Influence of Processing Variables on the Rheological and Textural Properties of Lupin Protein-Stabilized Emulsions

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This paper deals with the influence that the agitation speed and emulsification time exert on the droplet size distribution and some rheological and textural parameters of the final product. With this aim, oil-in-water emulsions were prepared in a rotor-stator turbine under different processing conditions (8000−20500 rpm, 3−10 min) using a vegetable protein, isolated from *Lupinus albus* seeds, as emulsifier. Oscillatory and steady-state measurements, as well as droplet size distribution and textural profile tests, were performed. The study was carried out according to an experimental design based on the response surface methodology. From the experimental results it may be concluded that an increase in the energy input during the emulsification process decreases the mean droplet diameter of the emulsion and increases the values of the rheological and textural parameters, although the evolution of these properties cannot be explained only by the modification of the droplet size distribution.

Keywords: *Emulsion; lupin protein; processing; rheology; texture; viscoelasticity*

INTRODUCTION

Mayonnaise and salad dressing-type emulsions are typically stabilized by an adsorbed layer of protein at the oil−water interface. Many authors have studied the emulsifying capacity of different animal or vegetable proteins that may replace the traditional egg yolk. Thus, whey protein (Jost et al., 1986, 1989; Yost and Kinsella, 1992; McClements et al., 1993; Dickinson and Yamamoto, 1996a), pure β-lactoglobulin (Mulvihill and Kinsella, 1988; Paulsson et al., 1990; Matsudomi et al., 1991, 1992; Dickinson and Hong, 1995), caseins (Dickinson et al., 1989; Fang and Dalgleish, 1993; Dickinson and Yamamoto, 1996b; Euston et al., 1996; Dickinson and Golding, 1997a,b), soy protein (Tornberg, 1978; Toro-Vazquez and Regestein, 1989; Morr, 1990; Elizalde et al., 1996), or sunflower protein derivatives (Rossi et al., 1985) have been satisfactorily used as emulsifiers.

Vegetable proteins have some additional advantages. They do not contribute to the cholesterol increase, they are technologically easier to handle as coagulation occurs at higher temperatures, and they are agriculturally strategically more interesting because they miss the animal conversion step in the food chain. In this sense, protein isolates from *Lupinus albus* seeds may be another important alternative to egg products, encouraging in this way the use of a food additive ingredient from a legume plant with a positive agricultural impact. Previous studies (Sousa et al., 1996) have shown the poorer gelation and thickening properties of lupin protein in comparison to the soy protein, the most widely used as emulsifier vegetable protein. However,

at sufficiently high temperatures, heat-set lupin protein seems to be a good emulsifying (Raymundo et al., 1998) agent.

The structural and rheological properties of oil-in-water emulsions are determined by the interactions among oil droplets, which depend on the protein structure and concentration, the physical and chemical interactions of adsorbed proteins with nonadsorbed proteins or with other components as surfactants or polysaccharides, and the processing conditions (Dickinson, 1997; Gallegos and Franco, 1998). Thus, in many of the above-mentioned studies, the stability and the physical properties of the emulsions have been improved by heating the protein solution prior to the addition of the oil phase or inducing a chemical or enzymatic reaction that increases the cross-links among the adsorbed, or nonadsorbed, protein molecules. However, little or no research has been developed on the optimization of the mechanical variables in the processing of vegetable protein-stabilized emulsions. As has been pointed out for emulsions stabilized by a mixture of egg yolk and a low molecular weight emulsifier (Franco et al., 1995), the processing variables determine the viscous and viscoelastic behavior of the emulsion, modifying by themselves some structural parameters as droplet size distribution or interdroplet interactions. Thus, an increase in the energy input during the emulsification enhances the formation of an entanglement network, which favors the emulsion stability. In addition to this, the geometry of the impellers or stirrers affects the efficiency of the disruption of oil droplets during the emulsification (Sánchez et al., 1998).

The main objective of this work was to study the effect that some mechanical processing variables (emulsification time and agitation speed) exert on the textural and rheological properties of lupin protein-stabilized emul-

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Table 1. RSM Matrix and Respective Responses for Lupin Protein-Stabilized Emulsions Manufactured under Different Processing Conditions

processing variable (exptl design)	Sauter diam (μm)	firmness (g)	adhesiveness	G_N^0 (Pa)	η_0 (Pa s)	$\dot{\gamma}_c$ (1/s)	s
8000 rpm, 6.5 min	7.7	20	15	162	5.55×10^3	3.6×10^{-4}	0.38
9800 rpm, 4 min	4.4	35	48	383	3.71×10^4	4.1×10^{-4}	0.41
9800 rpm, 9 min	3.9	45	67	465	1.86×10^5	1.6×10^{-4}	0.44
14250 rpm, 3 min	3.1	52	83	792	5.21×10^4	1.6×10^{-4}	0.39
14250 rpm, 6.5 min	3.0	53	75	1020	1.68×10^5	1.0×10^{-4}	0.42
14250 rpm, 6.5 min	3.0	54	80	1100	1.61×10^5	1.1×10^{-4}	0.41
14250 rpm, 10 min	2.6	77	123	1410	5.10×10^5	7.0×10^{-5}	0.42
18700 rpm, 4 min	2.8	106	167	1610	6.54×10^5	6.0×10^{-5}	0.43
18700 rpm, 9 min	2.6	117	175	2120	8.07×10^5	4.5×10^{-4}	0.51
20500 rpm, 6.5 min	2.7	203	201	2560	4.76×10^5	2.7×10^{-4}	0.47

sions, which were not previously submitted to any thermal treatment.

MATERIALS AND METHODS

Oil-in-water emulsions containing a sunflower oil concentration of 65 wt % were prepared. A lupin protein isolate, L9020, from Mittex Alangenbau GmbH (Germany) was used, at a constant concentration (4.5 wt %), as emulsifier. Protein isolate was dispersed in distilled water under magnetic stirring (30 min) at room temperature. Emulsification was performed, using an Ultra Turrax T-25 homogenizer from Ika (Germany), at different agitation speeds (8000–20500 rpm) and emulsification times (3–10 min).

Droplet size distribution (DSD) measurements were performed in a Malvern Mastersizer-X (Malvern, U.K.). Values of the Sauter mean diameter (Sprow, 1967), which is inversely proportional to the specific surface area of droplets, were obtained as follows:

$$d_{SV} = \sum_i n_i d_i^3 / \sum_i n_i d_i^2$$

Dynamic viscoelasticity and steady-state flow measurements were carried out in a controlled-stress rheometer (RS-75) from Haake (Germany). Oscillatory tests were performed, inside the linear viscoelastic region, using a cone-and-plate sensor system (35 mm, 2°) in a frequency range of 0.05–200 rad/s. Steady-state flow curves were obtained with a serrated plate–plate sensor system (20 mm) to prevent wall-slip phenomena (Barnes, 1995).

Textural variables were obtained from the textural profile analysis carried out in a texturometer TA-XT2 (Stable Micro System, U.K.). Penetration tests were performed with a cylindrical probe in a load cell of 5000 g and 2 mm/s of crosshead speed. From the force versus time texturograms, parameters such as firmness and adhesiveness can be calculated. Firmness was considered as the maximum resistance to the penetration of a cylinder with 38 mm diameter at 5 mm depth. This is recorded as the maximum force. Adhesiveness is a characteristic of sticky materials and can be defined as the resistance of the material when the probe is recessing. This parameter is recorded as a negative area and is evaluated as the work necessary to take the probe out of the material. All of these measurements were conducted at 20 ± 1 °C and replicated at least three times.

The study was carried out according to an experimental design based on the response surface methodology (RSM), using a central composite rotatable matrix. The independent variables considered were agitation speed (x_1) and emulsification time (x_2) tested at five levels. The dependent variables considered were the plateau modulus, G_N^0; the zero-shear-rate viscosity, η_0; firmness; adhesiveness; and the Sauter diameter, d_{SV}.

RESULTS AND DISCUSSION

The experimental design of experiences and the respective values of the DSD and textural and rheological parameters obtained can be seen in Table 1.
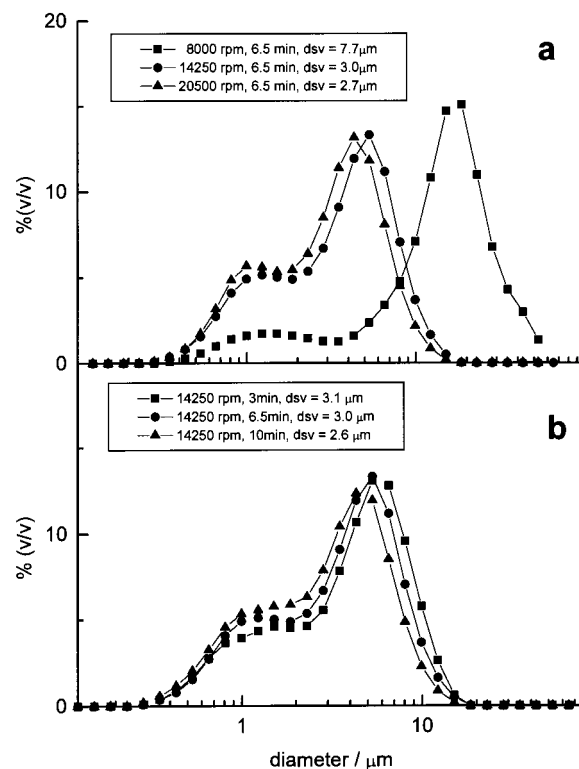

Figure 1. Evolution of the DSD with (a) agitation speed and (b) emulsification time for lupin protein-stabilized emulsions.

DSD. Figure 1 shows the effect of processing variables on the DSD curves for selected emulsions. As can be observed, all DSD curves show a bimodal shape with a secondary maximum at relatively low values of the droplet diameter and the maximum distribution value at higher sizes. An initial increase in the agitation speed (8000–14250 rpm) yields an important reduction in droplet size, the maximum of the distribution appearing at lower diameters and the secondary maximum becoming more pronounced. In other words, the larger particles are of a rather smaller size and less numerous, and, whereas the number of fine particles is appreciably increased, their size is not much altered. These results are in agreement with those found by Sánchez et al. (1998) for emulsions stabilized by low molecular weight nonionic surfactants. Nevertheless, a further increase in the agitation speed produces only a slight decrease in the mean droplet diameter (d_{SV}) and does not qualitatively modify the DSD curves. The emulsification time also reduces the droplet diameter, although this influence is less important, specially at higher agitation speeds, as previously reported by Sánchez et al. (1998) for surfactant-stabilized emulsions. Both influences are combined in Figure 2, where the RSM
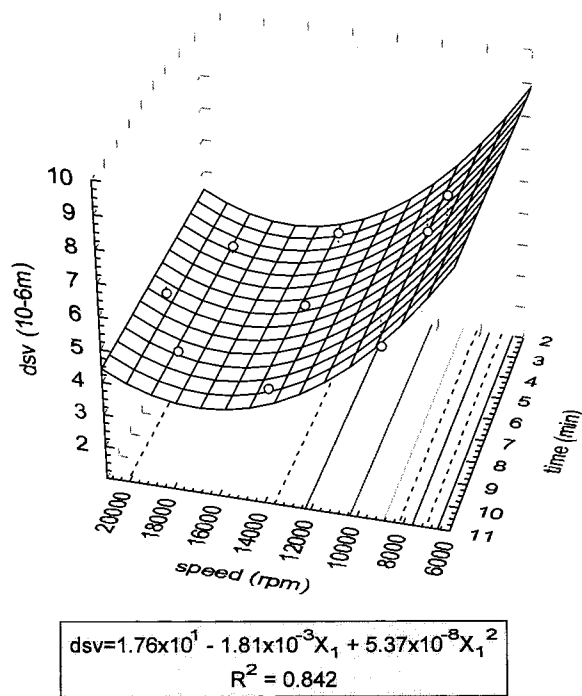
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Figure 2. Response surface for the Sauter diameter of lupin protein-stabilized emulsions prepared under different processing conditions.

analysis was applied. As can be observed, the statistical surface representing the Sauter diameter depends only on the agitation speed (x_1), predicting also a slight increase of this parameter at very high agitation speeds. This could be related to a coalescence process, occasionally observed with other similar emulsions when severe energetic conditions were applied during the emulsification process (Franco et al., 1995).

Rheological Measurements. The linear viscoelastic behavior shown by these types of emulsions is similar to that found for flocculated emulsions (Pal, 1995; Dickinson and Yamamoto, 1996b; Franco et al., 1997). The evolution of the storage modulus (G') with frequency shows a tendency to the development of a plateau region, accompanied by a minimum in the loss modulus (G'') as energy input during the emulsification process increases. Figures 3a and 4a show that there is a clear tendency to a crossover of both viscoelastic functions at low frequencies, at sufficiently low agitation speeds and/or emulsification times. On the contrary, an extended plateau region is observed as the values of both variables increase (Figures 3b and 4b). The plateau modulus, G_N^0, defined as the extrapolation of the entanglement contribution to G' at high frequencies (Baumgaertel et al., 1992), may be approximately calculated from the loss tangent (tan $\delta = G''/G'$) as follows (Wu, 1989):

$$G_N^0 = [G']_{\tan \delta \rightarrow \text{minimum}}$$

This parameter may be considered as a measure of the density of entanglements among polymeric molecules (Ferry, 1980) and has been related to the formation of a structural network in oil-in-water emulsions due to an extensive formation of entanglements among protein molecules located at the oil–water interface (Franco et al., 1995, 1997). As can be observed in Figure 5, the plateau modulus increases linearly with both agitation speed and emulsification time.
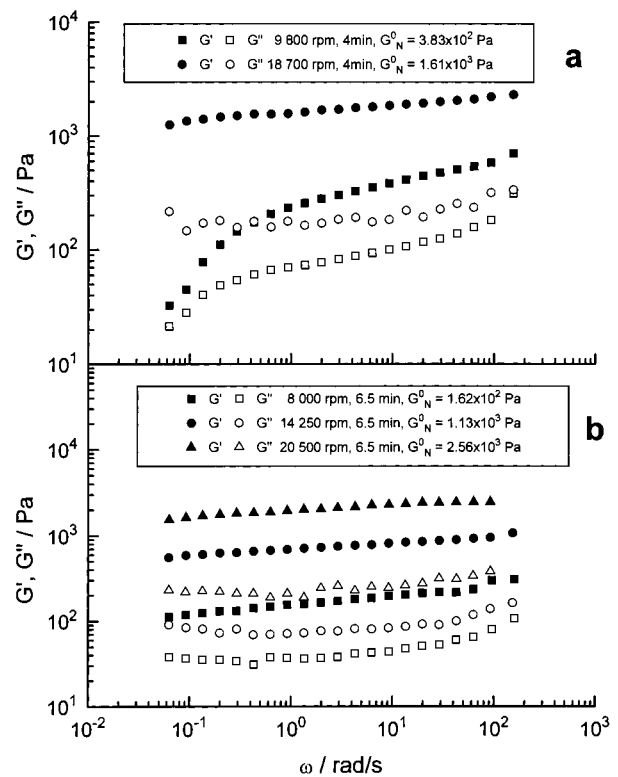

Figure 3. Evolution of the storage and loss moduli with frequency for lupin protein-stabilized emulsions prepared with different agitation speeds.
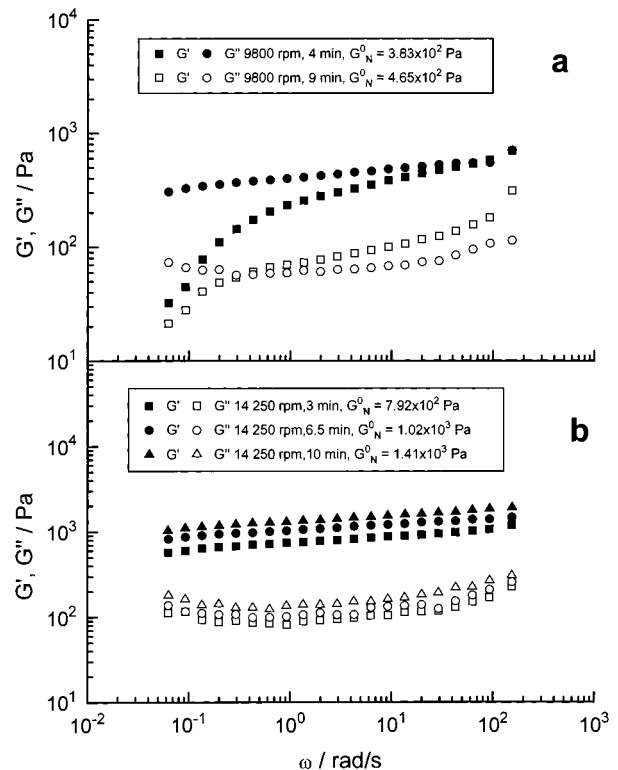

Figure 4. Evolution of the storage and loss moduli with frequency for lupin protein-stabilized emulsions prepared with different emulsification times.

The values of the linear viscoelasticity functions for vegetable protein-stabilized emulsions processed at the lowest agitation speeds are similar to those found with commercial mayonnaise (Gallegos et al., 1992). Nevertheless, the shape of the frequency dependence of
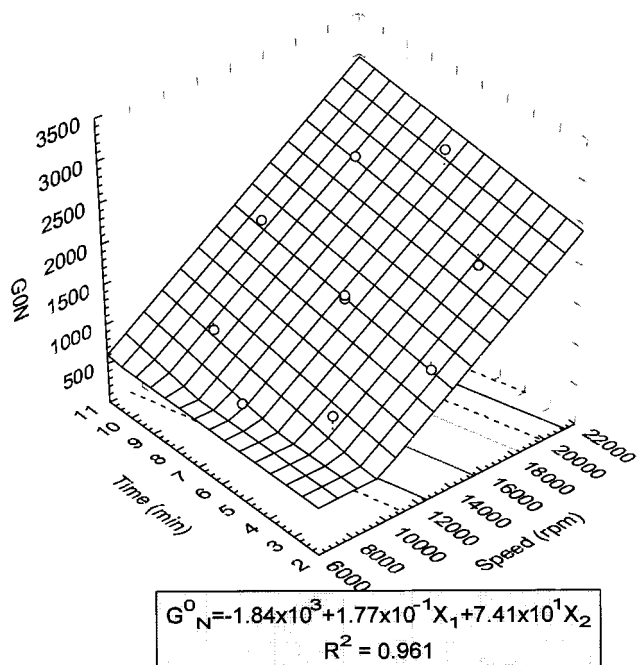
$$G^O_N = -1.84 \times 10^3 + 1.77 \times 10^{-1} X_1 + 7.41 \times 10^1 X_2$$

$$R^2 = 0.961$$

Figure 5. Response surface for the plateau modulus of lupin protein-stabilized emulsions prepared under different processing conditions.

Figure 6. Evolution of the steady-state flow curves with (a) agitation speed and (b) emulsification time for lupin protein-stabilized emulsions.

$$\text{lim. visc.} = -7.48 \times 10^5 + 4.92 \times 10^1 X_1 + 4.98 \times 10^4 X_2$$

$$R^2 = 0.844$$

Figure 7. Response surface for the zero-shear-rate-limiting viscosity of lupin protein-stabilized emulsions prepared under different processing conditions.

these functions may be different if the emulsification time is not long enough, favoring the appearance of a pseudo-terminal region at low frequencies. This region progressively disappears as emulsification time increases, yielding a linear relaxation spectrum with two characteristic regions, plateau and transition, which are the only two regions that appear in commercial mayonnaise. Both regions are also found for emulsions processed at larger agitation speeds, although in this case the values of the linear viscoelasticity functions are significantly higher than those shown by the commercial mayonnaise.

The presence of a pseudo-terminal region at low frequencies has been previously reported for emulsions stabilized by a mixture of egg yolk and a high HLB sucrose stearate (Franco et al., 1995), although, in this case, this region was always shown within the range of processing variables chosen (5000−8000 rpm; 3−10 min). For both types of emulsions, the plateau region in the relaxation spectrum spreads as energy input increases. In addition to this, the values of G' and G'', for emulsions processed at severe energetic conditions, are rather similar.

On the other hand, the values and the shape of the frequency dependence of G' and G'' of lupin-stabilized emulsions are comparable to those found for emulsions stabilized by a continuous-phase gel-forming emulsifier (Gallegos and Franco, 1998). However, with this type of emulsifier, high agitation speeds may produce a decrease in G' and G'' values. This is due to the fact that the gel-like structure tends to vanish, as a consequence of the formation of a larger interfacial surface related to a decrease in droplet size and polydispersity as the agitation speed increases.

Steady-state flow curves always show a shear-thinning behavior with a clear tendency to a zero-shear-rate-limiting viscosity, η_0, at very low shear rates. Thus, Figure 6 shows selected steady-state flow curves as a function of agitation speed (Figure 6a) and emulsification time (Figure 6b). As may be clearly observed, the
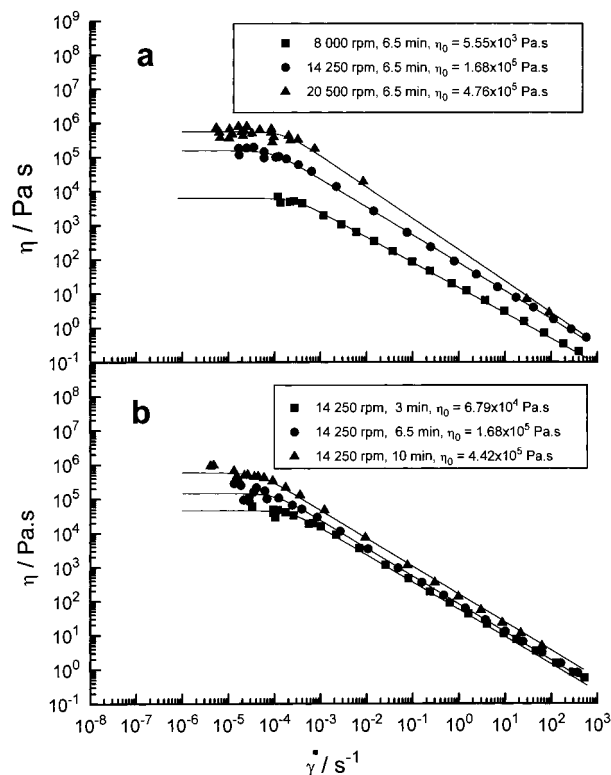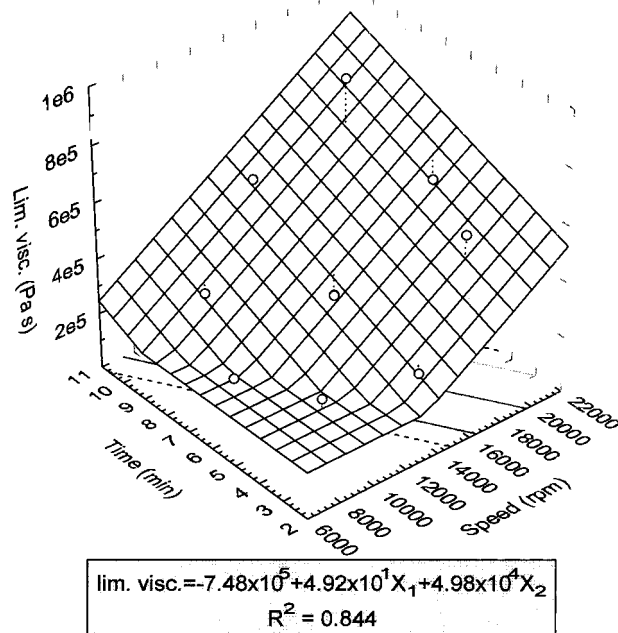
steady-state viscosity increases with both variables within the whole shear rate range studied, although the influence is more relevant at low shear rates. This fact explains the dramatic differences found in the values of the zero-shear-rate-limiting viscosity when emulsions prepared under mild and energetic conditions are compared (Figure 7).

This flow behavior can be fitted ($r^2 > 0.95$) to the Carreau model fairly well (Barnes et al., 1989)

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$$\eta = \eta_0/[1 + (\dot{\gamma}/\dot{\gamma}_c)^2]^s$$

where $\dot{\gamma}_c$ is the critical shear rate for the onset of the shear-thinning behavior and s is a parameter related to the slope of this region. The values of these parameters for all of the systems studied are also shown in Table 1. The differences found in the slope of the shear-thinning region make inappropriate the application of an empirical superposition method to obtain a master flow curve introducing the influences of both processing variables. In fact, agitation speed and emulsification time have a similar influence on the slope of the shear-thinning region, which increases with both variables. In other words, the influence of shear rate on the structural breakdown is much more important for emulsions processed at severe energetic conditions.

The results obtained from the rheological measurements may be explained on the basis of the development of an entanglement network as energy input during the emulsification process increases. This is due to bridging flocculation of oil droplets as a result of increased physical interactions among protein chains. As has been previously demonstrated (Raymundo et al., 1998), an increase in temperature modifies the hydrophobicity of lupin protein, which verifies that a thermal treatment improves the rheological properties of these emulsions. Likewise, the rise in temperature (up to 50−55 °C) induced by an intense agitation or long emulsification times may significantly affect the structure of the protein, increasing its hydrophobicity (Raymundo et al., 1998). This would explain why the values of the determined rheological parameters, G_N^0 and η_0, are much larger at high agitation speeds or long emulsification times, although droplet size may not be significantly influenced by these processing variables. As has been previously pointed out (Franco et al., 1995), an increase in the emulsification temperature enhances the formation of an entanglement network among oil droplets. Consequently, the rheological behavior cannot be explained only as a function of the variation of the mean droplet size and polydispersity. Thus, the rheology of the continuous phase and the interdroplet interactions should be taken into account (Gallegos and Franco, 1998), and both of them may be affected by a change in the protein conformation.

Textural Properties. Figures 8 and 9 show the response surfaces and the statistical models for firmness and adhesiveness of these emulsions as a function of the processing variables. As can be observed, both textural parameters show a similar evolution, increasing with emulsification time and agitation speed. Nevertheless, the statistical model predicts a dependence only on this last variable. Similarly to the variation found in the rheological parameters, firmness and adhesiveness mainly increase at high agitation speeds, which must be related to a decrease in droplet size. However, as the Sauter diameter mainly decreases at low agitation speeds (Figure 2), interdroplet interactions must also play a key role on the development of textural characteristics. A similar evolution of these textural parameters was found when lupin protein was submitted to a consecutively more energetic, previous thermal treatment (Raymundo et al., 1998), indicating that an important increase of the interparticle interactions by the formation of a heat-set protein network significantly affects the texture of these emulsions.

Figure 10 shows the relationship between the textural parameters studied and the zero-shear-rate-limiting
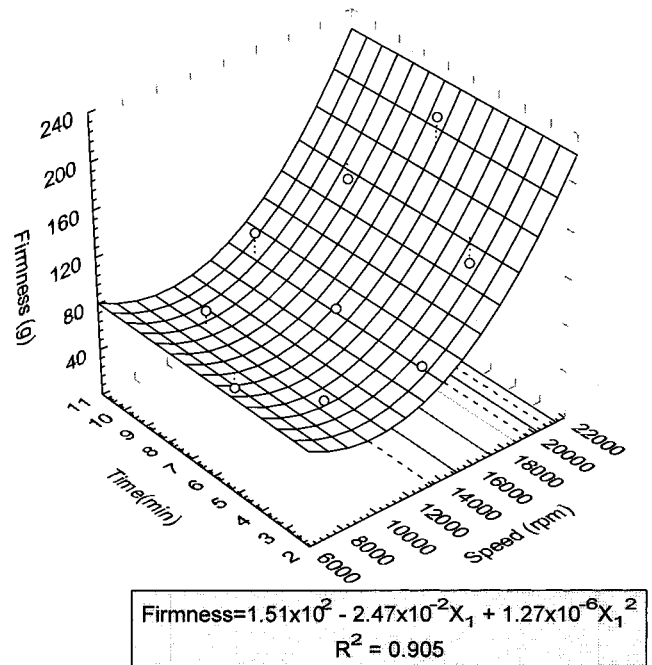

Firmness=1.51×10^2 - $2.47\times10^{-2}X_1$ + $1.27\times10^{-6}X_1^2$
$R^2 = 0.905$

Figure 8. Response surface for the firmness of lupin protein-stabilized emulsions prepared under different processing conditions.

Adhesiveness=1.40×10^2 - $2.43\times10^{-2}X_1$ + $1.45\times10^{-6}X_1^2$
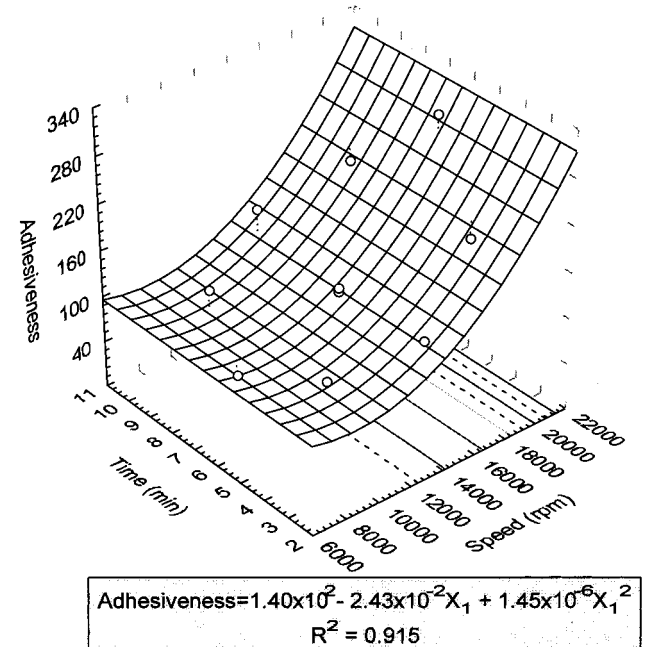$R^2 = 0.915$

Figure 9. Response surface for the adhesiveness of lupin protein-stabilized emulsions prepared under different processing conditions.

viscosity. As has been previously shown for other food systems (Richardson et al., 1989; Hill et al., 1995), a linear equation that relates the logarithm of textural parameters and the logarithm of the viscosity (Figure 10) can be fitted ($r^2 > 0.91$). These results support the view that the viscosity measured at very low shear rates is also a criterion for predicting sensorial parameters (Hill et al., 1995).

Concluding Remarks. From the experimental results it can be concluded that an increase in emulsification time and, especially, agitation speed produces a decrease in the Sauter diameter and favors the development of an entanglement network, as shown by the
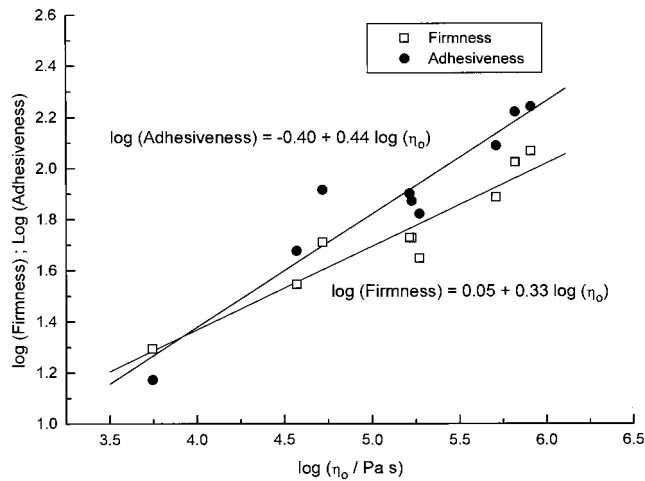
Figure 10. Correlation of the firmness and adhesiveness as a function of the zero-shear-rate-limiting viscosity.

appearance of the plateau region in the linear viscoelastic functions and a significant increase in the values of the plateau modulus. This also results in a significant increase of emulsion viscosity, firmness, and adhesiveness. The evolution of the rheological and textural parameters with processing variables cannot be explained only by the modification of the DSD. Thus, the increase in the temperature during the emulsification, induced by the application of a severe mechanical energy, must affect the protein hydrophobicity and, consequently, favor the interdroplet interactions.

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