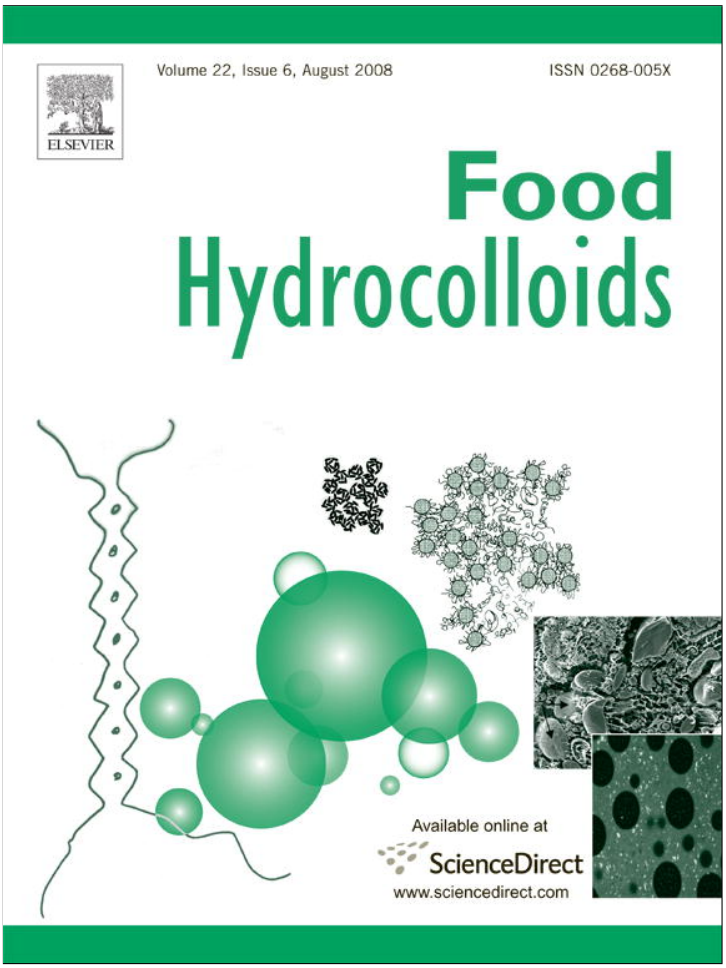

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Rheology and droplet size distribution of emulsions stabilized by crayfish flour

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Abstract

Highly concentrated oil-in-water (o/w) emulsions stabilized by crayfish flour at high pH were characterized by means of linear dynamic viscoelasticity and droplet size distribution (DSD) analysis. Power consumption and temperature were recorded as a function of emulsification time at different agitation speeds. The emulsions studied followed a gel-like behavior, characterized by G' being about one order of magnitude higher than G'' within the experimental frequency range. This behavior was characteristic of highly concentrated emulsions with a well-developed plateau region. Increase in both energy input and crayfish flour concentration yielded higher values of linear viscoelasticity functions and lower droplet size, which suggested an enhancement of the elastic network and an increase in emulsion stability. The evolution of plateau modulus and Sauter diameter was studied at different concentrations of crayfish flour (0.25–6.25 wt%) over storage time at 5 °C. The microstructure of these emulsions was characterized by using confocal laser scanning microscopy (CLSM).

Keywords: Oil-in-water emulsions; Crayfish protein; Backscattering; Linear viscoelasticity; Droplet size distribution; CLSM

1. Introduction

Food industry usually generates a big amount of by-products that may have some undesirable environmental impact. A way to get benefits from these by-products is to explore the functional properties of protein concentrates in order to obtain some added value products, as has been proposed for low-value by-products generated in the fish industry, or extract-specific protein fractions that could be utilized as functional ingredients in foods (Finch, 1977; Spinelli, Groninger, Koury, & Miller, 1975).

Crayfish flour from red crayfish (*Procambarus clarkii*) is an interesting source of high-quality protein (about 65%), rich in essential amino acids and lipids (ca. 19%), including long-chain polyunsaturated fatty acids from ω-3 and ω-6 series, and also containing other components of functional value such as astaxanthin that posses a high antioxidant capacity. The potentials of crayfish proteins as a functional ingredient in food products has been recently explored (Cremades, 2004). Three major groups of proteins are found in crustaceans: water-soluble sarcoplasmic proteins (about 30 wt%), which consist of albumins and lower amounts of myoglobin and enzymes; salt-soluble myofibillar proteins (60–70 wt%), containing myosin, actin and less amount of tropomyosin and troponin (Suzuki, 1981); and insoluble stromal proteins, which represent only between 3 and 10 wt% of total proteins.

Crayfish flour may be obtained as a by-product in the red crayfish industry, which typically produces important crayfish surpluses. Unfortunately, the manufacture of the crayfish flour produces a high degree of protein denaturation, which reduces its functional properties (i.e. emulsification ability).

In food emulsions, such as mayonnaise and salad dressings, egg yolk is the most widely used emulsifier.

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However, many efforts have been devoted for decades to replace egg yolk as emulsifier in order to avoid the presence of cholesterol from yolk or the development of salmonella in yolk-containing food products. Mixtures of macromolecular and low-molecular weight emulsifiers have been used in some cases (Clark, Wilde, Wilson, & Wunsteck, 1992; Franco, Guerrero, & Gallegos, 1995). Other authors have used alternative protein systems to replace the traditional egg yolk such as vegetable proteins (Bengoechea, Cordobés, & Guerrero, 2006; Elizalde, Bartholomai, & Pilosof, 1996; Gallegos, Franco, Madiedo, Raymundo, & Sousa, 2002; Tornberg, 1978). Fish proteins can also be used to stabilize oil-in-water (o/w) emulsions under certain conditions. Thus, for example, Cofrades, Carballo, Careche, and Colmenero (1996) showed that acto-myosin from hake had higher emulsifying activity and stability than the acto-myosin from chicken and pork, whereas Petursson, Decker, and McClements (2004) used protein fractions extracted from cod as emulsifiers to form fairly stable emulsions.

The main objective of the present work was to produce stable o/w emulsions containing crayfish protein as the only emulsifier. A previous requirement, particularly important in this product as a consequence of previous denaturation, was to establish the conditions necessary to enhance the interactions among droplets in order to obtain long-term emulsion stability. Characterization of both linear viscoelastic properties and particle size distribution (droplet size distribution (DSD)) was carried out in order to accomplish this objective. The microstructure of the emulsions obtained was also characterized at the colloidal level by means of confocal laser scanning microscopy (CLSM).

2. Experimental

Different o/w emulsions were prepared using 75 wt% sunflower oil (Koipesol S.A., Spain) in relation to the total weight, crayfish flour (0.25–6.25 wt%) and water. Crayfish flour (ca. 65 wt% protein content) was obtained from ALFOCAN S.L. (Isla Mayor, Sevilla, Spain). Before emulsification process, flour was sifted at 600 μm. The volume fraction of droplets is always about 0.77.

Emulsions were prepared in an Ultra Turrax T-50 homogenizer from IKA (Germany) at different agitation speeds (3000–10,000 rpm) and an emulsification time of 7 min. All the emulsions in this study were prepared at high pH, being far from the isoelectric point (p$I = 3.4$). The value selected for the pH was 11.5, obtained by adding NaOH 2 M to the corresponding protein dispersion. At pH lower than 11, it was not possible to produce any stable emulsion by direct emulsification. The emulsions were stored at 5 °C and were placed at 20 °C 1 h before taking any measurement.

Measurements of backscattering (BS) for some selected emulsions, prepared with an Ultra Turrax T-25 homogenizer from IKA (Germany) at 6000 rpm, were performed by means of a Vertical Scan Analyzer (QuickScan, Beckman Coulter). Each sample was put in a cylindrical glass measurement cell and the BS profile was recorded at 20 °C as a function of the sample height. Destabilization mechanism was analyzed from the evolution of BS profiles as a function of time. Determinations were conducted in duplicate. A description of the technique is reported by Mengual, Meunier, Cayré, Puech, and Snabre (1999) and Pan, Tomás, and Añón (2002).

Measurements of DSD were performed in a Malvern Mastersizer X (Malvern Instruments, UK). For this purpose, 0.5 ml of emulsion was taken and diluted in 11.5 mL of 0.05 M, pH 8 Tris–HCl buffer with 1% SDS, since previous results using water as solvent showed remarkably higher droplet sizes due to flocculation. The role of SDS is to produce displacement of protein molecules from the interface (Chen & Dickinson, 1998; Puppo et al., 2005). Each sample was carefully dispersed step-by-step applying gentle agitation in order to disrupt droplets flocs. Values of the Sauter mean diameter, $d_{3,2}$, which is inversely proportional to the specific surface area of droplets, were obtained as follows:

$$d_{x,y} = \frac{\sum n_i \, d_i^x}{\sum n_i \, d_i^y}, \tag{1}$$

where n_i is the number of droplets with a diameter d_i.

The uniformity ratio is an index of polydispersity of the different droplet sizes, defined by the following expression:

$$U = \frac{\sum V_i \left| d(v,0,5) - d_i \right|}{d(v,0,5) \sum V_i}, \tag{2}$$

where $d(v,0,5)$ is the median for the distribution, and V_i is the volume of droplets with a diameter d_i.

Dynamic viscoelasticity measurements were performed in a controlled-strain rheometer (ARES) from TA Instruments (USA) in the linear viscoelasticity region, so strain sweep were performed to establish the linear viscoelastic range. The geometry used was a plate and plate geometry (25 mm) with a rough surface and a gap between plates of 1 mm. Rheological tests were carried out at 20 °C, and at least two replicates were made. All the systems studied have the same thermorheological history.

Selected undiluted protein emulsions were analyzed by means of a CLSM (Leica Mycrosystems, Heidelberg, Germany). The CLSM was used in the fluorescent mode, and a wavelength of 488 nm from the laser was used. Wavelengths above 500 nm were analyzed. This technique provided images of dark, fat droplets and a bright water phase where proteins were found. A $\times 100$ objective was used. It was not necessary to stain the aqueous phase due to the autoflourescent properties shown by the proteins used.

A statistical analysis (ANOVA) was done in order to establish the influence of the different variables. This study was also performed using P-value. The significance level was established at 95%.

3. Results and discussion

3.1. Emulsification process

The emulsification process was carried out by adding sunflower oil to an aqueous dispersion of the emulsifier, at 20 °C, while processing at constant agitation speed. The addition of oil took always the same time (ca. 6 min) and followed by 1 min further processing. Power consumption and temperature were monitored along emulsification time. Fig. 1 shows the evolution of both variables at different agitation speed values. The percentage of oil to be added is also plotted as a function of emulsification time. This parameter was calculated as follows:

$$Y_o(\%) = \left(1 - \frac{M_o(t)}{M_{o,T}}\right) \times 100, \quad (3)$$

where $M_o(t)$ is the mass of oil added at time t to a continuous phase containing a mass M_c of crayfish aqueous dispersion and $M_{o,T}$ is the total amount of oil in the final emulsion.

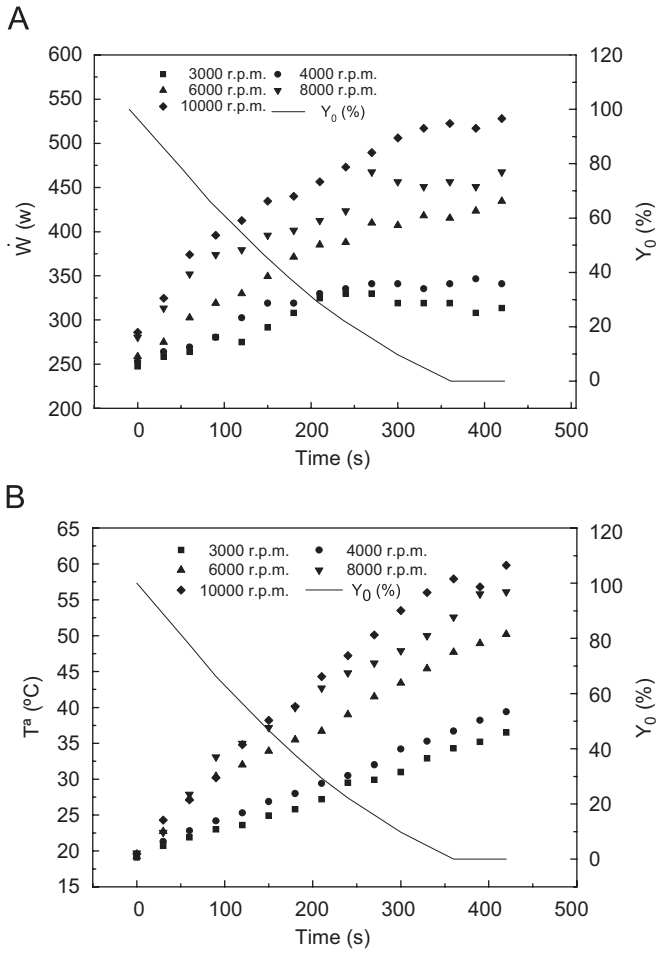

Fig. 1. Influence of emulsification time for emulsions containing 75 wt% oil and 5 wt% crayfish flour processed at different agitation speeds (3000, 4000, 6000, 8000 and 10,000 rpm): (A) evolution of power consumption and oil addition; and (B) evolution of Sauter diameter $d_{3,2}$ and oil addition.

An apparent increase in power consumption took place while the sunflower oil was added (Fig. 1A). After the addition of oil a plateau was typically obtained. An increase in the agitation speed used for the emulsification gave rise to a relevant increase in power consumption. The plateau obtained is more remarkable at the lowest values of the agitation speed. The total amount of energy supplied along the emulsification process is typically used to produce convective flow of oil and aqueous phases, high deformation and break-up of oil droplets, as well as dissipation of mechanical energy.

The emulsification process was carried out within a constant temperature bath. However, the energy dissipation taking place along the emulsification process was so important that a continuous increase in temperature was obtained. Furthermore, this increase became more pronounced as energy input was raised (Fig. 1B).

3.2. Short-term stability

Once the emulsification process was finished and the temperature was set at 20 °C, BS measurements were obtained in order to analyze the destabilization mechanism of crayfish flour emulsions. Fig. 2A shows BS profiles obtained as a function of time for emulsions stabilized by a small amount of crayfish flour (0.25 and 1 wt%). An apparent coalescence process, characterized by a reduction in the value of BS along time, is shown in both cases. At the lowest emulsifier concentration, an increase in the size of the aqueous layer, which displays a low BS value, takes place at the bottom part of the tube along time (Mengual et al., 1999; Palazolo, Sorgentini, & Wagner, 2005). This creaming process is not observed at the experimental time scale when the concentration of protein was raised. Fig. 2B shows the evolution of the relative BS as a function of time obtained within the middle part of the tube defined as follows:

$$BS_{rel} = BS(t)/BS_0, \quad (4)$$

where BS_0 and $BS(t)$ are the mean values for the BS profile obtained at the initial time and time t, respectively. An initial dramatic decrease followed by decaying up to a plateau value in relative BS takes place along time. This reduction is more relevant at the lowest concentration at which the coalescence among oil droplets is evident. An increase in concentration is followed by a remarkable reduction in the coalescence mechanism. Nevertheless, the coalescence mechanism seems to be limited to the first storage stage after emulsification.

Short-term emulsion stability was also analyzed by means of linear viscoelastic properties and DSD of the emulsions, obtained as a function of storage time for the first hours.

Fig. 3 shows the values obtained for the storage and loss moduli ($G'(\omega)$ and $G''(\omega)$) and DSD as a function of storage time at 20 °C. Emulsions stabilized by crayfish flour

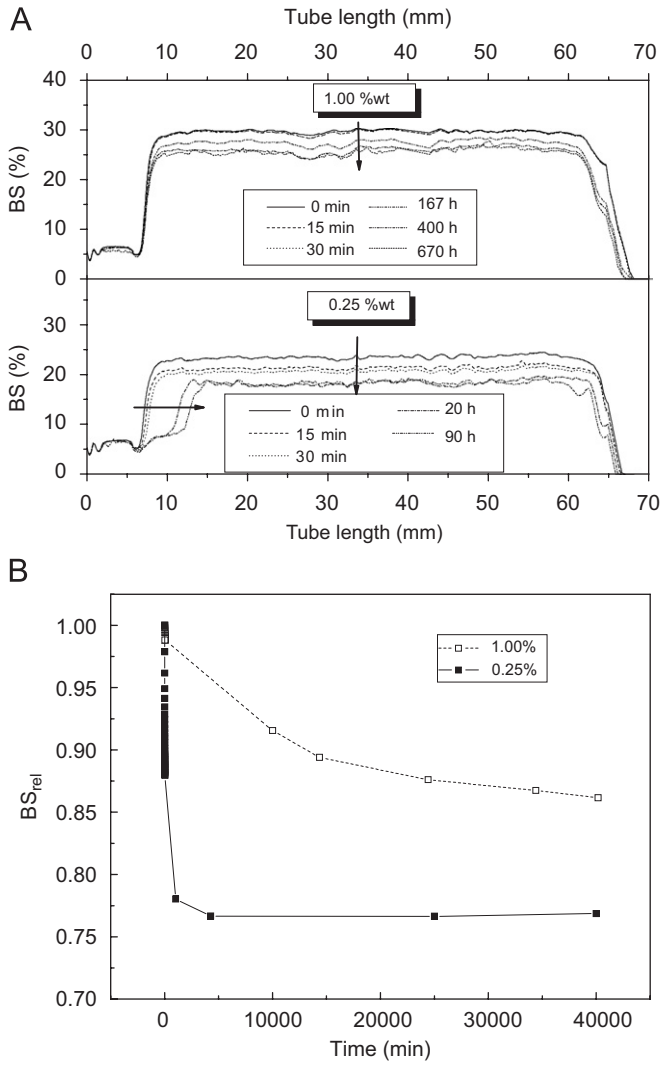

Fig. 2. Evolution of backscattering results obtained at 20 °C for emulsions containing 75 wt% oil and 0.25 or 1 wt% crayfish flour prepared at 6000 rpm (UT-T25) as a function of time: (A) BS% profiles; (B) BS_{rel} results. The arrows indicate the evolution of time.

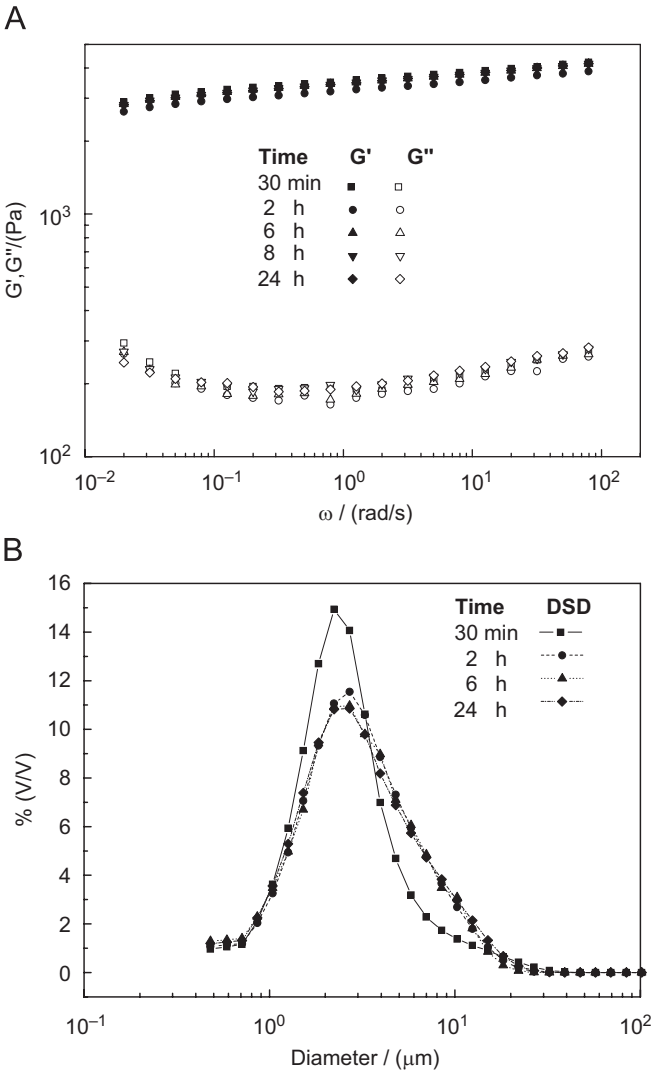

Fig. 3. Influence of time after emulsification for emulsions containing 75 wt% oil and 5 wt% crayfish flour prepared at 6000 rpm and 20 °C: (A) evolution of storage and loss moduli as a function of frequency; and (B) evolution of droplet size distribution.

followed a gel-like behavior, characterized by G' being about one order of magnitude higher than G'' within the experimental frequency range. This behavior has been generally found for highly flocculated o/w emulsions such as mayonnaise (Gallegos, Berjano, & Choplin, 1992; Guerrero & Ball, 1994), salad dressing stabilized by egg proteins (Franco et al., 1995; Moros, Cordobés, Franco, & Gallegos, 2003; Muñoz & Sherman, 1992) and concentrated emulsions stabilized by vegetable proteins (Bengoechea et al., 2006; Franco, Partal, Ruíz, Conde, & Gallegos, 2000; Franco, Raymundo, Sousa & Gallegos, 1998; Raymundo, Franco, Empis, & Sousa, 2002). Occurrence of a well-developed plateau region, where a very low-frequency dependence for G' can be observed, has been related to the formation of an elastic structural network that confers a high stability to the emulsion (Dickinson, 1989; Franco et al., 1995). As may be seen in this figure, the values of G' follows a power-law dependence on frequency while G''passes through a minimum with frequency. The loss tangent also showed a minimum, which was used to calculate an approximate value of the plateau modulus (G_{N^0}) as reported by Wu (1989).

Fig. 3B displays DSD profiles obtained after emulsification, showing a log-normal unimodal distribution. Much higher sizes were obtained when the emulsions were diluted in the buffer without SDS. In fact, the ratio between the volume mean diameter, $d_{4,3}$ in the absence of SDS and $d_{4,3}$ in 1% SDS buffer, defined as a flocculation index by Puppo et al. (2005), is in the order of 10 for 5 wt% CF emulsions. As may be observed in Fig. 3B, the DSD profiles are narrower at shorter time. A displacement toward higher sizes due to the above-mentioned coalescence mechanism as well as an increase in polydispersity may also be noted (i.e. the uniformity ratio undergoes an increase from 0.59 to 0.76 within 6 h after emulsification and storage at 20 °C). Moreover, this evolution seems to be restricted to a short

period of storage time since no further modification in DSD took place after a few hours.

Fig. 4shows the evolution of G_{N^0} and $d_{3,2}$ at 20 °C for two different storage temperatures. The emulsion was divided into two parts immediately after emulsification. The first part was stored at 5 °C. Aliquots of the emulsion stored at 5 °C were taken after different storage time lengths and kept at 20 °C for an equilibrium time (15 min) before the linear dynamic viscoelasticity measurement was performed. The second part was stored and measured directly at 20 °C. A sudden decrease up to a minimum value in G_{N^0} took place simultaneously to the above-mentioned DSD evolution for the two storage conditions. This decrease was followed by a significant increase in G_{N^0}, in absence of any further evolution of DSD, as well as an asymptotical evolution toward an equilibrium value.

Storage at low temperature may promote the development of a three-dimensional connective network among myofibrillar proteins, which eventually would prevent any further coalescence. Proteins from the bulk and from the o/w interface can participate in this process. As shown in Fig. 4, the evolution is similar when the emulsion was stored at 5 °C instead of 20 °C. However, the decrease in storage temperature lead to a significant decrease in parameter U (shifting from 0.76 to 0.67) as well as in the volumetric mean diameter ($d_{4,3}$). A slight decrease in the Sauter mean diameter was also found although was not statistically significant. Therefore, a reduction in the rate of coalescence was obtained by decreasing the storage temperature, leading to a larger specific interface area and to an increase in the number of droplet interactions. As a consequence, an enhancement of network structures was observed giving rise to a higher plateau modulus.

A consequence of the above-mentioned results is that the conditions for storage and measurements have to be previously established for each emulsion. The storage temperature selected for this study was 5 °C and the viscoelasticity measurements were carried out 24 h after emulsification, to allow enough time to finish the short-term evolution of the system.

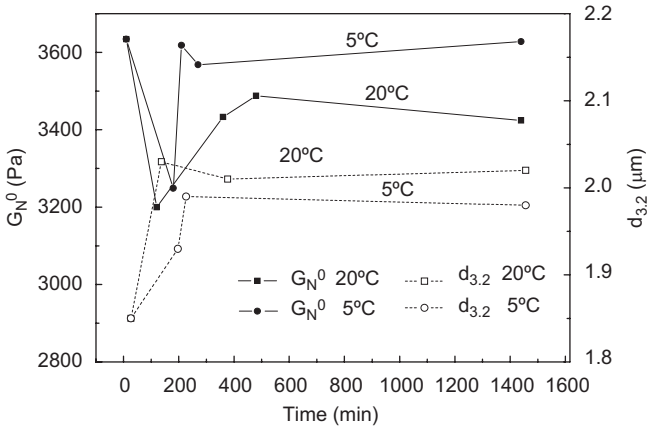

Fig. 4. Evolution of the plateau modulus and the Sauter mean diameter at 20 °C as a function of time after emulsification at different storage temperatures (5 and 20 °C). The emulsions contain 75 wt% oil and 5 wt% crayfish flour and were prepared at 6000 rpm.

3.3. Energy input

In order to obtain meaningful oscillatory shear data, the strain range for linear viscoelasticity has been defined by means of a dynamic strain sweep test performed at constant frequency. No significant differences for the critical strain value (γ_c) of emulsions processed at different agitation speeds were found (i.e. the critical strain is about 11% at 6.3 rad/s).

Fig. 5 shows the viscoelastic properties and DSD profiles for emulsions stabilized by 5% crayfish flour as a function of energy input, which may be expressed in terms of rotational agitation speed for the rotor element of the emulsifying equipment. An increase in energy input did not produce any change in the shape of the mechanical spectrum but led to an increase in both G' and G''. Thus, Fig. 5C shows an apparent increase in the plateau modulus, which is almost linear. Only the emulsion prepared at the highest agitation speed deviates from this tendency. An evolution toward smaller droplets was also found as can be deduced from the evolution of DSD curves (Fig. 5B) and from the values of the Sauter mean diameter (Fig. 5C). No significant differences were observed for the uniformity ratio, except at the lowest energy input conditions at which a higher polydispersity was obtained.

The widest used method to characterize the energy efficiency of homogenizers consists of evaluating the relationship between the Sauter mean diameter obtained after emulsification and the amount of energy supplied (McClements, 2004; Schubert & Engel, 2004; Walstra, 1983, Chapter 2).

The total amount of energy supplied to an emulsion during the homogenization process is often referred to as the energy density, which has been defined as the energy input per unit volume of emulsion, or the power input per unit volume flow rate of emulsion (Schubert, Ax, & Behrend, 2003). In general, the energy density is given by the following function (Walstra & Smulder, 1998, Chapter 2):

$$E_v = \int P_v(t)\,dt, \tag{5}$$

where P_v is the net power density and t is the emulsification time.

Droplet disruption does not take place unless P_v exceeds some critical value, which depends on the Laplace pressure of the droplets. If the net power density is below this critical value in any region within the homogenizer, then droplet disruption cannot occur and the energy is wasted (McClements, 2004).

In the present analysis, the energy density (E_v) has been calculated as follows:

$$E_v = \int \frac{\dot{W}(c,N,t)}{M_c/\rho_c(c,t) + M_o(t)/\rho_o(t)}\,dt, \tag{6}$$

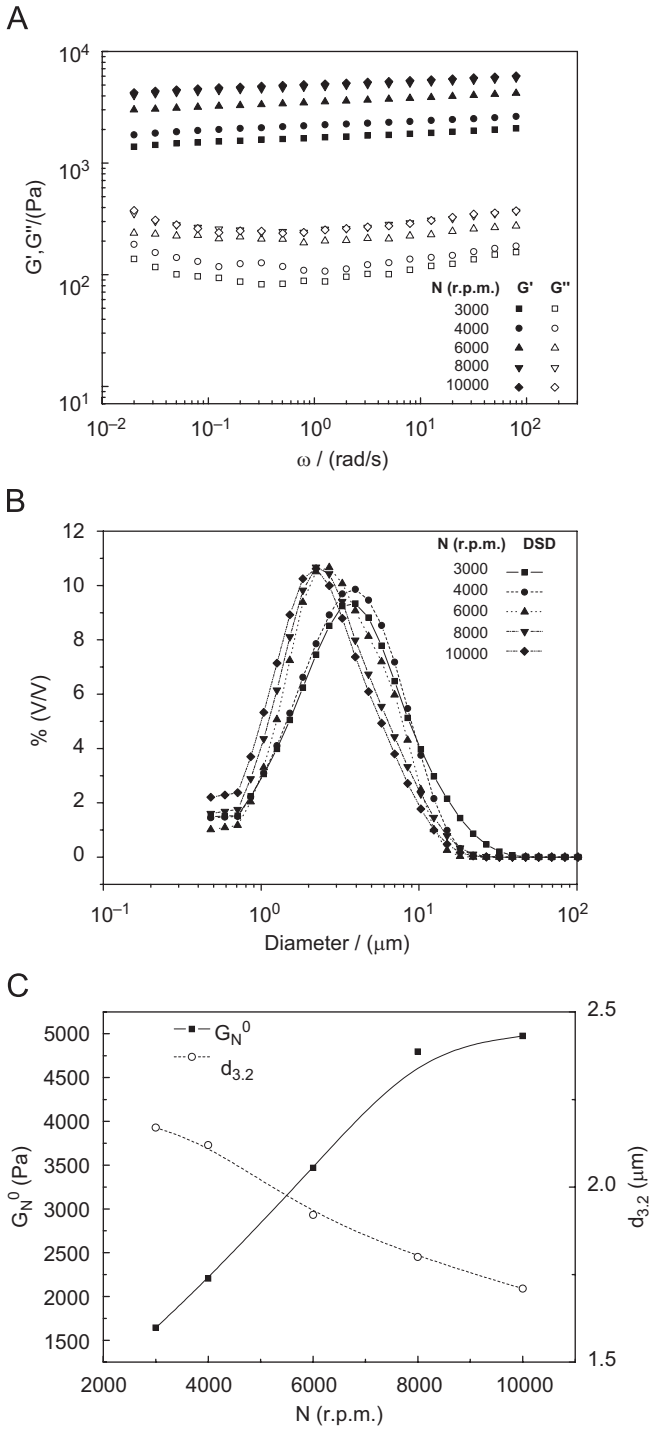

Fig. 5. Influence of the agitation speed for emulsions containing 75 wt% oil and 5 wt% crayfish flour: (A) evolution of the storage and loss moduli with frequency; (B) evolution of droplet size distribution; and (C) evolution of the plateau modulus and the Sauter mean diameter.

where c is the crayfish flour concentration, N is the agitation speed, $\dot{W}(c,N,t)$ is the power consumption and $\rho_c(c,\ t)$ and $\rho_o(t)$ are the densities of the continuous and oil phases, respectively. Both densities are time dependent due to the increase in temperature along emulsification. A volume additivity assumption was taken in Eq. (6).

A plot of the mean droplet diameter produced by the emulsification device as a function of energy density is shown in Fig. 6. As can be observed, a power-law relationship fits the results fairly well at constant protein content:

$$d_{3,2} = aE_v^{-b} \tag{7}$$

with the constants a and b depending on the type of emulsifier, the properties of the continuous and the disperse phase and the emulsification equipment used. This power-law equation has been used by other authors for a variety of mechanical homogenizers (Schubert & Engel, 2004; Walstra, 1983)

The value obtained for the power-law exponent, b, was 0.6 which is a little bit higher than the value of 0.4 reported by Walstra (1983) for batch emulsification using a rotor-stator homogenizer; however, much higher emulsification time was used in the present study.

The size of droplets in an emulsion is reduced by increasing the intensity or duration of disruptive energy supplied during homogenization as long as there is sufficient emulsifier available to cover the interfaces generated in the emulsification process (McClements, 2004). The final DSD of any emulsion results from the dynamic equilibrium between droplet disruption and coalescence, both of which are favored by an intense agitation (Walstra, 1983). An increase in energy input generally produces a displacement toward smaller sizes since droplet disruption becomes predominant over coalescence, although coalescence is also promoted (Sánchez, Berjano, Brito, Guerrero, & Gallegos, 1998). As a consequence, an increase in specific interface area takes place leading to a greater number of droplet interactions (Rahalkar, 1992) and to the spread of the plateau region because of enhancement of entangled network structures (Bengoechea et al., 2006; Franco et al., 1995).

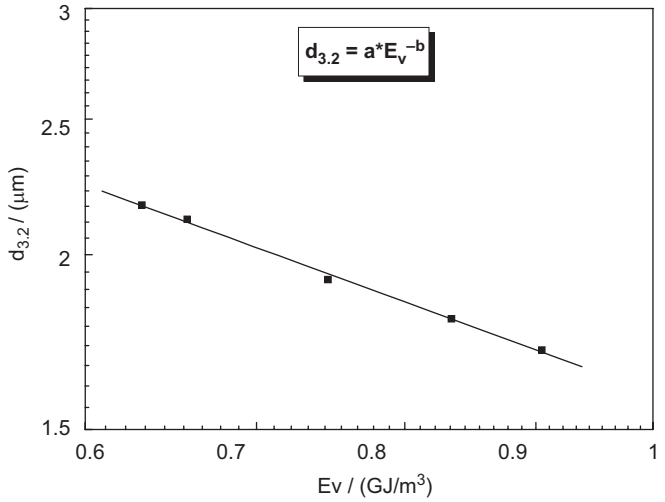

Fig. 6. Relationship between the Sauter mean diameter and energy density for emulsions containing 75 wt% oil and 5 wt% crayfish flour processed in Ultra-Turrax device at different rotation velocities (3000, 4000, 6000, 8000 and 10,000 rpm).

3.4. Crayfish flour concentration

The emulsions studied showed a wide linear viscoelastic range, which depends on the protein concentration level. The critical strain values for the onset of the linear viscoelasticity region, which are shown in Table 1, remained almost constant excepting for the two lowest protein contents which showed much lower values. As may be noted, it was possible to stabilize 75 wt% o/w emulsions by using a small amount of protein, under the experimental conditions. The minimum concentration of crayfish flour required for stabilization was 0.25 wt% that corresponds to ca. 0.16 wt% protein.

The influence of crayfish flour concentration on linear viscoelastic properties of the emulsion studied may be observed in Fig. 7A, which shows the evolution of the mechanical spectrum, and Fig. 8A, which displays the evolution of the plateau modulus. These figures show an apparent increase in both moduli as well as in the plateau modulus with increasing protein concentration. Fig. 7A also shows an initial decrease in the slope of G' vs. frequency, with CF concentration, up to a roughly constant value (ca. 0.04, starting at 0.5 wt%). A remarkable flattening in G'' at high frequencies also takes place as protein content increases, which suggests an enhancement of the plateau region. Fig. 7B shows an evolution from bimodal to unimodal emulsions, with the first peak located at 1–2 μm. A growth of this peak may be observed as protein content is raised. A displacement toward smaller sizes may be clearly detected for the second peak. Both peaks eventually overlap to give an unimodal DSD profile. Fig. 8B displays the evolution of the Sauter mean diameter, which reflects a change of behavior at 0.50 wt%. The lowest polydispersity corresponds to this value (U~0.4). At concentrations lower than 0.50 wt% a dramatic increase in droplet size takes place. Thus, 80% of the total reduction in diameter $d_{3,2}$ (from 0.25 to 6.25 wt%) takes place by duplicating the crayfish flour concentration from 0.25 to 0.50 wt%. Fig. 8B also shows the evolution of the energy density with crayfish flour concentration. As may be seen, an increase in energy density is required to produce emulsions with higher protein content as a consequence of the higher viscosity of the continuous phase. This increase in energy density corresponds to higher values in the plateau modulus and lower droplet sizes.

Some attempts of correlating rheology and microstructural parameters of high internal phase ratio emulsions may be found in the literature. Princen and Kiss (1986) proposed an equation that related the small-strain elastic shear modulus to the interfacial tension, the volume fraction of the dispersed phase, and the Sauter mean diameter, for highly concentrated polydispersed emulsions. Some authors have successfully used this equation to correlate structural and rheological parameters for emulsions stabilized by surfactants (Pal, 2006; Pons, Solans, & Tadros, 1995; Princen & Kiss, 1986; Sánchez et al., 1998) or polymeric surfactants (Perrin, 2000). Mason et al. (1997) working on monodisperse concentrated emulsions stabilized by SDS have obtained a universal master curve for the evolution of G' that obeyed the following relationship:

$$G' \propto \frac{2\sigma}{d_{3,2}}\phi(\phi - \phi_o), \tag{8}$$

where ϕ_o is the close packing volume fraction that has a value of 0.64 for randomly packed monodisperse spheres. The rheology of emulsions is governed by a three-dimensional network of interfacial films when Princen

Table 1
Critical strain values, γ_c, for emulsions stabilized by crayfish flour at different concentrations for emulsions containing 75 wt% oil processed at 6000 rpm

Concentration (wt%)	0.25	0.375	0.50	1.25	2.50	3.75	5.00	6.25
γ_c (%)	4.21	4.34	9.68	10.03	10.02	9.89	11.16	10.07

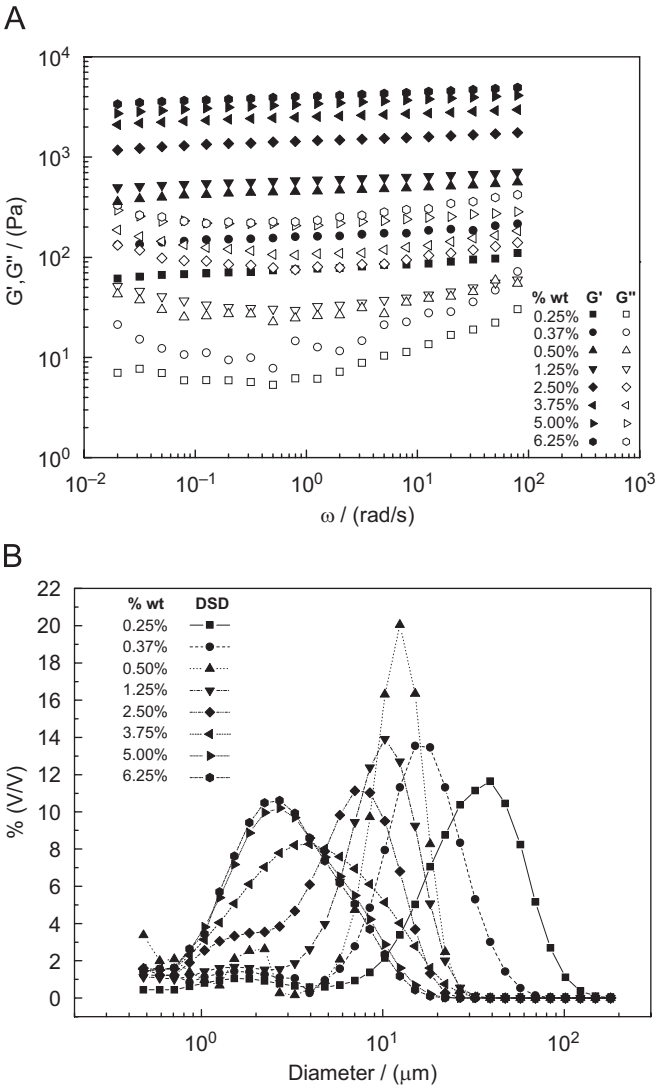

Fig. 7. Influence of crayfish flour concentration for emulsions containing 75 wt% oil processed at 6000 rpm: (A) evolution of the storage and loss moduli with frequency; and (B) evolution of droplet size distribution.

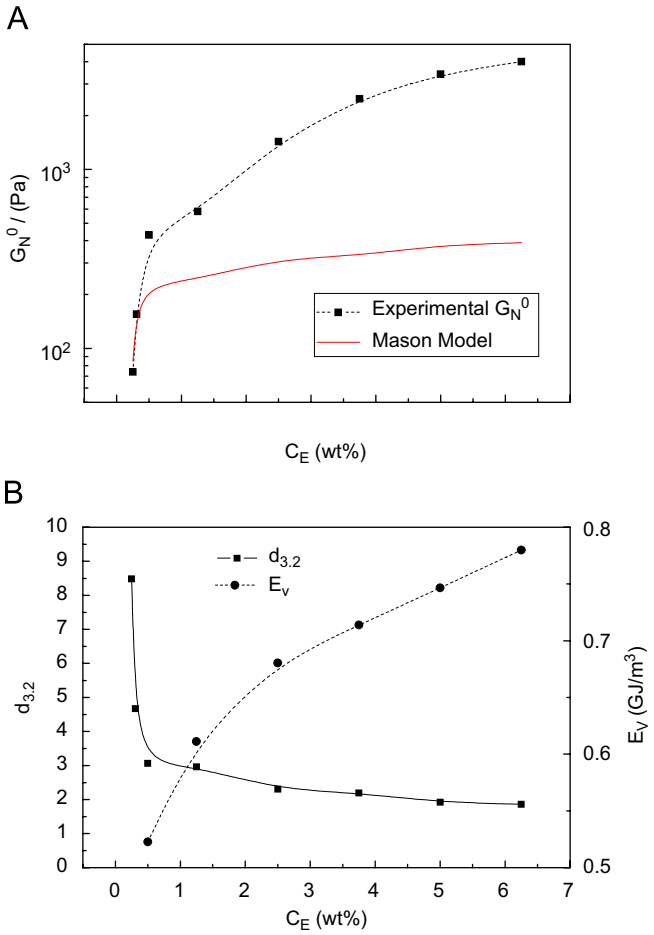

Fig. 8. Evolution of the plateau modulus, the Sauter mean diameter and energy density for emulsions containing 75 wt% oil processed at 6000 rpm, as a function of crayfish flour concentration (C_E).

and Kiss or Mason et al. equations were followed. Some authors have recently tried to use the scaling procedure reported by Mason and co-workers for protein-stabilized emulsions (Bressy, Hebraud, Schmitt, & Bibette, 2003; Dimitrova & Leal-Calderon, 2001, 2004). However, the elastic modulus may be much higher than that predicted by Mason's law. Thus, an additional source of elasticity should be considered. Previous studies on concentrated emulsions stabilized by soy protein isolate or gluten, used the Mason model of elasticity of compressed emulsions to correlate the plateau modulus and microstructural parameters, giving adequate fitting but underestimating the elastic properties obtained for the highest gluten content (Bengoechea et al., 2006). The values for G_{N^0} calculated according to Eq. (8) are gathered in Fig. 8A. As can be observed, the experimental values show apparent deviations from Mason's equation above 0.5 wt% CF. These deviations, which increase with protein content, cannot be explained solely by taking into account the effect of the interfacial film thickness through an effective volume fraction, as given by Princen and Kiss (1986). As a consequence, CF-based emulsions above 0.5 wt% do not follow the rheological behavior of high internal phase ratio emulsions. The protein layer adsorbed at the surface of the droplets contributes to the emulsion's overall elasticity as suggested by Dimitrova and Leal-Calderon (2004).

Different amounts of an anionic surfactant (SDS) have been added to an emulsion containing 2.5 wt% CF in order to displace protein molecules from the interface disrupting bridging flocs. Linear viscoelastic and DSD properties have been performed to analyze this process. Fig. 9 shows a remarkable decrease in G' and G'' with SDS content and may be attributed to a deflocculation process. In fact this emulsion shows a flocculation index about 6 that reduces to 1 (no flocculation) for the highest SDS concentration studied. The displacement of protein from the interface by a low molecular weight emulsifier is a well-known phenomenon as described by Chen and Dickinson (1998). The reduction in G_{N^0} for the highest SDS content is about 60%, however, it does not reach the level predicted by Mason et al. equation. This may be due to an increase in the continuous phase consistency produced by the displacement of protein from the interface, although depletion forces due to the excess of protein may also act within these systems.

Fig. 10 shows the CLSM images for crayfish emulsions prepared at different protein concentrations where the above-mentioned reduction in size may be clearly seen. Pictures of emulsions corresponding to each CLSM image are also shown (Fig. 10D–F). As may be seen, the extension of bright areas that corresponds to the presence of protein increases with crayfish flour concentration. Furthermore, the micrographs obtained at the highest protein content show some small units with high protein content located at the aqueous phase. This effect may be related to the aforementioned excess of protein above the concentration required to saturate the interface obtained for these emulsions. The microstructures shown in Fig. 10 are typical of highly concentrated emulsions with a close packed distribution of oil droplets, as reported in a

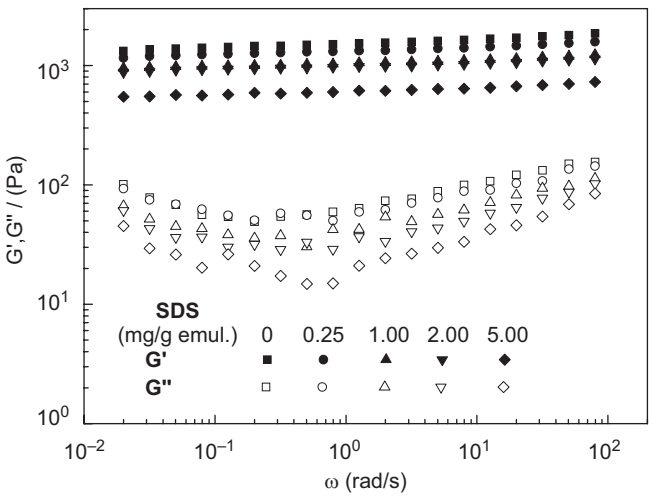

Fig. 9. Influence of SDS addition on the storage and loss moduli obtained at 20 °C as a function of frequency, for an emulsion containing 75 wt% and 2.5 wt% crayfish flour prepared at 6000 rpm.

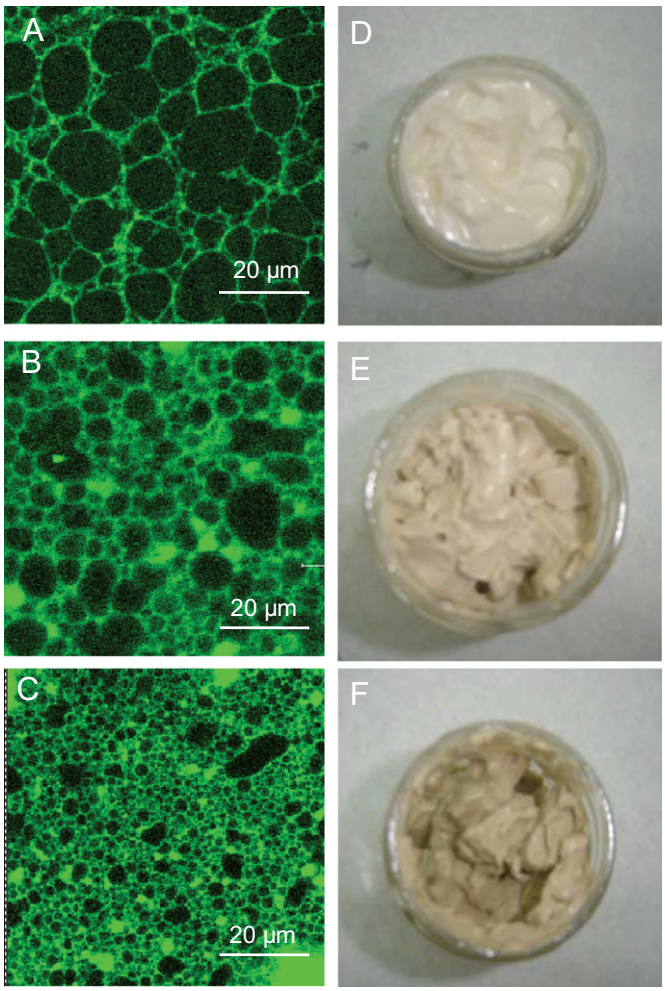

Fig. 10. Images for emulsions containing 75 wt% oil processed at 6000 rpm at different crayfish flour concentrations: CLSM images at (A) 0.5 wt% flour; (B) 2.5 wt% flour; (C) 5.0 wt% flour; Pictures at (D) 0.5 wt% flour; (E) 2.5 wt% flour; and (F) 5.0 wt% flour.

previous paper in which gluten or soya protein isolate was used as the emulsifier (Bengoechea et al., 2006). Langton, Jordansson, Altskär, Sorensen, and Hermansson (1999) reported similar CLSM images of undiluted samples of highly concentrated emulsions stabilized by yolk in which the morphology of the water phase was described in terms of a network of yolk aggregates.

A remarkable evolution in visual appearance of the emulsion may be also noted (Fig. 10D–F). The modifications associated with this change in visual appearance are an increase in G_{N^0} (about 1 order of magnitude) and a decrease in $d_{3,2}$ (about 50%).

3.5. Long-term stability

Physical stability is the most important property of emulsions. In order to study long-term stability, emulsions prepared at different crayfish flour concentrations were stored at 5 °C. Linear viscoelastic properties and DSD measurements were carried out along storage time for these emulsions. The parameters selected to follow the evolution

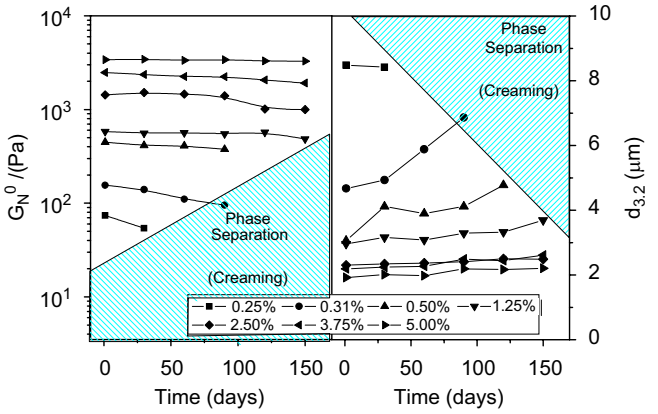

Fig. 11. Evolution of the plateau modulus and the Sauter mean diameter for emulsions containing 75 wt% oil, processed at 6000 rpm at different crayfish flour concentration, as a function of storage time at 5 °C.

along time were the plateau modulus and the Sauter mean diameter, which represent linear viscoelastic properties and DSD, respectively.

Fig. 11shows a long-term stability diagram in terms of the evolution of G_{N^0} and $d_{3,2}$over storage time. Both parameters provide a fairly consistent information. Thus, as can be observed, the emulsions studied are highly stable above a critical concentration (ca. 1.25%) since no significant evolution for G_{N^0} and $d_{3,2}$took place after 5 months. Any physical instability was also detected. Conversely, below the critical crayfish flour concentration a decrease in G_{N^0} as well as an increase in $d_{3,2}$over time occurred followed by the appearance of two phases due to a creaming process. As protein concentration decreases, a displacement toward lower values in G_{N^0} and higher values in $d_{3,2}$ takes place and as a consequence the creaming process becomes faster. Fig. 11 also shows a region where the probability of creaming is very high.

4. Concluding remarks

Crayfish flour was checked as a new emulsifier in the stabilization of highly concentrated o/w emulsions, showing fairly good ability to produce long-term stability at high pH provided that the flour content is above a critical value. Thus, these emulsions followed a gel-like behavior, characterized by G' being about one order of magnitude higher than G'' within the experimental frequency range. This behavior was characteristic of highly concentrated emulsions showing bridging flocculation with a well-developed plateau region.

An increase in energy input yielded higher values of linear viscoelasticity functions and lower droplet size as has been generally found in the literature on emulsion rheology. This behavior leads to an enhancement of the elastic network as well as an increase in emulsion stability.

A remarkable change in behavior was obtained for emulsions above 0.50 wt% CF, which suggests that a saturation of the oil–water interface takes place. This

change is reflected by the values of critical strain, droplet size and linear viscoelastic functions.Moreover, significant deviations from the Mason et al. model of elasticity of compressed emulsions were found. Above this concentration, the amount of protein exceeding the concentration necessary to saturate the interface tends to be located at the aqueous continuous phase, as may be deduced from CLSM images.

From the long-term stability diagram it may be concluded that crayfish flour-based emulsions with $G_{N^0} > 1$ kPa and $d_{3,2} < 3$ μm show long-term stability (at least over 5 months). In terms of energy efficiency, this means that highly stable crayfish flour emulsions may be produced even at the lowest energy input studied at which the aforementioned conditions were fulfilled. Thus, the experimental values obtained were $G_{N^0} \geqslant 1.6$ kPa; and $d_{3,2} \leqslant 2.2$ μm.

Further processing would be required in order to establish the actual potentials of these emulsions for food applications. Thus, these emulsions cannot be directly used as a food product due to the high pH value. Although some alkaline food products may be found the pH is always lower than 8.5. Therefore, future research on these emulsions should focus on the study of the modifications that would allow industrial commercialization of crayfish flour-stabilized emulsions for food applications (change in the pH of the final emulsion, thermal processing to enhance gel strength, processing conditions for the crayfish flour manufacture, etc.).

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