Rheology and Flocculation of High-Pressure-Treated β-Lactoglobulin-Stabilized Emulsions: Comparison with Thermal Treatment

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High-pressure treatment (HPT) has been shown to induce significant levels of flocculation in model oil-in-water emulsions stabilized by β-lactoglobulin at neutral pH, as indicated by changes in droplet size distribution and rheological behavior. Light microscopy has also provided additional evidence of extensive droplet flocculation following severe treatment. The proportion of unadsorbed protein greatly influences the extent of flocculation, and this may explain in part the nature of these pressure-induced effects. It has been observed that severe HPT (800 MPa for 60 min) is equivalent to relatively mild thermal treatment (TT) (65 °C for 5 min) in terms of the associated changes in emulsion gel rheology. Since HPT destabilizes these emulsion systems to a much lesser degree than TT, it can be considered to be a gentler processing operation in comparison. Emulsion flocculation is more sensitive to pressure and temperature at pH values closer to the isoelectric point and at higher ionic strength. That is, conditions favoring a loss of electrostatic stability tend to cause an increase in sensitivity toward pressure and temperature, although HPT consistently appears to be a gentler process than TT under all conditions studied.

Keywords: *High-pressure treatment; thermal treatment; protein functionality; flocculation; rheology; emulsion stability; emulsion gel*

INTRODUCTION

The effect of high-pressure treatment (HPT) (0−1000 MPa) in modifying the macromolecular structure and functional properties of proteins is now well recognized (Weber and Drickamer, 1983; Balny et al., 1989; Silva and Weber, 1993; Heremans, 1995; Messens et al., 1997). As with thermal processing, the pressure-induced denaturation of globular proteins in concentrated solutions can lead to extensive aggregation and gelation (Johnston, 1992; Dumay et al., 1994; Kanno et al., 1998). In the case of β-lactoglobulin or whey protein concentrate, this pressure-induced unfolding and aggregation is associated with a reduction in emulsifying capacity of the milk protein under conditions of neutral pH and high oil/protein ratio (Galazka et al., 1995, 1996). Structural studies of β-lactoglobulin following HPT have shown (Iametti et al., 1997) that intermolecular disulfide bond formation is essential for the irreversible formation of protein aggregates and that the extent of protein aggregation is highly dependent on protein concentration. The polymerization of partly unfolded monomers (following dimer dissociation) into stable aggregates, as a result of exposure to high pressure, appears in many respects to resemble thermal denaturation of β-lactoglobulin (Cairoli et al., 1994; Iametti et al., 1995, 1996; Roefs and De Kruif, 1994).

There may be advantages in the generation of pressure-induced gels over heat-treated gels in certain food protein applications. Overall, pressure-induced gels are generally considered to be better at retaining color and flavor and softer in texture, in comparison with heat-induced gels. A study involving a selection of different proteins, including soy and egg white proteins, has shown (Okamoto et al., 1990) that gels produced by HPT become harder and less adhesive on increasing the applied pressure.

For the potential of HPT to be fully realized for the rheological control of protein-based food systems, it is necessary to have more detailed information on the comparative effects of HPT and the more traditional thermal treatment (TT). Some significant structural differences between temperature-induced and pressure-induced whey protein gels made from concentrated protein solutions have already been identified (Van Camp and Huyghebaert, 1995). Evidence suggests that TT induces a stronger gel, possessing a greater number of permanent cross-links between the polypeptide chains. Pressure-induced whey protein gels seem to contain a higher proportion of weaker intermolecular bonds and are therefore considered comparatively weak or fragile. Differing influences of HPT and TT on globular protein gelling behavior may reflect different mechanisms of denaturation and aggregation for the two sets of conditions. The conformational structure of the β-lactoglobulin gel network appears to share some features in common with a flocculated emulsion system (Doi, 1993). A substantial increase in the viscosity of concentrated β-lactoglobulin-stabilized emulsions following HPT, including the generation of gel-like characteristics, has recently been demonstrated (Dumay et al., 1996; Dickinson and James, 1997).

In this paper we compare the effects of HPT and TT on the flocculation and rheology of model oil-in-water emulsions stabilized by β-lactoglobulin. We are particularly interested in determining the precise thermal processing conditions that can give changes in the state of aggregation and the viscoelasticity of the emulsions

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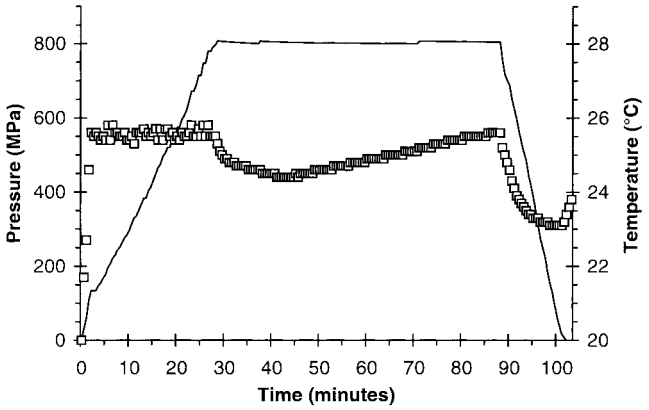

Figure 1. Change in pressure (—) and temperature (□) during a typical HPT run with a set treatment pressure of P_{max} = 800 MPa for a duration of 60 min.

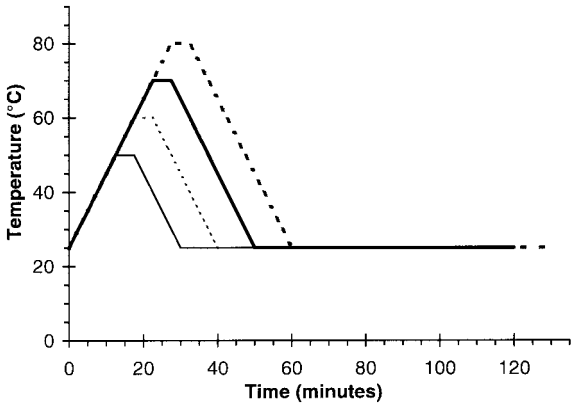

Figure 2. Individual temperature−time profiles for TT processes with different values of set temperature T_{max}: thin solid line, 50 °C; thin dashed line, 60 °C; heavy solid line, 70 °C; heavy dashed line, 80 °C.

similar to those induced by applied pressures in the range 200−800 MPa.

MATERIALS AND METHODS

Reagents and Chemicals. β-Lactoglobulin (3× crystallized and lyophilized) (L-0230, lot 114H7055), imidazole, poly(dimethylsiloxane), and research-grade *n*-tetradecane were purchased from Sigma Chemical Co. (St. Louis, MO). Citric acid and sodium chloride were purchased from BDH Chemicals Ltd. (Poole, England). All buffer solutions were prepared with double-distilled water.

Emulsion Preparation. Three separate sets of *n*-tetradecane-in-water emulsions (20 mmol dm^{-3} imidazole buffer, pH 7.0), containing 0.25, 0.50, and 1.0% β-lactoglobulin (by weight of emulsion), together with 10, 20, and 40 vol % oil, respectively, were prepared at 20 °C using a laboratory homogenizer at an operating pressure of 40 MPa. (The lowest protein/oil ratio giving a fine oil droplet size was adopted to maximize the proportion of the protein emulsifier present that was adsorbed at the oil−water interface.) Freshly prepared emulsions were characterized with respect to droplet size distribution using a Malvern Mastersizer MS20 laser light-scattering particle size analyzer. Fast protein liquid chromatography (FPLC) was employed to estimate the amount of protein present in the aqueous phase. It was found that, at each volume fraction, a mere 5 ± 3% of the total protein used to make these emulsions remained in the serum phase of the emulsion sample following centrifugation.

High-Pressure Treatment. Emulsion samples (8 mL) were hermetically sealed in polyethylene bags and exposed to pressures of 200, 400, 600, or 800 MPa for 60 min in a laboratory-scale high-pressure rig (Stansted Fluid Power Ltd., Stansted, U.K.). The rates of compression/decompression were carefully controlled to avoid significant temperature excursions arising from adiabatic heating/cooling. The temperature was maintained at 25 ± 1 °C during the 60 min period at the set treatment pressure, and it was prevented from rising above 26 °C or falling below 20 °C during the periods of compression and decompression. A typical pressure/temperature profile, obtained for an HPT run at the set treatment pressure of 800 MPa, is shown in Figure 1.

The influence of changing the proportion of unadsorbed protein on the properties of the HPT emulsions was considered by investigating additional systems of higher protein/oil ratio. That is, some samples were prepared with 40 vol % oil + 1.0, 1.5, or 2.0 wt % protein and were subjected to a single pressure treatment of 800 MPa for 60 min.

Immediately after completion of the HPT, each emulsion sample was characterized with respect to its droplet size distribution. Untreated emulsion samples were stored at 25 °C for the duration of the HPT run, and their droplet size distributions were determined again at the same time following preparation as for the pressure-treated samples. This procedure enabled appropriate allowance to be made for changes occurring simply as a result of emulsion aging over a period of equal length to that of the high-pressure processing. [The extent of flocculation of stored whey protein isolate emulsions has been shown to increase over time (McClements et al., 1993).]

Emulsion Rheology. Pressure-treated emulsions (and untreated emulsions of the same age) were characterized in terms of their rheological properties. Small-deformation shear viscoelastic measurements (strain < 0.5%) were made using a controlled-stress Bohlin CS-50 rheometer with a concentric cylindrical cell (inner diameter = 14 mm, outer diameter = 15.4 mm, sample volume ~ 2 mL). Oscillatory measurements were made at 25 °C, and the storage (G'), loss (G''), and complex shear (G^*) moduli were determined over the frequency range from 10^{-3} to 10 Hz. For the purpose of comparing values of G^* for the different samples, the reference frequency of 0.01 Hz was arbitrarily chosen.

Thermal Treatment. The thermal processing was carried out in situ in the cell of the Bohlin CS-50 rheometer. The presence of a thin layer of low-viscosity poly(dimethylsiloxane) on top of the sample was necessary to prevent moisture loss through evaporation at the higher temperatures. Fine *n*-tetradecane-in-water emulsions were prepared with 0.25 or 1.0 wt % β-lactoglobulin, together with 10 or 40 vol % oil, respectively, under the conditions previously specified. Emulsion samples were heated at 2.5 °C min^{-1} from an initial temperature of 25 °C to a variable set temperature in the range 35−80 °C. Following a 5 min period at the set temperature, samples were cooled to 25 °C at a rate identical to that in the heating stage. Figure 2 shows individual temperature−time profiles for set temperatures of 50, 60, 70, and 80 °C. The oscillatory rheology measurements were made as a function of frequency at 25 °C.

Variation of pH and Ionic Strength. The sensitivity to pH and ionic strength of the effect of HPT and TT on emulsion properties has been studied for the system containing 40 vol % oil + 1.0 wt % protein. Emulsion samples were made after the pH of the aqueous phase (20 mmol dm^{-3} citric acid) was adjusted to 6.0, 6.5, or 7.0. In another set of experiments, the ionic strength at neutral pH was adjusted to 0.01, 0.02, 0.04, 0.06, or 0.08 M by addition of an appropriate amount of NaCl to the aqueous phase prior to emulsification. Separate samples of these emulsions were subjected to HPT (800 MPa for 60 min) and TT (65 °C for 5 min).

RESULTS AND DISCUSSION

For all three sets of emulsion systems investigated (10, 20, and 40 vol % oil), it has been found that the HPT, especially at the higher set treatment pressures, causes a substantial increase in average *effective* droplet size d_{43} (see Figure 3). The average particle size

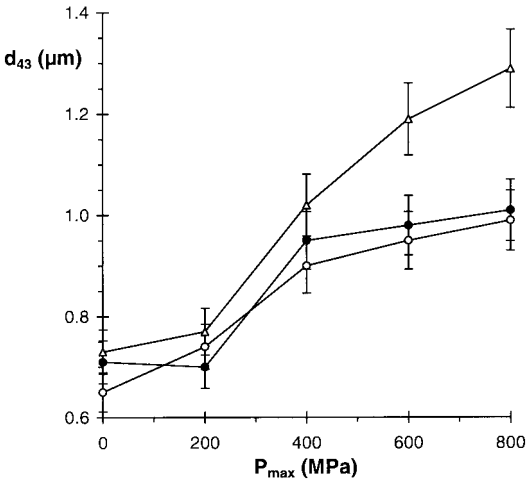

Figure 3. Influence of HPT (60 min at set pressure P_{max}) on the average droplet diameter d_{43} for β-lactoglobulin-stabilized emulsions (pH 7.0) containing oil volume fractions of 10% (○), 20% (●), and 40% (△).

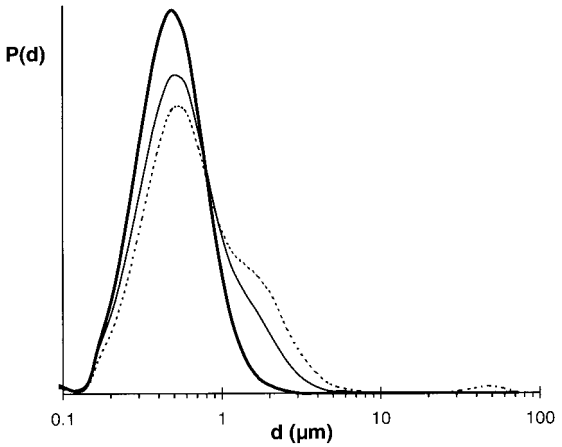

Figure 4. Oil droplet size distributions $P(d)$ for the fresh (heavy solid line), aged (thin solid line), and the most severely pressure-treated (800 MPa for 60 min) (dashed line) emulsion containing 40 vol % oil + 1.0 wt % β-lactoglobulin.

parameter d_{43} is defined by

$$d_{43} = \sum_i n_i d_i^4 / \sum_i n_i d_i^3$$

where n_i is the number of droplets of diameter d_i. The d_{43} value for the freshly prepared emulsion represents the average size of the primary (unflocculated) emulsion droplets.

The d_{43} value quoted in Figure 3 for the untreated emulsion (i.e., one kept at 0.1 MPa) represents the value for the sample stored for an equivalent length of time (105 min) to that of the high-pressure-processing procedure. Changes in d_{43} for stored and pressure-treated emulsions reflect changes in effective particle size of the emulsion systems. (The word "effective" is used here because the changes are predominantly due to the aggregation of the primary droplets and not to their coalescence.) We confirm here gradual changes in average droplet size, and in the droplet size distribution as a whole, that are in agreement with previous work by McClements et al. (1993), which demonstrated significant levels of flocculation in whey protein emulsions during storage of a few hours. Figure 3 indicates that the extent of flocculation is substantially enhanced by HPT at $P_{max} > 400$ MPa.

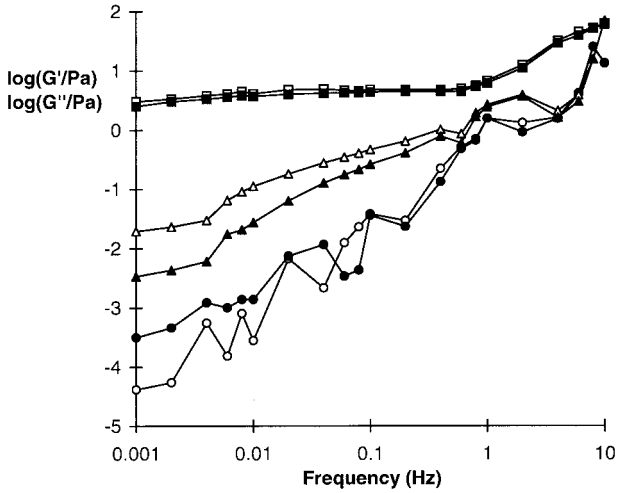

Figure 5. Storage modulus G' (open symbols) and loss modulus G'' (solid symbols) as a function of frequency for untreated and pressure-treated emulsions (pH 7.0, 25 °C) containing 40 vol % oil and 1.0 wt % β-lactoglobulin: ○, ●, untreated; △, ▲, HPT at 400 MPa for 60 min; □, ■, HPT at 800 MPa for 60 min.

Table 1. Average Droplet Size d_{43} in Emulsions Containing 40 vol % Oil with β-Lactoglobulin Concentrations of 1.0, 1.5, and 2.0 wt % before and after HPT of 800 MPa for 60 min

	d_{43} (μm)		
protein concn (wt %)	fresh sample	aged sample	pressure-treated sample
1.0	0.54 ± 0.05	0.73 ± 0.05	1.29 ± 0.10
1.5	0.53 ± 0.05	0.75 ± 0.05	1.66 ± 0.15
2.0	0.55 ± 0.05	0.77 ± 0.05	2.04 ± 0.20

It was suggested by McClements et al. (1993) that the flocs of whey protein-coated droplets are formed initially by noncovalent bonding or bridging flocculation and are subsequently stabilized through the formation of disulfide bonds arising from the reaction of free sulfhydryl groups exposed on adsorbed protein layers. This suggestion is supported by previous observations of time-dependent polymerization of β-lactoglobulin and mixed whey proteins at the oil−water interface via sulfhydryl−disulfide interchange (Dickinson and Matsumura, 1990; Monahan et al., 1993).

The most concentrated emulsion system studied here, containing 40 vol % oil, is the most strongly influenced by HPT. The similarity in d_{43} values in Figure 3 for the untreated samples and those subjected to relatively low intensity HPT (P_{max} = 200 MPa) indicates that pressure-induced changes are negligible at such processing levels. The extent of pressure-induced flocculation for the samples subjected to a high value of P_{max} is indicated by substantial changes in measured droplet size distributions. Figure 4 compares distributions for the freshly prepared emulsion containing 40 vol % oil, the untreated aged emulsion (stored for the duration of the HPT), and the most severely pressure-treated emulsion (800 MPa for 60 min).

The relative contributions of free and unadsorbed protein to the effect of HPT on droplet flocculation have been investigated by altering the total protein present during emulsion preparation. The fractions of unadsorbed protein in fresh emulsions (40 vol % oil) made with 1.0, 1.5, and 2.0 wt % β-lactoglobulin were found to be 5 ± 3, 29 ± 3, and 48 ± 3%, respectively. The effect on d_{43} is shown in Table 1. With increasing

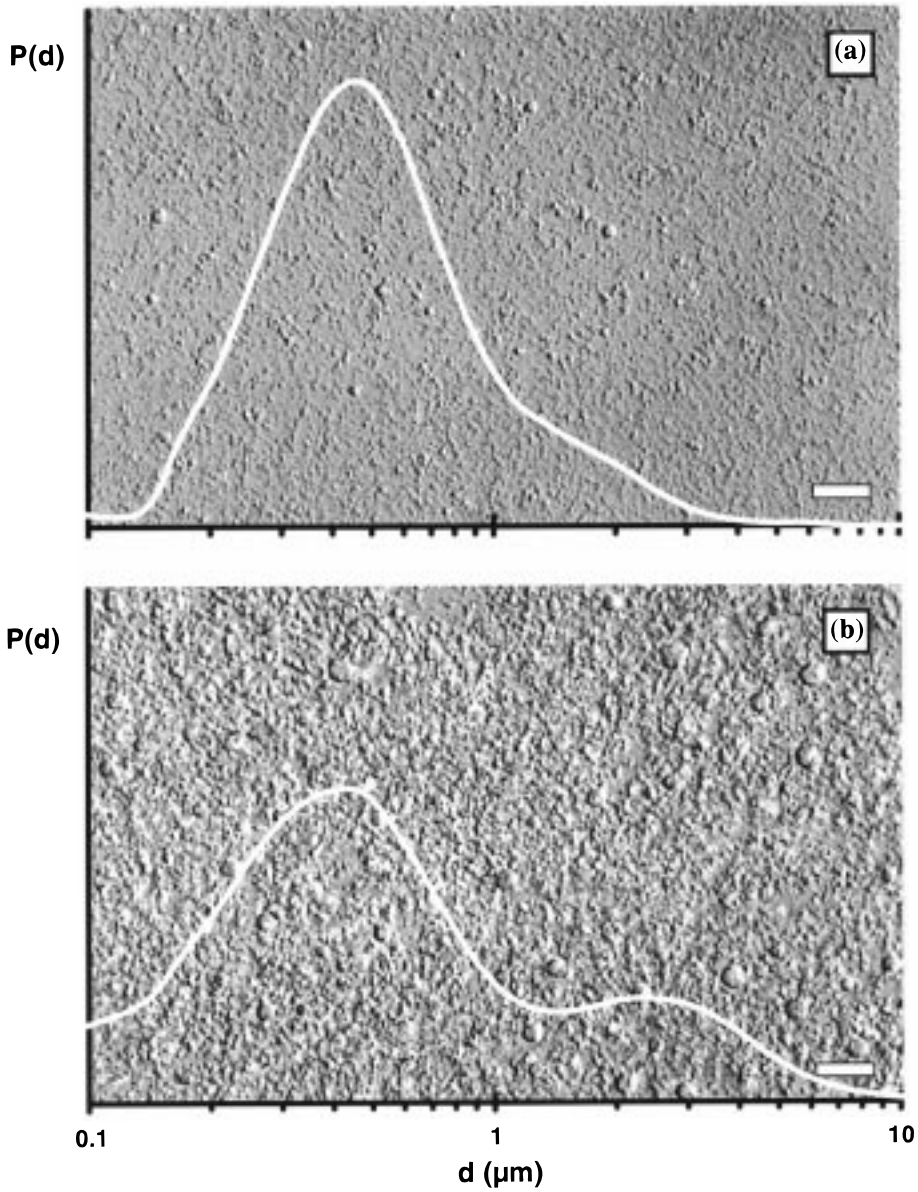

Figure 6. Pressure-induced flocculation of oil droplets in β-lactoglobulin-stabilized emulsions (10 vol % oil, 0.25 wt % protein, pH 7.0) as indicated by light microscopy (a) *before* treatment ($d_{43} = 0.65 \pm 0.05$ μm) and (b) *after* severe HPT (800 MPa for 60 min) ($d_{43} = 0.99 \pm 0.07$ μm); scale bar = 10 μm. The corresponding droplet size distributions of the samples as determined by light scattering after dilution are superimposed.

protein emulsifier content, there is a significant increase in the average effective particle size after HPT, but not in that for the untreated emulsion before or after aging. This would seem to suggest that the presence of unadsorbed protein greatly enhances the degree of pressure-induced flocculation. The behavior can be explained in terms of binding of some of the free protein, in a partially unfolded state, with the adsorbed protein layer, thereby facilitating the formation of direct protein links between the oil droplets. Evidence has been provided (Galazka et al., 1996) to suggest that severe HPT of β-lactoglobulin in concentrated solution causes unfolding and extensive aggregation of the globular protein molecules, as indicated by a reduction in emulsifying efficiency. A separate study by Pittia et al. (1996) has noted that the capacity for protein−protein interactions in the adsorbed state is greater for pressure-treated β-lactoglobulin than for the native protein.

Various rheological characteristics of the *un*treated emulsion are indicative of a typical viscoelastic liquid—a substantial increase in G' and G'' with increasing frequency, the predominance of the viscous component ($G'' > G'$) at low frequency, and the predominance of elastic character ($G' > G''$) at high frequency. The HPT of the 20 and 40 vol % oil emulsion systems was found to induce significant changes in the rheology: the storage and loss moduli become less frequency dependent, and G' remains slightly higher than G'' over the entire frequency range. Figure 5 shows the effect of HPT at 400 and 800 MPa on the rheology of the 40 vol % oil system. Strong gel rheological behavior of the 800 MPa treated emulsion is indicated by the relatively weak frequency dependence of the moduli. In considering the influence of HPT on the rheology of the most liquidlike initial emulsion studied (containing 10 vol % oil), negligible changes in the frequency dependence of the moduli are evident, despite the induction of significant levels of droplet flocculation as indicated by changes in droplet size distribution and the modified appearance in light micrographs (Figure 6).

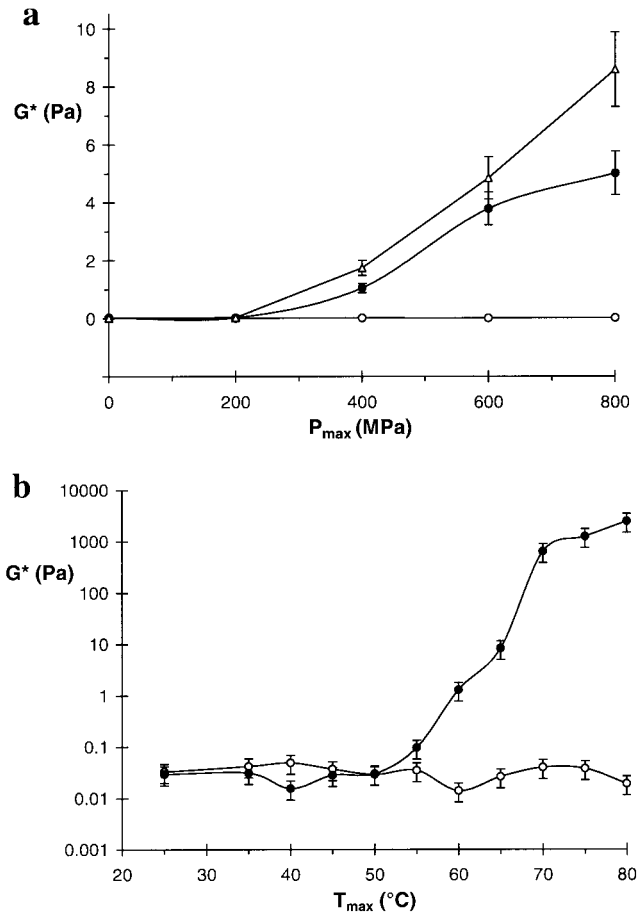

Figure 7. Comparison of influences of HPT and TT on complex modulus G^* (at 0.01 Hz) of β-lactoglobulin-stabilized emulsions (pH 7.0, 25 °C). (a) G^* is plotted against set treatment pressure P_{max}: ○, 10 vol % oil + 0.25 wt % protein; ●, 20 vol % oil + 0.5 wt % protein; △, 40 vol % oil + 1.0 wt % protein. (b) G^* is plotted against set treatment temperature T_{max}: ○, 10 vol % oil + 0.25 wt % protein; ●, 40 vol % oil + 1.0 wt % protein.

The relationship in Figure 7a between G^* at 0.01 Hz and the set treatment pressure P_{max} provides an indication of the sensitivity of emulsion viscoelasticity to processing conditions. Although the values of G^* are not as high as those associated with strong heat-set emulsion gels (see Figure 7b), the plot still serves to illustrate the substantial potential for HPT as a processing tool for texture control in concentrated emulsions (20 and 40 vol % oil). The pressure independence of G^* for samples containing 10 vol % oil is again noteworthy.

One might suspect that β-lactoglobulin in its adsorbed state would be less susceptible to pressure processing, since the molecules are already partially unfolded and are interacting strongly with neighboring molecules in the interfacial layer. On this basis, the adsorbed globular protein molecules could be viewed as existing perhaps in more pressure-resistant macromolecular conformations than their native counterparts. The observed increase in the level of flocculation with increase in protein concentration suggests that protein in the unadsorbed state is a major contributor to the mechanism of pressure-induced flocculation.

We turn now to the temperature-treated emulsions. As with high-pressure processing, there is a negligible change in the small-deformation rheology of the 10 vol % oil emulsion even under the most severe conditions

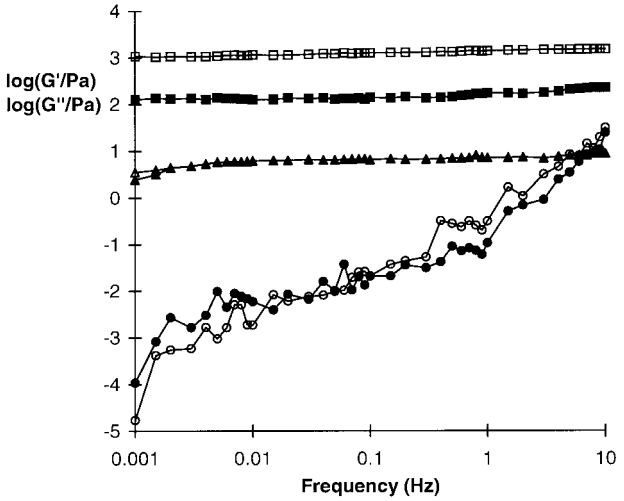

Figure 8. Storage modulus G' (open symbols) and loss modulus G'' (solid symbols) as a function of frequency for untreated and heat-treated β-lactoglobulin-stabilized emulsions (pH 7.0, 25 °C) containing 40 vol % oil and 1.0 wt % protein: ○, ●, untreated; △, ▲, TT at 65 °C for 5 min; □, ■, TT at 80 °C for 5 min.

employed (80 °C for 5 min). In sharp contrast, the emulsion made from 40 vol % oil undergoes very substantial changes in rheological behavior at the higher treatment temperatures. Treatment at 80 °C and subsequent cooling to room temperature leads to "strong gel" rheology as indicated by $G' \gg G''$ and frequency-independent moduli (Figure 8). On the other hand, the warming of emulsion samples to ≤50 °C over the time scale described has a negligible effect on the rheological behavior. Any increase in the induced level of flocculation as a result of this comparatively mild TT is therefore insufficient to be detectable rheometrically. In the intermediate temperature region (around 65 °C), which can be regarded as being close to the "gel point" ($G' \sim G''$), there is a strong sensitivity of the final gel strength to the processing temperature. This is illustrated in Figure 7b by the plot of complex shear modulus G^* at 0.01 Hz as a function of the set temperature T_{max}. The sharp rise in G^* with T_{max} in these 40 vol % oil emulsions corresponds to a dramatic increase in viscoelasticity as a result of strong gel formation at the higher treatment temperatures. Emulsion gels produced at $T_{max} = 65$ °C have G' and G'' values that are already relatively independent of frequency (Figure 8), although the state of aggregation of oil droplets is presumably less extensive than in systems prepared at higher processing temperatures. Figure 7b also shows $G^*(T_{max})$ data for the equivalent 10 vol % oil emulsions. The relative insensitivity of the rheology of the moderately dilute emulsion to thermal processing mirrors the behavior found with HPT (Figure 7a).

The comparison in Figure 7 of the rheological changes induced by HPT and TT indicates that the former is a much gentler treatment than the latter. Maximum values of elastic moduli can be some 2−3 orders of magnitude larger for emulsion gels made by thermal processing ($T_{max} = 80$ °C) than for those made by high-pressure processing ($P_{max} = 800$ MPa). In the experiments reported above, the concentrated emulsion gel (40 vol % oil) generated by *intense* high-pressure processing (800 MPa for 60 min) can have viscoelastic properties that are approximately the same as those for the equivalent emulsion gel generated by *mild* heating (65 °C for 5 min). To facilitate further comparison of factors

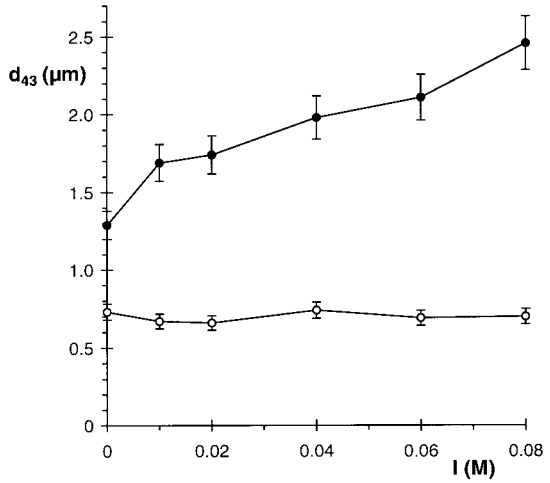

Figure 9. Influence of ionic strength I on average effective diameter d_{43} of β-lactoglobulin-stabilized emulsions (pH 7.0) containing 40 vol % oil and 1.0 wt % protein: ○, untreated sample; ●, pressure-treated sample (800 MPa for 60 min).

Table 2. Influence of pH on the Change in Average Droplet Size d_{43} and Complex Modulus G^* (at 0.01 Hz) of β-Lactoglobulin-Stabilized Emulsions Containing 40 vol % Oil + 1.0 wt % Protein following HPT (800 MPa for 60 min) and TT (65 °C for 5 min)

	d_{43} (μm)			G^* (Pa)	
pH	fresh	aged	HPT	HPT (800 MPa, 60 min)	TT (65 °C, 5 min)
6.0	0.52 ± 0.05	0.76 ± 0.05	1.58 ± 0.15	19.3 ± 3.0	23.2 ± 3.0
6.5	0.53 ± 0.05	0.71 ± 0.05	1.39 ± 0.15	13.6 ± 2.5	15.8 ± 2.5
7.0	0.54 ± 0.05	0.73 ± 0.05	1.29 ± 0.10	8.61 ± 2.0	12.1 ± 2.0

influencing the properties of the HPT and TT emulsion gels, these two sets of conditions have been chosen for investigating effects of pH and ionic strength on the extent of flocculation and the rheology.

Influences of treatment pressure (P_{max}) and temperature (T_{max}) were investigated at pH values for which similar fine emulsions could be prepared (i.e., 6 ≤ pH ≤ 7). The G^* data in Table 2 show that there is an increase in gel strength as pH is reduced from 7 to 6 for both pressure-set and heat-set concentrated emulsion gel systems. This can be interpreted in terms of an increased intermolecular association between β-lactoglobulin molecules during processing when the pH is lowered toward pI. Consistent with this trend is the increase in d_{43} with decreasing pH for the emulsion gel following high-pressure processing (see Table 2). Figure 9 shows that the degree of flocculation following HPT, as measured by the parameter d_{43}, is significantly enhanced by the addition of salt to the aqueous continuous phase. The bimodal droplet size distribution of the pressure-treated emulsion gel made at ionic strength I = 0.08 M (Figure 10) indicates a higher level of flocculation in comparison with the broad shoulder on the distribution for the heat-treated emulsion gel made in the absence of salt (Figure 4). A dramatic increase in the viscoelasticity of emulsion gel samples with increasing ionic strength is evident following either HPT or TT (Figure 11). In the presence of added salt, the processing of an emulsion by either treatment clearly induces an enhanced state of oil droplet aggregation. The sensitivity of the gel strength to ionic strength does seem slightly greater, however, for the emulsion gels produced by thermal processing.

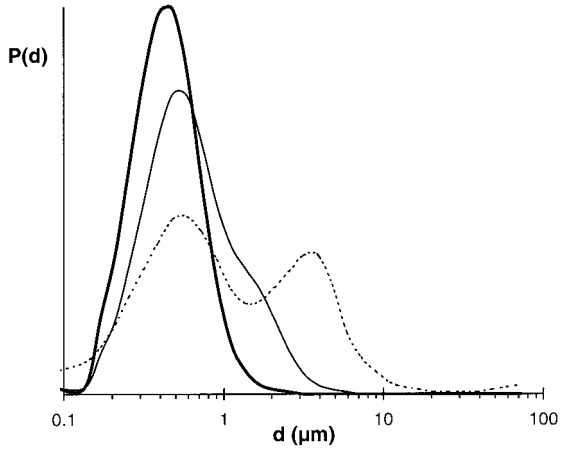

Figure 10. Oil droplet size distributions $P(d)$ for the fresh (heavy solid line), aged (thin solid line), and the most severely pressure-treated (800 MPa for 60 min) (dashed line) emulsion (pH 7.0) containing 40 vol % oil + 1.0 wt % β-lactoglobulin at an ionic strength of 0.08 M.

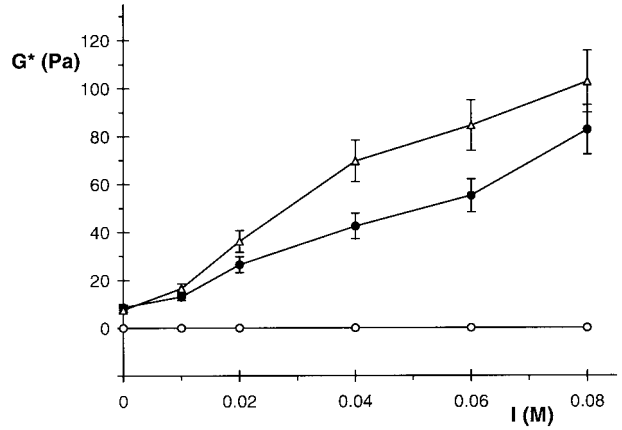

Figure 11. Influence of ionic strength I on the complex modulus G^* of β-lactoglobulin-stabilized emulsion gels (pH 7.0) containing 40 vol % oil and 1.0 wt % protein: ○, before treatment; ●, after HPT (800 MPa for 60 min); △, after TT (65 °C for 5 min).

CONCLUSIONS

Moderate thermal processing has been shown to have a far greater effect than high-pressure processing on the state of flocculation of β-lactoglobulin-coated emulsion droplets. Therefore, generally speaking, HPT can be regarded as a *milder* processing operation than TT. At neutral pH, strong emulsion gels are produced from concentrated emulsion samples (40 vol % oil + 1.0 wt % protein) following heat treatment at ≥70 °C for 5 min. The increase in viscoelasticity caused by mild heating (i.e., 65 °C for 5 min) resembles the change in rheological behavior induced by rather severe pressure processing (800 MPa for 60 min).

Although both treatments induce droplet flocculation, the rheological properties of moderately dilute β-lactoglobulin-stabilized emulsions (10 vol % oil + 0.25 wt % protein) are unaffected by HPT (800 MPa for 60 min) or TT (80 °C for 5 min). This is because of the insensitivity of small-deformation rheological analysis to the state of flocculation of droplets in a dilute emulsion such as this.

Under conditions of lower pH or higher ionic strength, β-lactoglobulin-stabilized emulsions become more flocculated following temperature or pressure processing. The extent of emulsion flocculation following HPT is

seen to be greater when there is a larger proportion of free protein present in the aqueous continuous phase. Previous work has shown that severe HPT causes partial unfolding of β-lactoglobulin molecules in solution, resulting in the exposure of reactive hydrophobic sites. Therefore, one would expect unfolded protein molecules to aggregate in solution, as well as to bind to adsorbed molecules at the surface of oil droplets. The cross-linking or flocculation of droplets by unadsorbed protein, following HPT, provides an explanation for the observed behavior. However, the observation of significant levels of droplet flocculation, even in the presence of minimal unadsorbed protein, suggests that changes in adsorbed protein structure also contribute significantly to the overall effect of pressure treatment.

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