The Emulsifying Properties of Hydrolyzates of Whey Proteins

A. M. SINGH* and D. G. DALGLEISH†,1
*New Zealand Dairy Research Institute,
Private Bag 11029, Palmerston North, New Zealand
†Department of Food Science, University of Guelph,
Guelph, ON, Canada N1G 2W1

ABSTRACT

A commercial range of hydrolyzates of whey proteins with degrees of hydrolysis ranging from 8 to 45% was used to make emulsions with soybean oil (3% wt/wt); the range of the hydrolyzate concentrations used was 0.02 to 5% (wt/wt). The stability of these emulsions was measured by determining the average sizes of the emulsion droplets and their size distribution both immediately after formation and after storage. The effects of heating on the stability of the emulsions were also determined. As estimated by the particle sizes, the maximum emulsifying capacity was obtained from hydrolyzates with a 10 or 20% degree of hydrolysis. Higher hydrolysis resulted in peptides that were too short to act as effective emulsifiers, and, at lower proteolysis, the somewhat reduced solubility of the hydrolyzates slightly decreased their emulsifying power. All of the emulsions were unstable when they were subjected to heat treatment at high temperatures (122°C for 15 min), but emulsions prepared from the less hydrolyzed peptide mixtures were stable to heat treatment at 90°C for 30 min. To a limited extent, emulsion stability could be altered by mixing the different peptide preparations after an emulsion was formed using one of them.
(**Key words**: emulsions, whey proteins, peptides, emulsion stability)

Abbreviation key: **DH** = degree of hydrolysis, **DLS** = dynamic light scattering, **SALLS** = small angle laser light scattering.

INTRODUCTION

Emulsions are widely used in foods, and many of these emulsions are stabilized by proteins, partly for nutritional reasons, but partly also because of the highly functional nature of the proteins, which makes them good surfactants and also stabilizing agents against flocculation or coalescence of the emulsion droplets during storage (6, 13). It is sometimes necessary or desirable, however, that the proteins be partially hydrolyzed before being used as emulsifiers because the products may need to be easily digested (formulas for parenteral nutrition), hypoallergenic (infant formulas), or possibly both. The hydrolysis of the surfactant proteins, either before or after the formation of the emulsions, can affect the stability of the emulsion system (1, 2, 17) by making the emulsion inherently unstable or by altering its sensitivity to outside influences (e.g., calcium ions, reduced pH, or high temperature). In some cases, hydrolysis may even promote stability, as has been observed in the increase in calcium stability of caseinate emulsions treated with trypsin (2) or the enhanced emulsifying properties of β-LG when treated with trypsin (12, 20, 21, 22).

Relatively little information is available on the effects of peptides, compared with the effects of native proteins, on the stability of oil-in-water emulsions. Proteins stabilize emulsion droplets by adsorbing to the oil-water interface as it is formed in the homogenizing device (3). The adsorption arises because of hydrophobic interactions between sections of the protein and the surface of the oil and creates an interfacial layer that is generally charged (because proteins contain charged amino acids) and that can also sterically stabilize the droplets because the protein molecules protrude some distance from the surface (7). In addition, at least some proteins (e.g., whey proteins), once they are adsorbed, can interact to form a strong interfacial layer that stabilizes the emulsion against coalescence (9). If hydrolyzed proteins are used as emulsifiers, there is a risk that the stabilizing effect of the protein will be lost, for a number of reasons. First, hydrolysis produces a range of peptides, some of which are unlikely to adsorb because they have insufficient hydrophobicity. Second, the peptides that have minimal hydrophobicity are likely to be the most charged, and, if these remain in solution, the emulsion droplets can possess

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[1]Corresponding author. Present address: Danone, CIRDC, 15 avenue Galilée, 92350 Le Plessis-Robinson, France.

TABLE 1. The properties of the hydrolyzate preparations used in the emulsions.[1]

Hydrolyzate	DH[2]	Mean molecular mass	Solubility
	(%)	(Da)	
ALATEL 916	8	1400	Good
ALATEL 917	10	1100	Good
ALATEL 926	20	520	Very good
ALATEL 931	28	460	Excellent
ALATEL 1010	45	NA[3]	Very good

[1]Taken from technical bulletins, New Zealand Milk Products (Wellington, New Zealand).

[2]Degree of hydrolysis.

[3]Not available.

only small charges. Third, if the adsorbing peptides are short, there is less possibility of steric stabilization. Against these factors must be set the possibility that the disruption of the protein structure may permit more efficient adsorption of some peptides (12, 16, 20, 21, 22). It has indeed been suggested that specific peptides from β-LG may have enhanced stabilizing effects on emulsions (12, 20).

This study was undertaken to determine the properties of a range of hydrolyzates of total whey protein with respect to their ability to form and maintain stable emulsions. The hydrolyzates used had degrees of hydrolysis (**DH**) varying from 8 to 45%. The object of the research was to determine the minimal length of peptides that could be used to produce a stable oil-in-water emulsion system and to define the effects of heating on the stability of the emulsions.

MATERIALS AND METHODS

A range of proprietary hydrolyzates of different DH (i.e., the fraction of the total peptide bonds that have been broken) were obtained from New Zealand Milk Products (Wellington, New Zealand). The hydrolyzates used in this research had mean DH of 8, 10, 20, 28, and 45%. These values do not indicate that the peptides in any hydrolyzate are all of the same length, because each preparation contains a range of lengths, depending on the extent of hydrolysis and the particular enzymes used. The properties of the materials are summarized in Table 1.

Oil-in-water emulsions were made using a Microfluidizer (M110S, Microfluidics Inc., Newton, MA). Each protein hydrolyzate was dissolved in deionized water to a defined concentration and heated to 60°C. Soybean oil was then added to a concentration of 3% (wt/wt), and the mixture was prehomogenized using a high shear mixer (Dia-Med, Mississauga, ON, Canada). The preemulsion was then passed through the Microfluidizer for five strokes of the pump at an input pressure of 40 MPa, was collected, and was then subjected to a further four cycles of the pump before being again collected. The concentrations of hydrolyzates used in the emulsions ranged from 5 to 0.02% (wt/wt); however, most attention was paid to emulsions containing 4% of the hydrolyzates, because these are of the appropriate concentration for parenteral formulations. The Microfluidizer also had a beneficial effect in solubilizing some of the low DH hydrolyzates, which tended to have somewhat lower solubility than did the others (Table 1).

Mixed emulsions were also studied to determine whether competitive effects could be beneficial in determining the stabilities of the emulsions. Small amounts (0.25%) of low DH hydrolyzates were mixed with oil, and the emulsion was prepared as before. Extensively hydrolyzed fractions were then added to the emulsion, and the stability, particularly during heat treatment, was determined.

The stabilities of the emulsions were determined by measuring the sizes of the particles in the suspensions, which gives an indication of the emulsifying capacity of the hydrolyzates and also provides information about the tendency of the emulsions to coagulate or coalesce; we did not attempt to distinguish between the two types of instability. Two methods of measuring particle size were used. The first, which also gives a measure of the distribution of particle sizes from 0.1 to 80 μm, was small angle laser light scattering (**SALLS**; Mastersizer X; Malvern Instruments, Southboro, MA). The measurement also permitted the determination of the specific surface area of the emulsion droplets. Results from this measurement allowed the determination of the weight mean diameter (d_{43}), as well as allowing the shape of the distribution (monomodal or bimodal) to be ascertained; this latter measure gives important information on the state of aggregation of the emulsions. For any measurement, the emulsions were diluted 1:100 (vol/vol) in deionized water.

The second method of particle size measurement was dynamic light scattering (**DLS**). Diffusion coefficients and, hence, apparent diameters of the particles were calculated from the measurement of the correlation functions of light scattered from diluted suspensions (1:1000 vol/vol in deionized water) of the emulsions. The measurements were made at a scattering angle of 90° (Malvern Instruments 4700 optical system attached to a 7032 correlator). The diffusion

coefficients were calculated by the method of cumulants, which gives an average value. We did not attempt to measure the distribution of particle sizes using this method. The SALLS and DLS give different results because of the different angles at which the measurements are taken and the scattering and hydrodynamic properties of the particles under investigation.

To determine the stability to heat treatment of the emulsions containing 4% of the different hydrolyzates, two methods were employed. In the first, 5-ml aliquots of emulsion were heated in test tubes in a water bath for 30 min at 90°C. They were then removed and cooled to room temperature in ice before the particle sizes were measured. In the second method, intended to be an analog of retort sterilization, 10-ml samples of the emulsions were placed in a beaker in an autoclave and heated at 121°C for 15 min. The samples were removed from the autoclave as soon as it had cooled to 60°C and were further cooled in ice. The particle sizes in the emulsions were then measured.

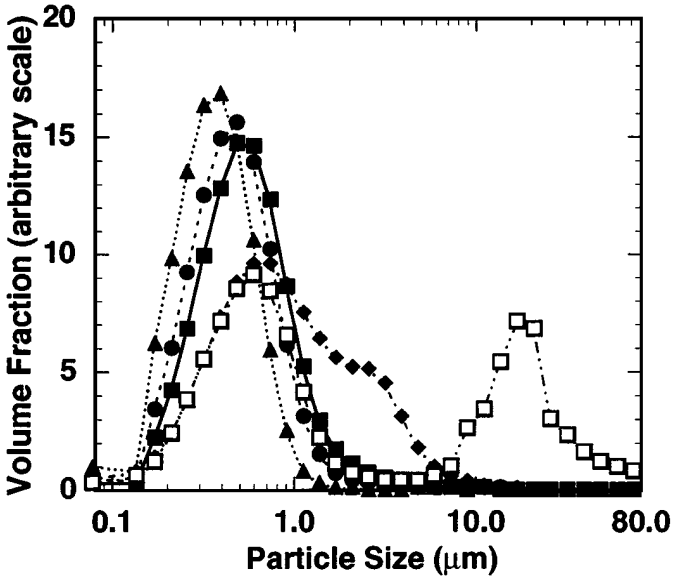

Figure 1. Size distributions measured by small angle laser light scattering for emulsions (3% oil and 4% protein) containing hydrolyzates of different degrees of hydrolysis (DH). Symbols are for hydrolyzates of 8% (■), 10% (●), 20% (▲), 28% (♦), and 45% (□) DH.

RESULTS AND DISCUSSION

Formation of Emulsions

Because all conditions for forming the emulsions were kept constant (apart from the DH and concentrations of the hydrolyzates), the particle size in the emulsion can be taken as being indicative of the emulsifying capacity of the hydrolyzates. The emulsions prepared using the least hydrolyzed material had mean particle sizes of about 0.5 μm, almost independently of the protein concentration, as measured by SALLS. The distributions of particle size in these emulsions showed a single peak with most of the particles being <1 μm, but with a tail toward higher particle sizes. As the DH of the protein increased, this tail became less apparent, until, at DH of 20%, the narrowest peak, showing optimal emulsifying capacity of the hydrolyzate, was obtained (Figure 1). For the preparations that had DH >20, the emulsifying capacity was conspicuously worse, because mean particle sizes were larger and the amounts of material >1 μm were greater. Indeed, the emulsions made from the material of DH 45% were very coarse, having bimodal distributions of particle size and containing some particles with diameters in the tens of microns. This situation was generally worse at lower concentrations of the hydrolyzates, although, for some unexplained reason, the emulsions that were prepared with hydrolyzate concentrations <1% did not share in this general behavior. The results differ from those on heated hydrolyzates of Britten and Gaudin (3), which suggested that the emulsifying capacity and coalescence stability were independent of DH under most conditions.

The dependence on DH of the average particle sizes in the emulsions is shown in Figure 2 for a range of different overall concentrations of the hydrolyzates and demonstrates that, at low DH, the emulsion particles were small; at DH >20, the emulsifying capacity of the hydrolyzates was less. The droplet size depended strongly on concentration at these high values of DH; the oil was more effectively emulsified in the presence of 5% hydrolyzate than 1% hydrolyzate, as estimated by the particle size. This situation may well arise from the small content of larger peptides present in the hydrolyzates, even those with large DH. If these larger peptides are present in sufficient concentration (as occurs at the highest concentrations of hydrolyzate), they may be sufficient to cover the interface completely and to provide a reasonably stable emulsion.

At lower concentrations of hydrolyzate (<1%), the behavior was different because the particle size did not greatly increase, even at DH >20%. Only at the lowest value of the concentration of hydrolyzate (0.02%) was there a significant effect of DH on the droplet size.

The measurements of particle size using DLS confirmed the general impression gained from the

SALLS measurements and gave some impression of steric effects in the emulsions (Figure 3). Two general types of behavior were observed, depending on the concentrations of the hydrolyzate used to make the emulsions. For the higher concentrations of hydrolyzates (>1%), the particle sizes in the emulsions first decreased and then increased as DH increased; the minimum particle size was at DH of 20%, and all emulsions gave similar particle size. Above and below that value, the particle size depended both on the concentration of hydrolyzate and DH. At low DH, the largest concentration of hydrolyzate gave the largest particles. This result can probably be explained by the reduced solubility of the low DH hydrolyzates used to make the emulsions (Table 1). It should be remembered that the diameters that are measured by the DLS technique are hydrodynamic diameters; these are sensitive to the sizes and structures of the materials that make up the surface layer of the emulsion droplets (5, 8). Adsorption of peptide aggregates produces diameters bigger than those of the original droplet core. If the oil droplets are assumed to be much the same size (as suggested by SALLS), the peptide aggregates with the lowest DH will project more from the interface than will the short peptides at larger DH, which explains the decrease in diameter between DH 8 and 20 (Figure 3).

The second behavior pattern evident in the DLS results (Figure 3) was for hydrolyzate concentrations <1%. As was observed with SALLS, the diameters of the droplets in these emulsions were generally larger than in those made with concentrations of ≥1%. For DH <30%, the particle sizes changed little with changing DH, but the particle sizes increased as the concentration of the hydrolyzate was decreased. This relationship is as expected: in any emulsion, the particle size increases as the concentration of the emulsifier decreases. For the highest DH and the lowest concentrations, no measurements were possible because the sizes of the emulsion droplets were too large to be measured using the DLS system.

We calculated that, in most of the emulsions, there was more than sufficient protein or peptide material to cover the oil-water interface. Because of the low concentration of oil, the total surface area of the droplets in the emulsion was approximately 0.4 m^2 if the mean droplet size was 0.5 μm. Because saturation monolayer coverage of the surface by whey protein or casein is known to be approximately 2 to 3 $mg \cdot m^{-2}$)

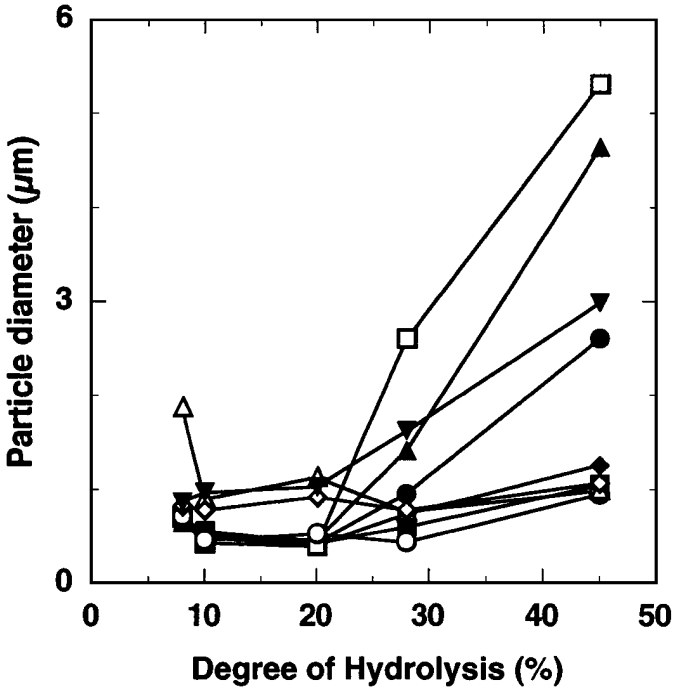

Figure 2. Particle sizes (d_{43}) measured by small angle laser light scattering for the particles in the emulsions (3% oil) prepared from hydrolyzates of different degrees of hydrolysis. The emulsions contained concentrations of peptides of 5% (■), 4% (●), 3% (▲), 2% (♦), 1% (□), 0.5% (○), 0.25% (△), 0.1% (◊), and 0.02% (▼).

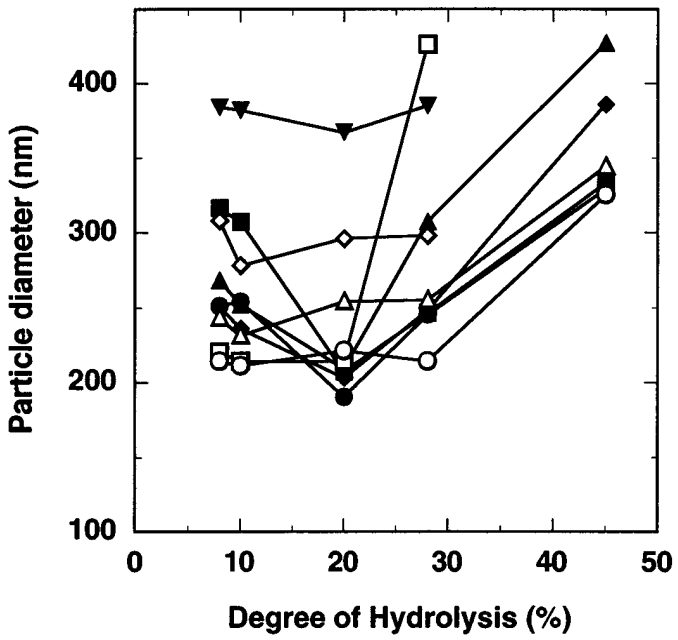

Figure 3. Mean particle sizes in the emulsions measured using dynamic light scattering at 90° scattering angle for the suspensions shown in Figure 2. The emulsions contained concentrations of peptides of 5% (■), 4% (●), 3% (▲), 2% (♦), 1% (□), 0.5% (○), 0.25% (△), 0.1% (◊), and 0.02% (▼).

(10, 11, 12), no more than 1.2 $mg \cdot ml^{-1}$ of protein (i.e., 0.12%) is necessary to form the emulsion. The excess protein either may remain in solution or may form multilayers (4, 19). For even the lowest concentration of the hydrolyzate used (0.02%), there was still enough peptide or protein present, assuming all was adsorbed, to cover the surface to the extent of 1 $mg \cdot m^{-2}$, because, as shown in Figure 2, the particle diameter in these emulsions is 1 μm, and the surface area is correspondingly reduced. Measurement of the adsorption of the peptides from hydrolyzates has shown that, as with intact proteins, the load on the surface depends on the amounts of oil and protein available and is in the range of 0.5 to 3.0 $mg \cdot m^{-2}$ (Agboola et al., 1998, unpublished data). It seems likely that the coarseness of the emulsions prepared from hydrolyzates of low DH is not simply caused by the lack of potential surfactant.

The efficiency of the homogenization step in the manufacture of the emulsions depends on the mechanical properties of the homogenizer and the concentration and surfactant properties of the constituents of the emulsion. Thus, as the size of the peptides in the hydrolyzates is decreased, the surfactant properties of the peptides increase initially, followed by a decrease. The increase in surfactant ability from low to moderate DH presumably arises from the hydrolysis of the original proteins. As the DH is increased by increasing hydrolysis, peptides of steadily decreasing length are generated, until they appear to reach an optimal length (or, more properly, length distribution) for the production of the emulsions (21). However, as the peptide length is decreased further, the emulsifying ability is decreased either because some of the peptides are sufficiently hydrophilic to bind more weakly or not at all to the oil-water interface or because they do not provide an interfacial layer strong enough to prevent recoalescence of the oil.

Stability of the Emulsions During Short-Term Storage

All of the emulsions prepared from hydrolyzates of DH ≤20% were stable during storage for up to 5 d at 5°C; no creaming was observed in any of the preparations, nor was there an increase in particle size, which would suggest that aggregation or coalescence had occurred. There was no apparent influence of either protein concentration or DH on the stability of these emulsions. However, the emulsions made with hydrolyzates of DH >20% were not stable. Within a few hours after they were formed, the emulsions began to show that aggregation and coalescence were occurring, as evidenced by the presence of large particles (developing a bimodal size distribution), and the emulsions were completely destabilized over the course of a few days of storage (the particle sizes were too large to measure, i.e., >80 μm). Neither high nor low concentrations of hydrolyzate were capable of producing long-term stability in these emulsions. The instability probably arises from weak adsorption of the short peptides in these mixtures, giving a poorly stabilizing surface layer that lacks cohesion and offers little barrier to coalescence.

In attempts to modify the instability of the emulsions prepared with hydrolyzates with high DH, mixed emulsions were prepared using mixtures of hydrolyzates of high and low DH. The emulsions were first prepared using 0.25% of one of the low DH hydrolyzates (DH of 8 or 10%), and the high DH hydrolyzate was added to give a final concentration of 4%. The emulsions showed smaller droplet sizes than the emulsion made with the high DH hydrolyzate alone, showing that the larger peptides of the low DH mixtures were not displaced by the small peptides of the added high DH hydrolyzate. This observation seemed to be confirmed by the fact that even an emulsion made with high DH hydrolyzate to which low DH hydrolyzates were added also became more stable, suggesting that the large peptides could actually displace the smaller ones from the oil-water interface.

Effects of Heating on the Emulsions

It is known from previous studies that emulsions prepared using unproteolyzed whey protein isolates are generally stable when heated, provided that the concentration of protein is not too high or that the pH is not in the range of 3.5 to 5.5 (14). In such cases, the emulsions can form gels (15). For the emulsions formed using hydrolyzates (4%) and oil (3%), the results are shown in Figure 4. The unheated control emulsions, as has been shown, contain small particles until the hydrolyzate used has DH >20%, after which the droplets in the emulsion become larger. Heat treatment at 90°C caused some increase in the particle size at moderate DH (≤10%), a considerable increase at DH of 20%, and a large increase in particle size at DH >20%. The most stable emulsions to the effects of heat were made from the hydrolyzates of DH 8 or 10%, which were only slightly affected by the heating at 90°C.

All of the emulsions showed some instability during autoclaving. Although the effects were moderate

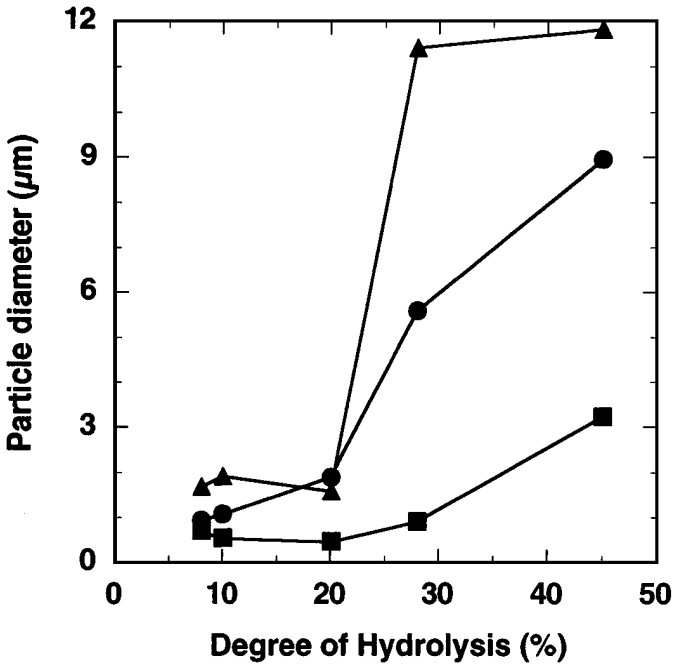

Figure 4. Mean particle diameters (d_{43}) measured by small angle laser light scattering as functions of the degree of hydrolysis in emulsions (3% oil and 4% hydrolyzate) that were untreated (■), heated at 90°C for 30 min (●), or heated at 121°C for 10 min (▲).

at the lower DH values, they were still appreciable, and the high DH emulsions destabilized completely. Both heating regimens, therefore, accelerated the instability of the high DH emulsions and introduced some level of instability into the lower DH preparations. The extent of aggregation induced in these emulsions by autoclaving would render them capable of creaming very rapidly, which might lead to coalescence of the emulsion droplets during storage of the emulsion.

To attempt to improve their stability to heating, the high DH emulsions were mixed with other hydrolyzates as described in the previous section. Emulsions made with small amounts of low DH hydrolyzates and then made up to concentration with high DH hydrolyzates had increased stability with both forms of heating but were in no case as stable as the corresponding emulsions made with the low DH hydrolyzates alone. Similarly, the addition of the low DH hydrolyzates to emulsions made with high DH hydrolyzates also improved the stability, but not to the level of the emulsions made with low DH hydrolyzates alone.

The mechanism of coagulation during heating is likely to arise from a mixture of reactions. Simply, the emulsion droplets are given enough energy to overcome any energy barrier between them. The lack of steric effects (and probably the low charge) of the emulsion droplets made with high DH hydrolyzates would allow close approach to be rather easy. However, it is possible for other interactions, such as the formation of disulfide bonds (18), to be important. The relatively small increases in diameter caused by heating in the low DH emulsions can be taken as an indication that the whey protein fragments do not form an extensive network between the oil droplets because they lack the free sulfhydryl groups to do so.

CONCLUSIONS

These observations make it evident that there is a minimum length for effective stabilization of emulsions by peptides. The mean length of the peptides in the hydrolyzates that were most effective was about 5 amino acids (Table 1). However, the active peptides in the preparation may be longer, because the mixtures of peptides in the hydrolyzates were polydisperse. The functional behavior of such hydrolyzates also depends on the particular enzymes that are used to hydrolyze the original protein, since many proteases do not attack proteins randomly, but at specific sites.

Although the peptides in the low and medium DH hydrolyzates were sufficiently surface-active to provide stable emulsions, they were generally incapable of preventing coagulation of the emulsion during heating. Indeed, they encourage the coagulation of emulsion droplets compared with unmodified whey proteins. This instability may severely limit the application of these hydrolyzates in products that require sterilization by in-can retort heating. Such products are likely to require other ingredients to impart enhanced stability to the emulsion droplets.

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