large proportion of the peptides in the WPH (total peptides) were hydrophobic, suggesting that many of the low molecular weight species were hydrophobic. It is therefore probable that the low molecular weight species detected at the interface were associated with the hydrophobic side of the oil/water interface. While these small peptides would be easily washed away if exposed to the water phase (loosely adsorbed), they would remain at the interface if they can penetrate the oil phase to an extent. Although this concept has not been

well researched, it may explain the ability of hydrolysates to stabilize water-in-oil emulsions and mayonnaise-type oil-in-water emulsions containing high oil volume fractions (Muschiolik et al., 1996). Turgeon et al. (1996) have also reported good performance of WPH in salad dressing formulations containing ~65% oil. Studies are needed to further explore this hypothesis.

Conclusion. We have shown that fairly stable emulsions could be formed with highly hydrolyzed whey proteins, especially at high peptide concentrations and at low homogenizing pressures. The mechanism of destabilization in these emulsions was found to be mainly coalescence, and the calculation of the apparent rate of coalescence agreed with the measurement of creaming stability under gravity. The use of microscopy proved complementary to the light scattering techniques in studying the destabilization mechanisms. Our results also highlight the importance of high molecular weight peptides in the stability of emulsions formed with hydrolysates. Research continues on the stability of these emulsions under retort conditions.

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