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Stability of concentrated emulsions measured by optical and rheological methods. Effect of processing conditions—I. Whey protein concentrate

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Abstract

The effect of processing conditions (Ultra Turrax (UT) and valve homogeniser (VH)) and protein concentration (0.37–2.93% w/w) on stability of 50% o/w emulsions prepared with commercial WPC were investigated by optical and rheological methods. The UT emulsions were slightly or not flocculated and presented Newtonian behaviour. The D_{43} constantly decreased with increasing protein concentration, the UT emulsions becoming more stable to the creaming process. The VH emulsions presented flocculation and an appreciably smaller particle size, which also decreased with WPC until reaching stability. These emulsions were Newtonian at the lowest concentration but presented shear thinning and hysteresis at the other concentrations. The low-shear viscosity increased with WPC, which suggested that the extent of flocculation was protein concentration dependent. The VH emulsions showed more stability to the creaming process and presented a delay phase which, surprisingly, increased with protein concentration. These results could be explained by a structure formation through a flocculation mechanism, as a consequence of a depletion mechanism and bridging flocculation.

Keywords: Emulsion; Whey proteins; Stability

1. Introduction

Concentrated oil-in-water emulsions find numerous applications in food industry, for example: mayonnaise, infant food, coffee cream, ice cream, sauces, etc. Usually, there is a significant density difference between the oil and the aqueous phases, which, over time, results in the accumulation of the droplets at the top of the container; a phenomenon known as creaming. Emulsions can undergo a number of different instabilities. Those that involve the changes in primary droplet size, such as coalescence, ripening, inversion and breaking, which have knock-on effects on the creaming behaviour, and those that involve the spatial rearrangement of the droplets with respect to each other and with respect to an external frame of reference, such as flocculation and creaming. The commercial importance of creaming is very high, since consumer perception of quality is influenced by appearance (Dickinson, 2001; Robins, 2000a).

Emulsifying characteristics and the stability of the resulting emulsion depend on the properties of the surface-active components in the system. The most important surface-active components in foods are proteins and low–molecular weight emulsifiers (lipids, phospholipids, surfactants, etc.). Milk proteins in soluble and dispersed forms are widely valued as food ingredients with outstanding surface-active and colloid-stabilising characteristics. During emulsion formation, the various protein molecules and aggregates become rapidly adsorbed on the newly formed oil–water interface. The resulting steric-stabilising layer immediately protects the oil droplets against coalescence and thereafter provides physical stability to the emulsion during processing and storage (Carrera Sanchez & Rodríguez Patino, 2005; Dalgleish, 1996; Dickinson, 1998).

The two main types of milk proteins are caseins and whey proteins. As they have a disordered structure, caseins

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are thought to adsorb at the oil–water interface as flexible chains, while whey proteins at the interface form close-packed globular protein monolayers. Contrary to caseins, whey proteins are soluble at any pH and show extensive denaturation at temperatures above 70 °C. Whey protein ingredients may be purchased in several different forms, the most common of which are whey protein isolate (WPI) and whey protein concentrate (WPC). WPI ingredients have higher protein concentrations and fewer impurities than WPC ingredients and, consequently, are more expensive because they require more processing. For this reason, WPI tends to be used in more specialized applications than WPC (Dickinson & Golding, 1997; Dickinson, Golding, & Povey, 1997; Foegeding, Davis, Doucet, & McGuffey, 2002; Sliwinski et al., 2003).

There are many ways to produce an emulsion from two liquid phases that are not mutually or only slightly soluble. It is usually achieved by applying mechanical energy. First, the interface between the two phases is deformed to such an extent that droplets form. These droplets are mostly far too large, and they are subsequently broken up or disrupted into smaller ones. Hence, the disruption of droplets is a critical step of emulsification. Particularly if the volume fraction of the future disperse phase is high, the formation and disruption of droplets may be considered to be at least initiated by making films of the continuous phase, sandwiched between the disperse phase (Walstra, 1983). Droplets can be deformed and disrupted by viscous or inertial forces. Viscous forces generate tangential and normal stresses at the drop surface. Inertial forces generate pressure differences. In practice, it is useful to distinguish three situations: laminar flow, where viscous forces are predominant; turbulent flow, where inertial forces are usually predominant, but viscous forces may be involved, hence, there is often only a gradual difference with conditions in laminar flow; and cavitation, where small vapour bubbles are formed which subsequently collapse extremely fast, causing heavy shock waves in the liquid (a pre-requisite is that the pressure falls below the vapour pressure). The occurrence or preponderance of any of these mechanisms does not depend only on the type of apparatus but also, on continuous phase viscosity and constructional details (Floury, Desrumaux, & Legrand, 2002; Walstra, 1983).

The importance of the emulsifying procedure in the evaluation of a protein as an emulsifier has previously been shown by authors like Tornberg and Lundh (1978). Previous works have shown that processing parameters influence, in a decisive way, on structural parameters such as particle size distribution, inter-droplet interactions, continuous phase rheology, etc; and hence, affect the emulsion rheological characteristics. Thus, an increase in power input during emulsification normally reduces the droplet size distribution of the emulsion (Tornberg, Olsson, & Persson, 1990; Gallegos, Sánchez, & Franco, 2003). Mixers of different types (simple mixers, turbo-mixers, etc.), colloid mills, pressure homogenisers and ultrasonic devices are the main tools for emulsification in the food industry. Various mixers find wide application in emulsion technology, but the high-speed turbo-mixer is among the most efficient. To produce highly dispersed emulsions both the valve homogeniser (VH) and the ultrasonic device can be useful. Tornberg (1980), using different emulsification systems (soybean protein isolate, WPC and sodium caseinate), found that the emulsifying efficiency of the turbo mixer is poorest in terms of creaming stability of the emulsion formed. The ultrasonic device was generally the best choice of equipment at lower-power input, whereas at increased power consumption the VH is an equally good, or even better alternative. In general, all these works have been made on the basis of particle size analysis and protein load determination. However, few works were concerned about the effect of the emulsifying procedure on emulsion stability. Consequently, the need for better understanding and control of the emulsification process is of vital importance.

The food ingredient industry is still concerned with studying commercial WPC in real systems. In this contribution, the creaming and flocculation of concentrated oil-in-water emulsions prepared with different amounts of commercial WPC as the sole emulsifier and stabiliser, using two emulsifying procedures: the Ultra Turrax (UT) and the VH were studied. Particle size analysis, optical characterisation and viscosity were the experimental methods used here to investigate the effect of processing conditions and WPC content on the stability of a concentrated emulsion system.

2. Materials and methods

2.1. Materials

Commercial powdered WPC with the following composition: protein 82.38% (dry weight basis), lactose 5%, fat < 7%, ash 4.33% and moisture 5.56%, was supplied by Milkaut S.A. (Frank, Santa Fe, Argentina).

Commercial corn oil (Mazzola, Refinerías de Maíz, Escobar, Argentina) was used as dispersed phase.

2.2. Protein solutions

Whey protein solutions were prepared by dissolving the WPC powder under gentle stirring in distilled water at 20 °C. The solution pH was 6.7. The protein concentrations considered in this study were from 0.37 to 2.93% w/w.

2.3. Emulsion preparation

Fifty percent oil-in-water emulsions were prepared at 20 °C following two procedures:

***Procedure* 1.** The WPC solution and the corn oil were emulsified with an UT (T25 IKA-Labortechnik, Germany)

using an S25 N-10 G dispersing tool (7.5 mm rotor diameter) at 25,000 rpm for 3 min.

***Procedure* 2.** The pre-emulsion prepared by Procedure 1 was then emulsified in a VH (Stanted—DRG, Stanted Fluid Power Ltd., Essex, UK) (VH), using a single pass at 40 MPa.

2.4. Particle size

The droplet size distribution of the emulsions was measured with a Coulter Counter LS-230 (a laser diffraction-based particle size analyser). The volume mean diameter D_{43} was determined as follows:

$$D[4,3] = \sum_{i=1}^{N}(n_i d_i^4) \Big/ \sum_{i=1}^{N}(n_i d_i^3)$$

where d is droplet diameter, N is total number of droplets, and n_i is number of droplets of d_i diameter.

Fresh emulsions were observed with a Leica DMLB optical microscope (0.1% SDS solution) operated with a transmitted light and phase contrast. Micrographs were taken with a Leica DG 100 camera.

2.5. Viscosity

Rheological measurements were performed at 20 ± 0.1 °C, using a stress controlled rheometer Rheostress 600 (Haake, Germany) with a plate–plate fixture (60 mm diameter). Ascending and descending flow curves of shear stress versus shear rate were carried out in the range of 1–100 s^{-1} during 300 s each. Two or three replicates were performed. The samples were covered with a thin film of silicone oil to prevent water vaporisation during measurements.

2.6. Optical characterisation

A QuickScan (Beckman–Coulter Inc., Fullerton, CA, USA) instrument was used to analyse the emulsion stability. The basic features of this equipment are the following. The sample to be analysed is contained in a cylindrical glass measurement cell. Near the cell is a reading head composed of a pulsed near infrared light source ($\lambda = 850$ nm) and two synchronous detectors. The transmission detector receives the light, which goes through the sample (0°), while the back-scattering (BS) detector receives the light backscattered by the sample (135°). The Quickscan head scans the entire height of the sample (about 65 mm).

The transmission (T) and the BS profiles are obtained as a function of the sample height and time. No change in these profiles would take place during the observation time for a total stable system. The BS profile at $t = 0$ was considered as the reference to analyse the stability of the system.

2.6.1. Kinetics of destabilisation

Particle migration kinetics such as creaming was evaluated by registering peak thickness variation at a threshold value versus time, i.e., the creaming rate was determined from the slope of a plot of the height of the lower layer versus time in the initial stages of creaming (Carrera Sanchez & Rodríguez Patino, 2005).

3. Results and discussion

3.1. Emulsions prepared with UT

3.1.1. Particle size

The size distribution and D_{43} dependence on WPC protein concentration for oil-in-water emulsions prepared with UT is shown in Fig. 1a and b. When WPC concentration was increased, a significant variation in size distribution and in D_{43} was noted. The size distributions of the emulsions prepared with UT presented three droplet

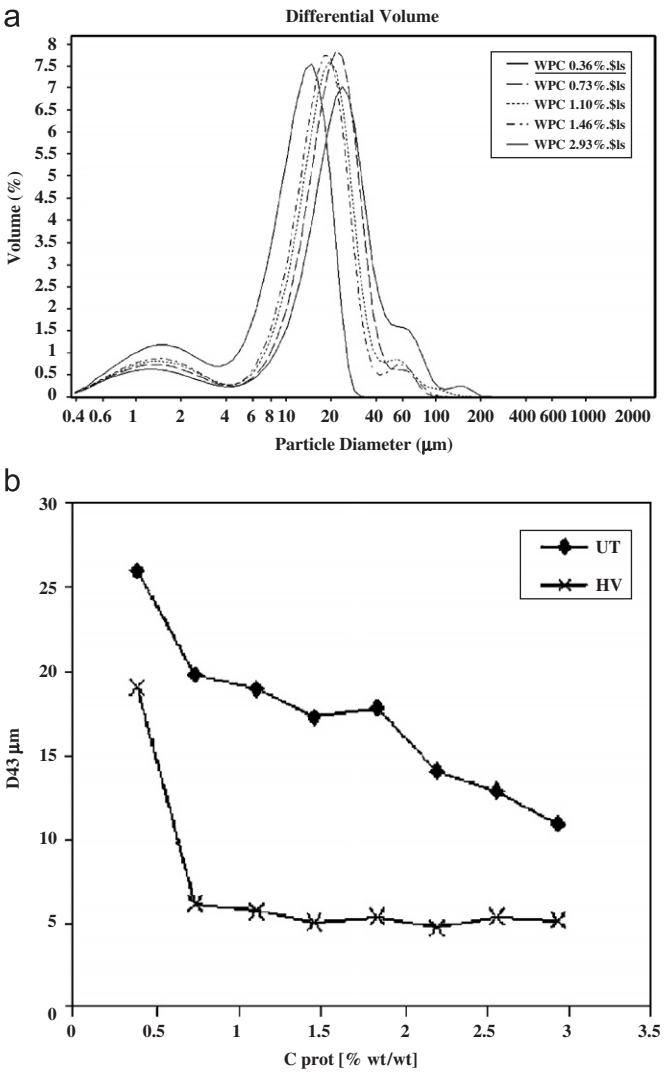

Fig. 1. (a) Effect of protein concentration on size distribution in emulsions prepared in Ultra Turrax (UT). (b) Effect of WPC concentration on D_{43} in emulsions prepared in UT and valve homogeniser.

populations: one main population of about 20 μm and two smaller populations (a bigger one and a smaller one in size). With increasing protein concentration, the main population moved down to smaller sizes (of about 10 μm), the bigger population decreased until it disappeared and the smaller population increased. Hence, the D_{43} decreased constantly with WPC concentration, being about 26 μm for 0.37% and 11 μm for 2.93% w/w of protein. Previous works (Lizarraga, De Piante Vicin, González, Rubiolo, & Santiago, 2006) have shown that, at the WPC protein concentrations studied, the behaviour of the continuous phase is essentially Newtonian and presents slight changes with protein concentration. Therefore, the UT results suggested that a slight increase in continuous phase viscosity would have generated an increased power input but also, the droplet size distribution being modified would probably have changed the shear stress exerted by the overall fluid on each of the droplet. Hence, the size distribution probably decreased as a result of a combined effect of continuous phase viscosity and droplet crowding effects. As was expected, although protein concentration was high enough to cover a larger oil droplet surface area, the UT homogeniser was incapable of creating it.

The emulsions prepared with UT treated with and without 0.1% SDS showed no appreciable changes either in D_{43} or in size distributions (Fig. 2b), which suggested that these emulsions were little flocculated; except for the lowest concentration of protein (0.37%) in which the addition of SDS solution lead to a reduction in the population of large particles (>100 μm). Bridging flocculation may be present under these conditions of low-power homogeniser and low concentration of protein.

3.1.2. Viscosity

Fig. 3 shows the steady-state rheological behaviour of UT oil-in-water emulsions and the shear stress versus shear rate at different WPC protein concentrations. It can be appreciated that the rheological behaviour was essentially Newtonian for all protein concentrations and, as was expected, the shear stress increased with increasing WPC. These results corroborated the fact that the emulsions prepared with UT were not flocculated, as was previously assumed.

3.1.3. Optical measurements and destabilisation kinetics

BS of light from emulsions with different WPC protein concentrations and prepared by the two different methods was measured as a function of height over a period of time. Initially, BS is fairly constant across the entire height of the emulsions due to an even distribution of droplets

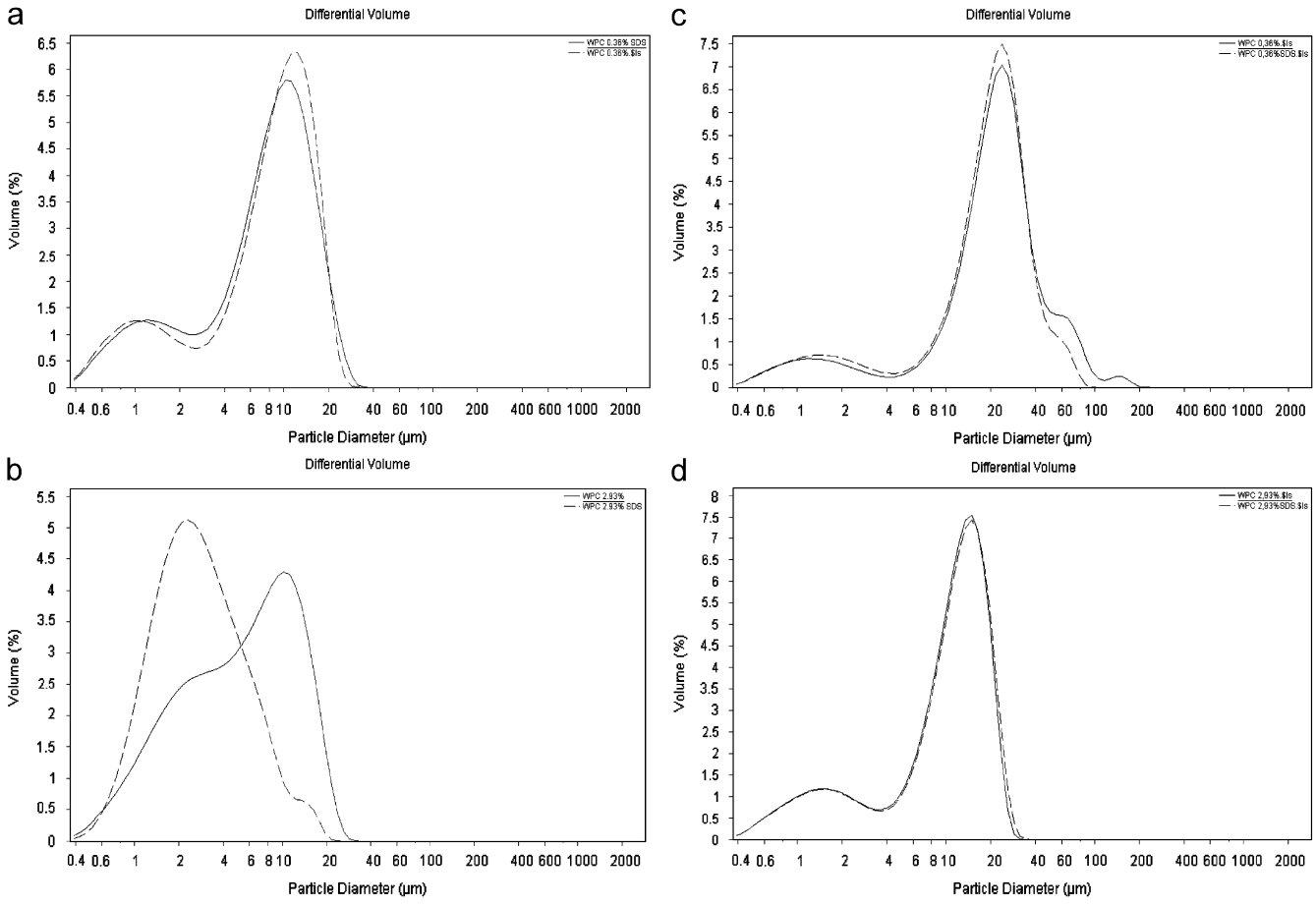

Fig. 2. (a) D_{43} of emulsions prepared with VH of concentrations 0.37% w/w (a) and 2.93% w/w (b), with (-----) and without SDS (—). (b) D_{43} of emulsions prepared with UT of concentrations 0.37% w/w (c) and 2.93% w/w (d), with (-----) and without SDS (—).

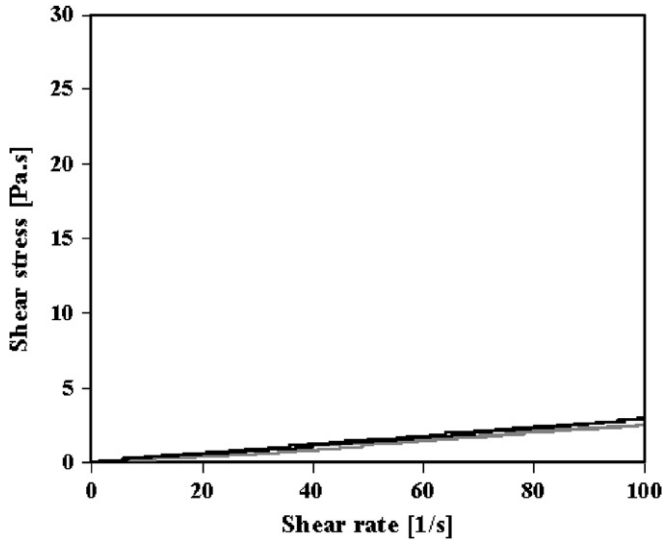

Fig. 3. Shear stress versus shear rate for the UT emulsions from 0.37 (——) to 2.93 (——) % w/w of protein concentration.

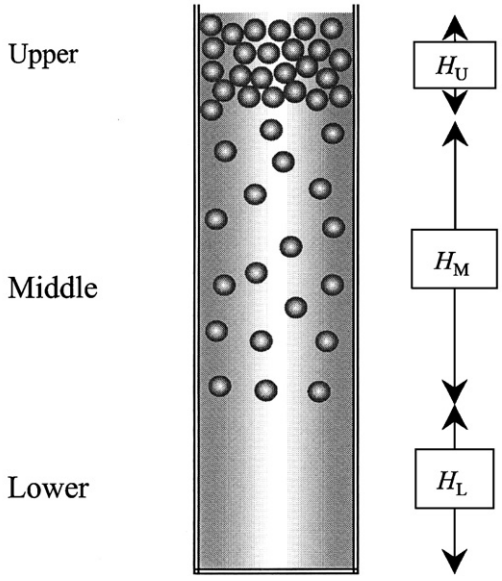

Fig. 4. Emulsion behaviour with time: a lower region depleted of droplets, a middle region with a droplet concentration close to the original one, and an upper region containing close-packed droplets.

throughout the system. As the droplets moved upwards due to gravity there was a decrease in BS at the bottom of the emulsions because the droplet concentration decreases (Fig. 4) (Carrera Sanchez & Rodríguez Patino, 2005; Chanamai & McClements, 2000a, 2000b; Robins, 2000b).

Fig. 5a and b shows the ΔBS profiles corresponding to WPC emulsions prepared with UT. At 0.37% w/w of protein (Fig. 5a), it can be appreciated that an important creaming process took place at the bottom of the sample as evidenced by an increase of the peak thickness of the backscattered light as it became droplet-depleted with time. The behaviour associated with the same type of emulsion prepared with 2.93% w/w of protein is shown in Fig. 5b. A

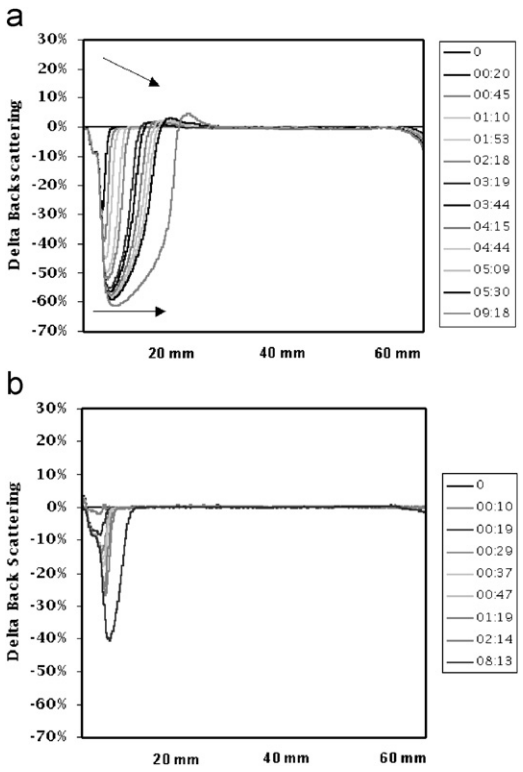

Fig. 5. Delta backscattering profiles of UT emulsions of concentrations (a) 0.73% w/w and (b) 2.93% w/w.

less relevant creaming process was observed at the bottom of the sample tube as compared with that of 0.37% w/w protein emulsion. As can be appreciated at the ΔBS profiles, in the lower portion of the cell tube there was an aqueous phase separation of about 10 mm for the 2.93% w/w emulsion, whereas for the 0.37% w/w emulsion the separation was of 20 mm when observed after 8 h.

The creaming process kinetics for the UT oil-in-water emulsions is shown in Fig. 6. When WPC protein concentration was increased, the emulsion became more stable to the creaming process. As can be observed at the profiles, the creaming kinetics can be divided in two stages: a very high creaming rate process at short times and then, a slower creaming process. At low WPC concentration, a rapid separation of the cream phase took place at the early stage and then, the creaming process became slower. With increasing WPC concentration, the early stage became less significant and did not follow any pattern in time. The second stage rate became slower with concentration, indicating that the emulsion was more stable to this destabilisation process. The concentration dependence of creaming rate (second-stage slope) is shown in Fig. 7. An exponential reduction in the creaming rate was found when the WPC protein concentration was increased ($R^2 = 0.9635$).

According to Robins (2000a), the creaming rate behaviour observed corresponds to droplets poly-disperse in size and not flocculated or forming very small aggregates,

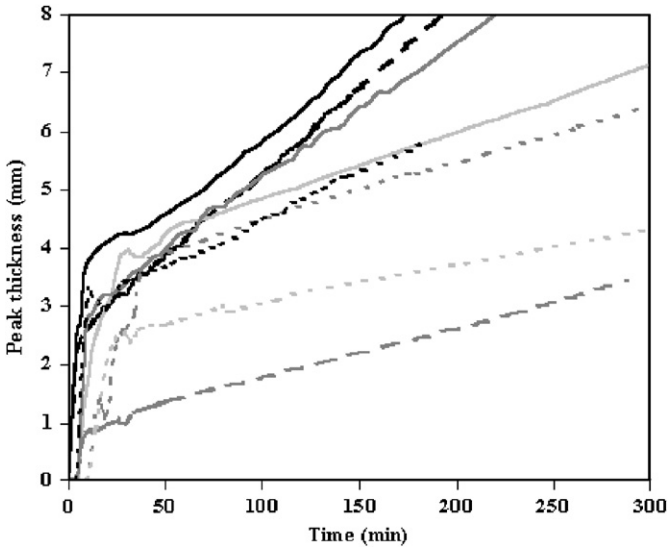

Fig. 6. Creaming kinetics of oil-in-water emulsions prepared with UT of concentrations: 0.37% (——), 0.73% (— — —), 1.10% (——), 1.46% (▪▪▪▪▪▪), 1.83% (——), 2.20% (▪▪▪▪▪▪▪▪), 2.56% (— —) and 2.93% (▪▪▪▪▪▪▪▪).

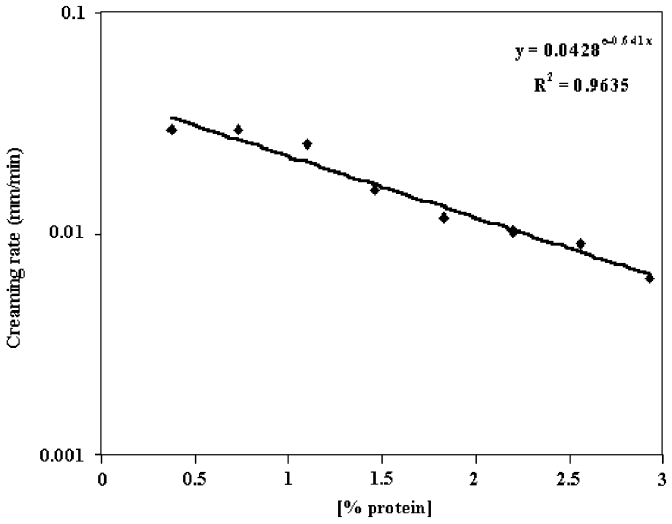

Fig. 7. Concentration dependence of creaming rate for UT emulsions.

which behave as single particles. In emulsions of this type, a diffuse boundary separates the emulsion phase from the serum layer and this boundary is difficult to monitor visually, although within the emulsion, the droplets are still moving at a constant speed, depending on their size. When the droplets reach the top of the emulsion they form a uniform cream layer of volume fraction ϕ_m and, in the poly-disperse case, the cream layer can rearrange to a higher packing fraction than in mono-disperse emulsions.

These results are in accordance with the droplets size distributions shown in Fig. 1(a) where the poly-dispersal in size of UT emulsions can be appreciated. The two creaming stages at the kinetic profiles correspond to the two main populations of droplets that creamed at different times depending on size. With increasing WPC protein concentration, the population of the bigger droplets decreased in size and the proportion of the smaller ones increased, as was previously analysed; hence, this would explain the reduction of the early stage at the creaming kinetics and the decrease of the creaming rate at the second stage. The close-packed cream layer stage was not observed in either concentration and this could be due to the high-volume fraction evaluated.

The correlation between particle size and creaming rate is given by Stoke's law. The creaming rate of an isolated rigid spherical particle in an ideal liquid is given by

$$U_{\text{Stoke}} = \frac{-2gr^2(\rho_2-\rho_1)}{9\eta_1},$$

where U is the creaming velocity, η the shear viscosity, r the particle radius, g the acceleration due to gravity (9.8 m s^{-2}), ρ the density and the subscripts 1 and 2 refer to the continuous and dispersed phases, respectively. For finite droplet concentrations, this equation must be modified. Where there is a significant number of other droplets of volume fraction ϕ, the presence of neighbouring droplets and the consequent backflow of continuous phase against the droplet movement causes a reduction in the terminal velocity of the droplets, so that they cream at a velocity $U = U_S \cdot f(\phi)$. The hindrance factor $f(\phi) < 1$ and, in the ideal case, depends only on the local concentration of droplets (Chanamai & McClements, 2000a, 2000b; Robins, 2000a). When the data obtained were applied to the Stoke's creaming rate equation the following results arose: since particle size decreased about one-half from 0.37% to 2.93% w/w (Fig. 1b) and the continuous phase viscosity had little contribution, the emulsion creaming rate would be expected to decrease about six times, however, it decreased about eight times (Fig. 7). Differences between theoretical and experimental data could be explain by the elevated volume fraction evaluated and the inter-droplet interactions.

3.2. Emulsions prepared with VH

3.2.1. Particle size

The D_{43} dependence on WPC protein concentration for oil-in-water emulsions prepared with VH is shown in Fig. 1b. The D_{43} decreased with WPC concentration in both emulsification methods, the mean droplet sizes for the VH emulsions being significantly lower than those obtained with UT. According to Floury et al. (2002), when studying the effect of ultra-high pressure homogenisation on structure and on rheological properties of soy protein-stabilised emulsions, for droplet break up in a homogenising device, droplet size distribution is the result of an equilibrium between disruptive forces and viscous forces. In turbulent flow, it is known that mean droplet diameter is only a function of the ratio of inertial to surfaces forces, characterised by the Weber number. But for laminar and

transitional flow regimes, mean droplet diameter is also a function of viscous forces. It means that the effect of the parameters, which characterise disruptive forces, results from the relative influence of surface and viscous forces. Therefore, the effect of the homogenising pressure is dependent on precise operating conditions, that is, the Reynolds number (*Re*) and physical properties of the product. For a small homogenising valve, like the one used in this study, where the slit width may be of the order of micrometres, *Re* is rather small and the regime is laminar. It can be appreciated that, in UT emulsions, the D_{43} decreased constantly with protein concentration whereas in VH emulsions, the D_{43} was stabilised from 0.73% w/w of WPC protein. The VH results would be indicating that there existed a critical concentration of WPC protein (0.73% w/w) in the aqueous phase at which the emulsifying capacity was maximum and the D_{43} was minimum. At protein concentrations above 0.73% w/w the emulsifying capacity was constant and the droplet size distributions were practically mono-modal. From this WPC protein concentration, the interfacial film around the droplets may be saturated, as was found by Carrera Sanchez and Rodríguez Patino (2005) studying the interfacial, foaming and emulsifying characteristics of sodium caseinate. The excess of WPC protein would participate in the formation of multi-layers and/or would be present in the aqueous phase with repercussions in the stability of the emulsions. At WPC protein concentrations below 0.73% w/w, the emulsions presented two populations of droplets (Fig. 2a) and a high D_{43}. These results suggested that the interfacial film was probably not saturated and, at 0.37% w/w of protein, the VH emulsion might present flocculated droplets, as will be analysed later. According to Euston and Hirst (1999), the bimodal nature at low protein contents is a feature of the WPC size distributions indicating two separate populations of droplets. These authors, citing Rosenberg and Lee, attributed this feature as being due to clustering of droplets through bridging flocculation.

Fig. 2a also shows the size distributions of emulsions prepared with VH treated with and without 0.1% SDS. It can be observed that SDS had no effect on size distribution and on D_{43} in the emulsion with the lower concentration of WPC (0.37%) while, at the other protein concentrations, a decrease in D_{43} was observed when SDS was added. The results would be indicating that: (i) at concentrations below 0.73% w/w, the emulsion would not be flocculated; (ii) from 0.73% w/w of protein, whereas the UT emulsions would be slightly or not flocculated, the VH emulsions would be highly flocculated.

The results provided by the Coulter Counter measurements were in agreement with those obtained by microscopic analysis (not shown) for the UT and VH o/w emulsions. As was previously described, the effect of WPC concentration and the emulsification procedure on droplet size distribution and on D_{43} was microscopically observed.

3.2.2. Viscosity

The rheological behaviour associated with the oil-in-water emulsions prepared with VH is shown in Fig. 8. Under these conditions, the shear stress (and viscosity) was appreciably higher than the UT emulsion shear stress. The 0.37% w/w protein emulsion behaviour was practically Newtonian. As the protein concentration was increased to greater than 0.73%, shear-thinning behaviour and hysteresis were observed. A significant increase in the low-shear apparent viscosity, as well as an increasing shear thinning and hysteresis nature at higher applied stresses were appreciated in the following concentrations.

On the one hand, these results were expected since the particle size decreased, the volume fraction was not modified, and hence, there was a significant increase in droplet concentration as a result of the high-pressure homogeniser. According to Chanamai and McClements (2000a), when studying the dependence of creaming and rheology of mono-disperse oil-in-water emulsions on droplet size and concentration, the effective volume fraction of the smaller droplets is greater than that of the larger droplets which accounts for their higher viscosities, but also, the electrostatic repulsion plays an important role in determining the rheology of concentrated emulsions. Electrically charged droplets are not able to approach each other as closely as uncharged droplets, and consequently they are not able to take full advantage of the upward drag that occurs in the vicinity of neighbouring droplets. At low shear rates, the droplets are not able to approach closely together because of the electrostatic repulsion between them, therefore their effective volume fraction is greater than their actual volume fraction. The droplet concentration required to reach close packing in the emulsion is therefore decreased. This effect is less significant at high shear rates because the stresses are large enough to overcome the electrostatic repulsion between the droplets and force them together.

On the other hand, both the hysteresis and the shear-thinning behaviour are usually associated with the presence of any structure that is totally or partially broken down with increasing shear rate. According to Tadros (2004), the above behaviour can be explained from consideration of the structure of the system. If the emulsion is weakly flocculated, then, on applying a shear force on the system, this flocculated structure is broken down and this is the cause of the shear-thinning behaviour. Weakly flocculated emulsions usually show hysteresis and the change of hysteresis with applied time may be used as an indication of the strength of this weak flocculation. At higher protein concentrations, there was a stronger structuring of the droplets in the flocculated emulsions. As was previously analysed, the behaviour of the continuous phase was essentially Newtonian and presented slight changes with protein concentration. Hence, there was no significant contribution from the continuous phase viscosity to the

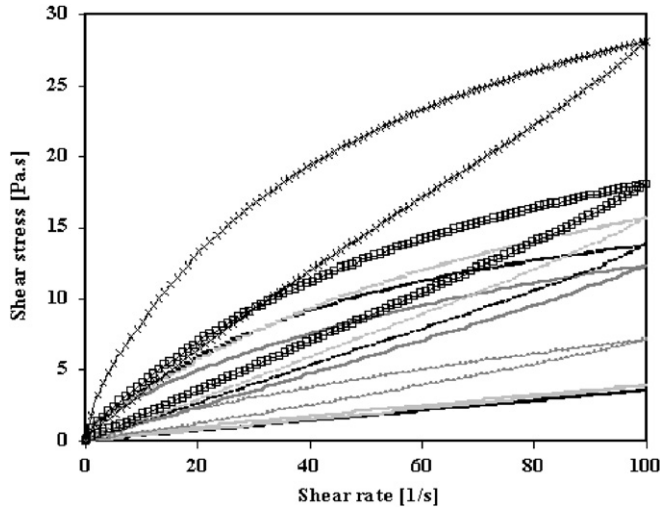

Fig. 8. Shear stress versus shear rate for the VH emulsions of concentrations: 0.37% (▬), 0.73% (▬), 1.10% (▪▪▪▪▪▪▪▪), 1.46% (▬▬▬), 1.83% (▪▪▪▪▪▪), 2.20% (▪▪▪▪▪▪▪▪), 2.56% (—) and 2.93% (×).

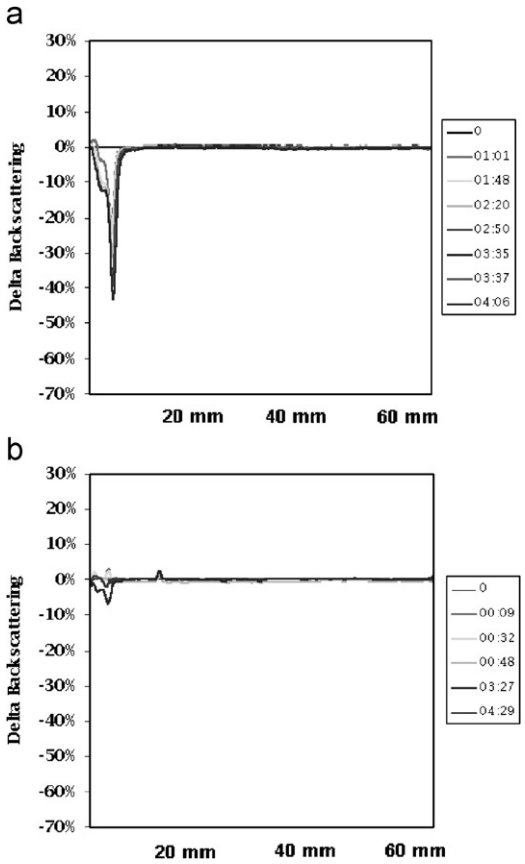

Fig. 9. Delta backscattering profiles of VH emulsions of concentrations (a) 0.37% and (b) 2.56% w/w.

behaviour observed at the VH oil-in-water emulsions. The increase in low-shear viscosity with increasing WPC protein concentration suggested that the extent of flocculation was protein concentration dependent.

3.2.3. Optical method and kinetic destabilisation

BS of light profiles for VH emulsions of 0.37% and 2.56% w/w of protein concentration are displayed in Fig. 9a and b. When the two methods were combined, a decrease of the peak thickness of ΔBS was observed corresponding to a relevant reduction of the creaming zone. These findings are in accordance with smaller particle sizes (smaller D_{43}) as compared with those obtained with UT alone. Under these conditions, the creaming process also decreased with increasing protein concentration, as was observed with UT, until the emulsion became stable during the observation time. The phenomenon of delay time was recorded from 2.20% w/w of the protein concentration.

The comparative evolution of the creaming kinetics for VH emulsions is shown in Fig. 10. A low concentration of WPC (0.37%) promoted a creaming process with both an initial rate and second-stage rates lower than those of the same emulsion prepared with UT alone. These results are consistent with the bimodal nature of this emulsion and the lower D_{43} observed in the VH emulsions (Fig. 1b). When WPC concentration was increased to 0.73% w/w a latency period appeared. The duration of the latency period, or delay time, was found to increase with WPC concentration, the 2.93% protein emulsion being completely stable for 300 min. When the delay time was plotted against WPC protein concentration (Fig. 11), a linear correlation was found ($R^2 = 0.9797$). We suggest that this phenomenon would be occurring since the flocculated emulsions would have formed a single network structure which was initially stress-bearing and behaved like a compacting cream then the network rose steadily until the cream layer was reached. According to Robins (2000a), these emulsions may be described as a particle gel. According to Dickinson et al. (1996), even at protein contents below that corresponding to full surface coverage, there is some unbound protein

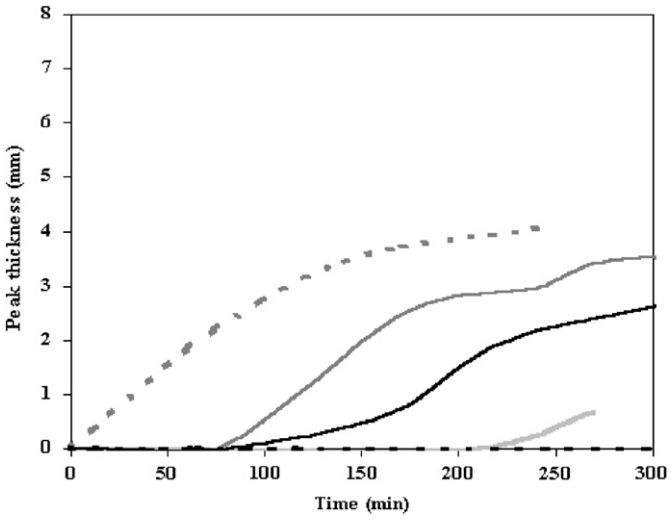

Fig. 10. Creaming kinetics of oil-in-water emulsions prepared with VH of concentrations: 0.37% (▪▪▪▪▪▪▪▪), 0.73% (▬▬▬), 1.46% (▬▬▬), 2.56% (▬ ▬ ▬) and 2.93% (▪▪▪▪▪▪).

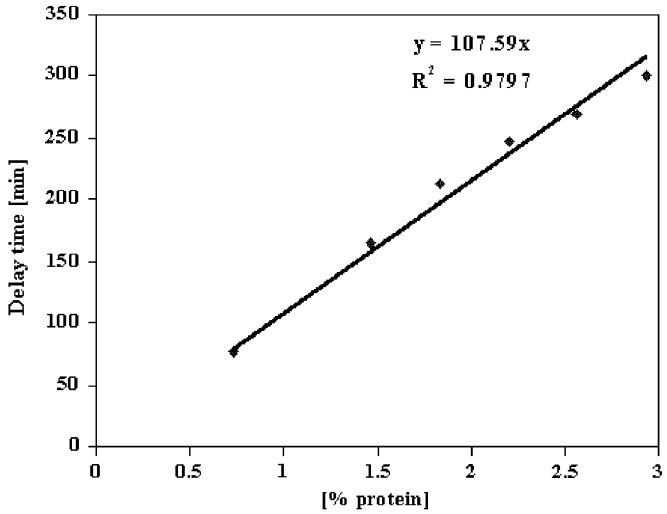

Fig. 11. Delay time of VH emulsions versus WPC protein concentration.

present in the aqueous phase. With increasing overall protein content, there is a steady increase in the concentration of unbound protein, resulting in a corresponding enhancement in the extent of flocculation of the droplets, from the initial formation of discrete individual flocs through the development of a more expansive droplet-network structure. In concentrated emulsions of the type investigated, separate droplet or floc movement to form a distinct cream layer becomes extremely restricted when the flocculation is extensive. This is because the droplets in the creamed layer are so tightly packed that they cannot move any further. Besides, the electrostatic repulsion also plays an important role in determining the creaming velocity in concentrated emulsions. The droplets become close packed, causing the emulsion to become rigid, at lower concentrations for smaller droplets because their effective volume fraction is greater (Dickinson, 2000).

The combined results obtained from the VH oil-in-water emulsion investigation, which are consistent with a flocculation process, could be explained analysing different mechanisms. Both the shear thinning, the hysteresis behaviour, the increase in low-shear viscosity, the re-stabilisation or reduced creaming and the protein concentration dependence are a typical evidence of an emulsion system undergoing flocculation via a depletion mechanism. The bibliography analysed has suggested that solutions of globular whey protein molecules do not cause depletion flocculation which is attributed to the more aggregated form of protein in these samples. This is reasonable since depletion flocculation is usually associated with non-adsorbing macromolecules or compact molecular assemblies (nano-particles) such as surfactant micelles, i.e., particles with more homogeneous characteristics than WPC constituents. Casein nano-particles are postulated to be excluded from the interstitial space and to cause depletion flocculation in approximately the same fashion as surfactant micelles (Berli, Quemada, & Parker, 2002; Radford & Dickinson, 2004). Blijdenstein, Veerman, and van der Linden (2004) proposed a depletion flocculation mechanism in β-lactoglobulin-stabilised emulsions induced by fibrillar protein assemblies. Taking into account that flocculation has been appreciated in VH emulsions but not in UT emulsions, the high-pressure effect on protein aggregation was considered. Authors like Funtenberger, Dumay, and Cheftel (1995) studying the pressure-induced aggregation of β-lactoglobulin in pH 7 buffers; and Galazka, Ledward, Dickinson, and Langley (1995), studying the high-pressure effects on emulsifying behaviour of WPC, found that the substantial change both in WPC and β-lactoglobulin (the major functional protein at WPC) at pH 7 was appreciated when they were subjected to higher pressure processing (200 MPa) and longer times (15 min) than the ones used in this study. Besides, high-pressure treatment of a WPC-stabilised oil-in-water emulsion made with untreated protein had little or no effect on droplet size, and hence, the author concluded that during homogenisation the protein becomes unfolded at the interface and thus, the subsequent high-pressure processing causes no significant further conformational change. Consequently, protein aggregation induced by the VH was discarded.

The following mechanism to be considered is the electrostatic repulsion. As was previously discussed, this mechanism plays an important role in concentrated emulsions. Since proteins are electrically charged, long-range repulsive forces arises due to the overlapping of electrical double layers surrounding the protein-covered droplets. The rheological behaviour of the UT emulsions was essentially Newtonian and would be indicating no interaction between particles, however, there was a strong shift to a shear-thinning behaviour in the VH emulsions, indicative of interaction and extensive flocculation (Floury et al., 2002). With increasing overall WPC content, there would be also a neutralising effect, resulting in an enhancement of the droplet flocculation in the VH emulsions. As the electrostatic repulsion is screened, hydrophobic attraction forces may dominate between the non-polar side groups of protein macromolecules, and thus, these forces may also contribute towards a bridging flocculation mechanism. According to the results obtained, i.e., the flocculation dependence on protein concentration would be indicating that a reduction on electrostatic repulsion between particles could be involved in the flocculation observed.

The electrostatic repulsion would probably be operating in the UT emulsions as it is in the VH emulsions, which was screened at higher concentrations of protein. The inability of the UT to reduce the D_{43} sufficiently is the ultimate reason for creaming of these emulsions, and the size of the droplets overwhelms any electrostatic repulsion that may be present.

Other mechanism that could be occurring at the emulsion flocculation would be bridging flocculation through disulphide bridges formed between different

protein macromolecules on the surface of different emulsion droplets. It is well known that the proteins evaluated in this study form multi-layers at the droplet interfaces that could link a protein at the bulk phase creating a bridge between two droplets. The results obtained at the droplet size distributions with and without SDS has conformed to this assumption. If the mechanism involved in emulsion flocculation was solely the reduction of electrostatic repulsion, dilution at the droplet-size measurement stage would have disaggregated the droplets and the flocculation would have not been observed. However, if bridging flocculation through covalent bridging were occurring, the dilution step would not have broken down the aggregates until the addition of SDS solution. The more surface-active SDS competitively displaces, the larger protein macromolecules between droplets and thus, breaking down any flocs (Floury et al., 2002). Emulsifier-limited bridging flocculation would probably not be occurring since, from 0.73% w/w of protein, the droplet size was stabilised and hence, there would be enough protein to cover the area created.

4. Conclusion

From the comparison of the stability of 50% o/w emulsions with commercial WPC at different concentrations, and prepared by two methodologies, the following conclusions arose: the limitations of the relatively low-powered shear homogeniser, the UT, were clearly shown in this study. Increased WPC concentrations were required to reduce the particle size sufficiently and even then, the emulsion was not truly stable in the long term. The distinction between the UT and VH to reduce the particle size was very clear. The high-pressure homogeniser was able to create dramatically higher nascent oil droplet surface area and showed the limitations of WPC as an emulsifier when using an efficient homogeniser.

Whereas the UT emulsions were slightly or not flocculated, the VH emulsions presented flocculation and the extent of flocculation was protein concentration dependent. Noticeably, these emulsions also showed a delay time or re-stabilisation which increased linearly with WPC concentration; the last being of great commercial importance since the delay phase can be designed to last for months or years. We propose that the structure formation influencing the stability of the VH emulsions at these protein concentrations would be attributed to a depletion-led flocculation event that may be occurring initially followed by a subsequent multi-layer/bridging floc stabilisation as the emulsion aged.

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