Optimization of the Composition of Low-Fat Oil-in-Water Emulsions Stabilized by White Lupin Protein

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ABSTRACT: The effect of composition (protein, xanthan gum, and oil content) of lupin protein-stabilized emulsions on their physical properties—droplet size distribution, rheological behavior, and texture—was studied. Droplet size distribution was measured by laser light-scattering experiments, rheological parameters were determined from oscillatory and steady-state flow measurements, and texture responses were obtained from texture profile analysis. Response surface methodology was used to optimize the emulsion composition using commercial mayonnaise parameters as a standard. An increase in protein, xanthan gum, or oil content produced, in the experimental range considered, an increase in the rheological and textural parameters studied, as well as a decrease in the average oil droplet diameter. It was possible to produce stable lupin protein-stabilized emulsions with physical properties similar to those of commercial mayonnaise for a wide variety of protein, xanthan gum, and oil concentration values, which may be designed to suit market specifications.

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KEY WORDS: Emulsion, low-fat, lupin protein, mayonnaise, rheology, texture, viscoelasticity.

Traditionally, food emulsions such as mayonnaise and salad dressing are stabilized by egg yolk lipoproteins. Nevertheless, a growing interest in alternative protein sources has been evident in the last few years. The emulsification properties of animal proteins such as whey protein, lactoglobulins, and casein have been extensively investigated by several authors (1,2). Moreover, vegetable protein isolates can be used effectively as food emulsion stabilizers because of their capacity to lower the interfacial tension between hydrophobic and hydrophilic components (3). Several vegetable proteins from soy (4), sunflower (5), pea (6), or tomato seed (7) have been tested successfully to stabilize oil-in-water (o/w) emulsions.

White lupin protein has been investigated in foods, and its capacity to produce stable emulsions has been proven (8–10). The emulsifying conditions (time and agitation speed) (11) as well as the previous protein thermal denaturation (12) play an important role in the resulting emulsion properties. But other important variables must be optimized (i.e., oil and emulsifier concentration, type of oil and emulsifier, presence of polysaccharide, pH, ionic strength, or salt content) to obtain vegetable protein-stabilized emulsions with properties similar to those found in commercial mayonnaises.

The increasing interest in developing low-fat products for human consumption prompted the use of thickening agents (xanthan gum, galactomannans, modified starches, propylene glycol alginate, and pectin) to act as fat replacers in the production of commercial mayonnaise and salad dressings (13). The addition of this type of polysaccharide affects emulsion stability through rheological modification of the aqueous phase and interactions with the protein films (14), thus retarding or even eliminating the processes leading to emulsion instability, such as creaming, sedimentation, flocculation, and coalescence. The widespread uses of xanthan gum in the food industry are related to its capacity to provide higher storage stability at low concentrations, its water-binding ability, and the improvement it gives in mouthfeel/texture. The amount of xanthan gum required is inversely related to the oil content (15). The main goal of this work was to optimize the composition of low-fat oil-in-water emulsions stabilized by white lupin protein and xanthan gum. The impact of lupin protein, xanthan gum, and oil concentrations on the droplet size distribution (DSD), rheology, and textural properties of these emulsions was evaluated.

EXPERIMENTAL PROCEDURES

Lupin protein isolate (L9020; Mittex Alangenbau GmbH, Weingarten, Germany) dispersions with xanthan gum (Kelco, Surrey, United Kingdom) were prepared in distilled water with magnetic stirring for 30 min at room temperature (20–22ºC), at pH values between 6.0 and 6.5. These lupin dispersions were not thermally denaturated because of the possibility of achieving commercial mayonnaise-like properties using this isolate in its native state (12). Oil-in-water lupin protein-stabilized emulsions with different combinations of protein isolate (2–7% weight), xanthan gum (0–0.49% weight), and sunflower oil (25–60% weight) were prepared in an UltraTurrax T-25 rotor-stator homogenizer (IKA, Staufen, Germany). Protein, xanthan gum, and oil contents were programmed according to an experimental design based on the response surface methodology (RSM), using a central composite rotatable matrix (16). The three independent variables were tested at five levels according to the design matrix shown in Table 1. Processing variables (14,250 rpm during

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TABLE 1
Response Surface Matrix and Measured Parameters for Low-Fat Lupin Protein-Stabilized Emulsions with Different Contents of Protein, Xanthan Gum, and Oil Concentration[a]

Protein (%, w/w)	Xanthan (%, w/w)	Oil (%, w/w)	d_{SV} (μm)	G_N^0 (Pa)	η_0 (Pa s)	$\dot{\gamma}_c$ (s^{-1})	*s*	Firmness (g)	Adhesiveness (g s)
4.5	0.25	25.0	4.0	2.5×10^1	3.6×10^2	3.0×10^{-2}	0.43	1.8×10^1	NE
3.0	0.10	32.1	6.0	NE	1.0×10^2	NE	NE	1.6×10^1	NE
3.0	0.39	32.1	3.5	6.0×10^1	1.0×10^3	4.1×10^{-3}	0.41	2.3×10^1	1.0×10^1
6.0	0.10	32.1	4.5	NE	3.5×10^2	NE	NE	1.7×10^1	1.2×10^1
6.0	0.39	32.1	2.8	9.5×10^1	2.6×10^3	4.5×10^{-3}	0.42	3.5×10^1	2.1×10^1
3.0	0.10	52.9	3.4	3.1×10^1	3.4×10^2	9.0×10^{-3}	0.33	2.1×10^1	1.5×10^1
6.0	0.10	52.9	2.5	1.1×10^2	2.5×10^3	2.5×10^{-3}	0.37	5.3×10^1	5.0×10^1
3.0	0.39	52.9	2.7	4.9×10^2	2.2×10^4	6.9×10^{-4}	0.39	5.6×10^1	6.0×10^1
6.0	0.39	52.9	2.4	1.1×10^3	7.6×10^4	6.4×10^{-4}	0.41	1.2×10^2	1.4×10^2
2.0	0.25	42.5	3.7	4.3×10^1	4.3×10^2	5.7×10^{-3}	0.36	2.0×10^1	1.7×10^1
7.0	0.25	42.5	2.5	1.3×10^2	3.1×10^3	3.0×10^{-3}	0.40	3.8×10^1	2.9×10^1
4.5	0.00	42.5	5.3	NE	2.2×10^1	NE	NE	1.6×10^1	NE
4.5	0.49	42.5	2.7	2.6×10^2	1.6×10^4	1.0×10^{-3}	0.40	5.0×10^1	4.6×10^1
4.5	0.25	60.0	2.6	1.5×10^3	1.3×10^5	4.5×10^{-4}	0.43	1.4×10^2	1.8×10^2
4.5	0.25	42.5	2.8	7.3×10^1	2.2×10^3	6.3×10^{-3}	0.40	3.3×10^1	2.4×10^1
4.5	0.25	42.5	2.8	6.2×10^1	1.7×10^3	3.5×10^{-3}	0.39	3.2×10^1	2.2×10^1
4.5	0.25	42.5	2.8	7.9×10^1	2.3×10^3	4.6×10^{-4}	0.37	3.4×10^1	2.5×10^1
4.5	0.25	42.5	2.8	7.7×10^1	2.1×10^3	2.8×10^{-3}	0.38	3.2×10^1	2.4×10^1
4.5	0.25	42.5	2.8	7.1×10^1	2.4×10^3	4.0×10^{-3}	0.39	3.4×10^1	2.3×10^1

[a]d_{SV}, Sauter diameter of oil droplets; G_N^0, plateau modulus of emulsion; η_0, zero-shear rate-limiting viscosity of emulsion; $\dot{\gamma}_c$, critical shear rate of emulsion flow; *s*, slope of viscosity decay with shear rate in emulsion flow; NE, nonexistent.

5 min) were selected according to the minimum value of mean droplet size found in similar systems (11). The dependent variables determined were the Sauter diameter (d_{SV}) of oil droplets, the plateau modulus (G_N^0), the zero-shear rate-limiting viscosity (η_0), firmness, and adhesiveness.

DSD measurements were performed in a Malvern Mastersizer-X (Malvern, United Kingdom). The Sauter mean diameter, which is proportional to the reciprocal value of the droplets' specific surface area, was obtained as follows (17):

$$d_{SV} = \sum_i n_i d_i^3 \Big/ \sum_i n_i d_i^2 \qquad [1]$$

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

Linear viscoelasticity and steady-state flow measurements were carried out in a controlled-stress rheometer (RS-75) from Haake (Karlsruhe, Germany), from which G_N^0 and η_0 were determined, respectively. Oscillatory tests were performed using a cone-and-plate sensor system (35 mm, 2º) in a frequency range of 10^{-1}–10^2 rad/s inside the linear viscoelastic region, 0.4–50 Pa, depending on the emulsion structure. Steady-state flow curves were obtained with a serrated-plate plate-sensor system (20 mm) to overcome the wall-slip effects observed (18) in the steady-state flow of food emulsions.

Textural variables were obtained from a textural profile analysis carried out in a TA-XT2 texturometer (Stable Micro Systems, Surrey, United Kingdom). Penetration tests were performed with a cylindrical probe in a load cell of 5,000 × *g* and 2 mm/s of crosshead speed. From the force vs. time texturograms, parameters including firmness and adhesiveness were calculated. Firmness was considered as the maximum resistance to the penetration of a 38-mm diameter cylinder at a 5-mm depth in a 60-mm diameter cylindrical glass flask filled with emulsion up to a 5-cm height. This value was 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 measurements were conducted at 20 ± 1ºC and replicated at least three times.

Statistical approach. The optimization of any process or formulation can be achieved systematically with a considerable reduction of time and resources by using RSM, a process that gives a mathematical model of the relationship between a dependent variable *Y*, or response, and the independent variables, x_i (e.g., Ref. 16). The function $Y = f(x_i)$ is represented by an equation that is an approximation of the true relationship.

The number of experimental trials required increases rapidly with the number of variables and with the complexity of the model to be fitted. Three independent variables were considered, i.e., protein, xanthan gum, and oil concentration. The physical properties of the emulsions were chosen as the dependent variables (d_{SV}, G_N^0, η_0, firmness, and adhesiveness).

Polynomial models are most frequently employed because they have the advantage of being easy to fit by using multiple regression. The use of a second-order polynomial of the form shown in the following equation is often chosen

$$Y = a + a_1x_1 + a_2x_2 + a_3x_3 + a_{11}x_1^2 + a_{22}x_2^2 + a_{33}x_3^2 + a_{12}x_1x_2 + a_{13}x_1x_3 + a_{23}x_2x_3 + E \qquad [2]$$

Use of this equation represents a good compromise between the conflicting requirements of providing a good fit to a complex surface, keeping the total number of experimental points down to an acceptable level, and using terms whose meaning can be readily appreciated.

The experimental design used was of the central composite rotatable type with three blocks. The first is a traditional factorial design with 2^p points [where p is the number of x_i] with levels coded +1 and −1 for each of the p variables. These 2^p points are the vertices of a p-dimensional cube centered at the origin of the coded system of reference. The distance of these points from the origin is $p^{1/2}$. The second set accounts for nonlinearity and is a star of $2p$ points coded as α and −α on the axis of the system at a distance of $2^{p/4}$ from the origin. The third set also accounts for nonlinearity and consists of the central points, which are replicated to provide an estimate of the experimental error. By choosing the appropriate value for α and repeating the central points a number of times, the design can be given the property of rotatability. This means that the standard error of Y will be the same for all points that are at the same distance from the center of the region.

RESULTS AND DISCUSSION

The experiments carried out according to RSM methodology and the results of each response are summarized in Table 1.

DSD. The influence of protein, xanthan gum, and oil con-

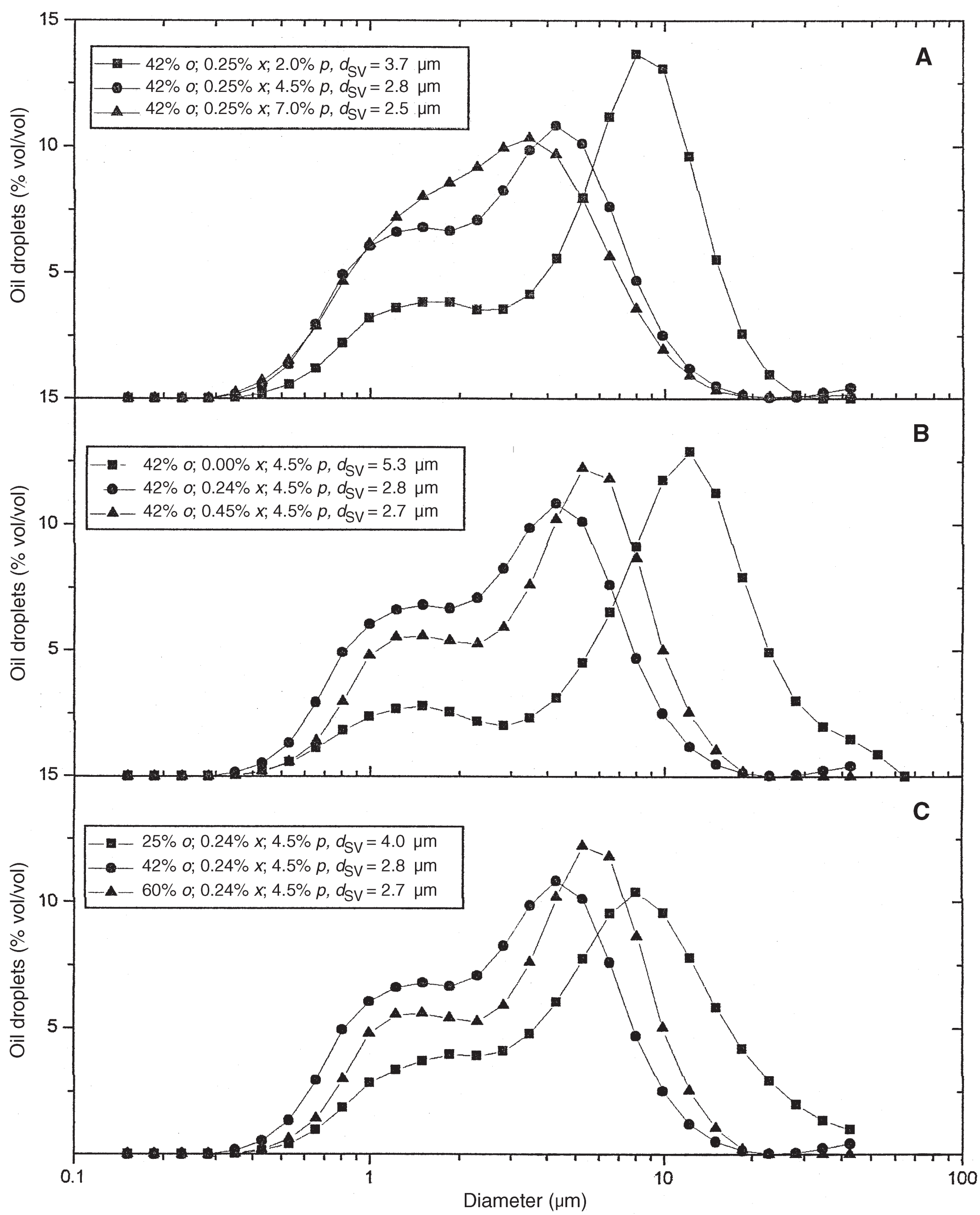

FIG. 1. Evolution of the droplet size distribution with changes in protein (A), xanthan gum (B), and oil concentrations (C) for lupin protein-stabilized emulsions. d_{SV}, Sauter diameter; *p*, protein; *x*, xanthan gum; *o*, oil.

centrations on DSD curves is shown in Figure 1 for selected emulsions. The DSD curves are generally bimodal in shape, showing a secondary maximum at low diameters. An increase in protein content (Fig. 1A) produced a significant decrease in oil droplet size, making the secondary maximum more pronounced. At constant protein (4.5% weight) and oil (42% weight) contents, a reduction in oil droplet size was observed when the xanthan gum concentration increased, followed by an increase in the secondary maximum and a displacement of the main maximum of the distribution into smaller sizes (Fig. 1B). The quantification of the droplet diameter used was the Sauter formula (Eq. 1), and this includes the n_i number of oil droplets. This explains the apparent reversed order of the last two curves of Figures 1B and 1C. The same effect, i.e., the reduction of oil droplet diameter, was observed when sunflower oil concentration (Fig. 1C) was increased. The tendency of the Sauter diameter to decrease with an increase in protein, xanthan gum, and oil content was observed for the whole range of compositions studied, which can be expressed in terms of their respective response surfaces or statistical model from the multiple regression for these three variables (*p,* protein; *x,* xanthan; *o,* oil):

$$d_{SV} = 2.9^{***} - 0.8^{***}p - 1.4^{***}x - 1.2^{***}o + 0.8^{***}x^2 + 0.3^{**}o^2 + 0.9^{***}xo \quad [3]$$

with $R^2 = 0.96$ (a mean absolute error of 0.0683) and significances of $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

Linear viscoelasticity. The evolution of the storage (G') and loss (G'') moduli with frequency for the emulsions prepared with different protein, xanthan gum, and oil concentrations are shown in Figures 2A, 2B, and 2C, respectively. In most of the emulsions studied, a plateau region in the frequency range studied may be found. This is a typical shape for flocculated systems, as previously found (11,12,19). This shape of curve results from the development of an entanglement network between adsorbed and nonadsorbed macromolecules, yielding a gel-like structure (20). From the mechanical spectrum shown by this kind of emulsion, it is possible to determine the plateau modulus, G_N^0, which can be easily estimated as the value of G' obtained for the minimum value of the loss tangent ($\tan\delta = G''/G'$), expressed as (21):

$$G_N^0 = [G']_{\tan\delta \to \text{minimum}} \quad [4]$$

As may be observed in Figure 2, G_N^0 increased with an increase of protein, xanthan gum, and oil content because of an increase in both viscoelastic functions. In most cases, G' values were higher than G'', indicating an important elastic component in these emulsions. Nevertheless, in Figure 2B a different behavior may be observed for the emulsion prepared with 4.5% of protein, 0% of xanthan gum, and 42% of oil. In this case, G'' was higher than G' for all frequencies studied, which is a typical behavior of nonflocculated or weakly flocculated emulsions. In this case, it was not possible to determine the plateau modulus, since $\tan\delta$ did not show a minimum in the experimental frequency range studied.

An increase of viscoelastic functions with oil and gum content also was found (22) for commercial mayonnaises with different compositions, showing an enhancement of shear sensitivity in the viscoelastic network for low concentrations of the dispersed phase. The reinforcement of the viscoelasticity because of the presence of the gum was explained in some cases by an increase in the interactions between protein and stabilizer molecules. Nevertheless, the addition of gum may increase the elasticity of the emulsion by itself as a result of the formation of a strong gel-like structure in the continuous phase, yielding also smaller oil droplet diameters because of a reduced coalescence process during emulsification.

A tendency of G_N^0 to increase with protein, xanthan gum, and oil concentration was found consistently and is reflected by the response surfaces and the statistical model for these dependent variables:

$$G_N^0 = 3.3\times10^{3***} - 5.2\times10^{2**}p + 4.4\times10^{3**}x - 1.8\times10^{2***}o - 9.0\times10^{3**}x^2 + 2.1\times10^{0***}o^2 + 9.1\times10^{0**}po + 6.0\times10^{2**}px \quad [5]$$

with $R^2 = 0.99$, a mean absolute error of 49.50 and significances of $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

Flow behavior. The steady-state flow curves are summarized in Figure 3 for selected emulsions. The effect of protein (A), xanthan gum (B), and oil concentration (C) on the zero-shear rate-limiting viscosity can be observed. All emulsions showed a shear-thinning behavior with a clear tendency to a zero-shear rate-limiting viscosity, η_0, at very low shear rates. This flow behavior can be fitted ($R^2 > 0.95$) to the Carreau model fairly well:

$$\eta = \eta_0 \Big/ \left[1 + (\dot{\gamma}/\dot{\gamma}_c)^2\right]^s \quad [6]$$

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 fitting parameters are shown in Table 1.

The zero-shear rate-limiting viscosity, η_0, clearly depends on emulsion composition. In Figure 3A a slight tendency of η_0 to increase with protein content can be noticed. An important increase in η_0 with both xanthan gum and oil concentrations, however, can be observed in Figures 3B and 3C, respectively. In fact, the increase of zero-shear rate viscosity with protein concentration was not statistically significant. But this dependent variable significantly increased with xanthan gum and oil content following the equation:

$$\eta_0 = 8.5\times10^{3*} + 4.8\times10^{4**}x + 6.2\times10^{4***}o - 6.0\times10^{4**}x^2 + 4.0\times10^{4***}o^2 \quad [7]$$

with $R^2 = 0.86$, a mean absolute error of 63.93 and significances of $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

Concerning the critical shear rate, this parameter decreased as protein, oil, and xanthan gum concentrations increased (Table 1), which is related to a higher shear sensitivity in the most structured emulsions. Parameter s was similar in all the

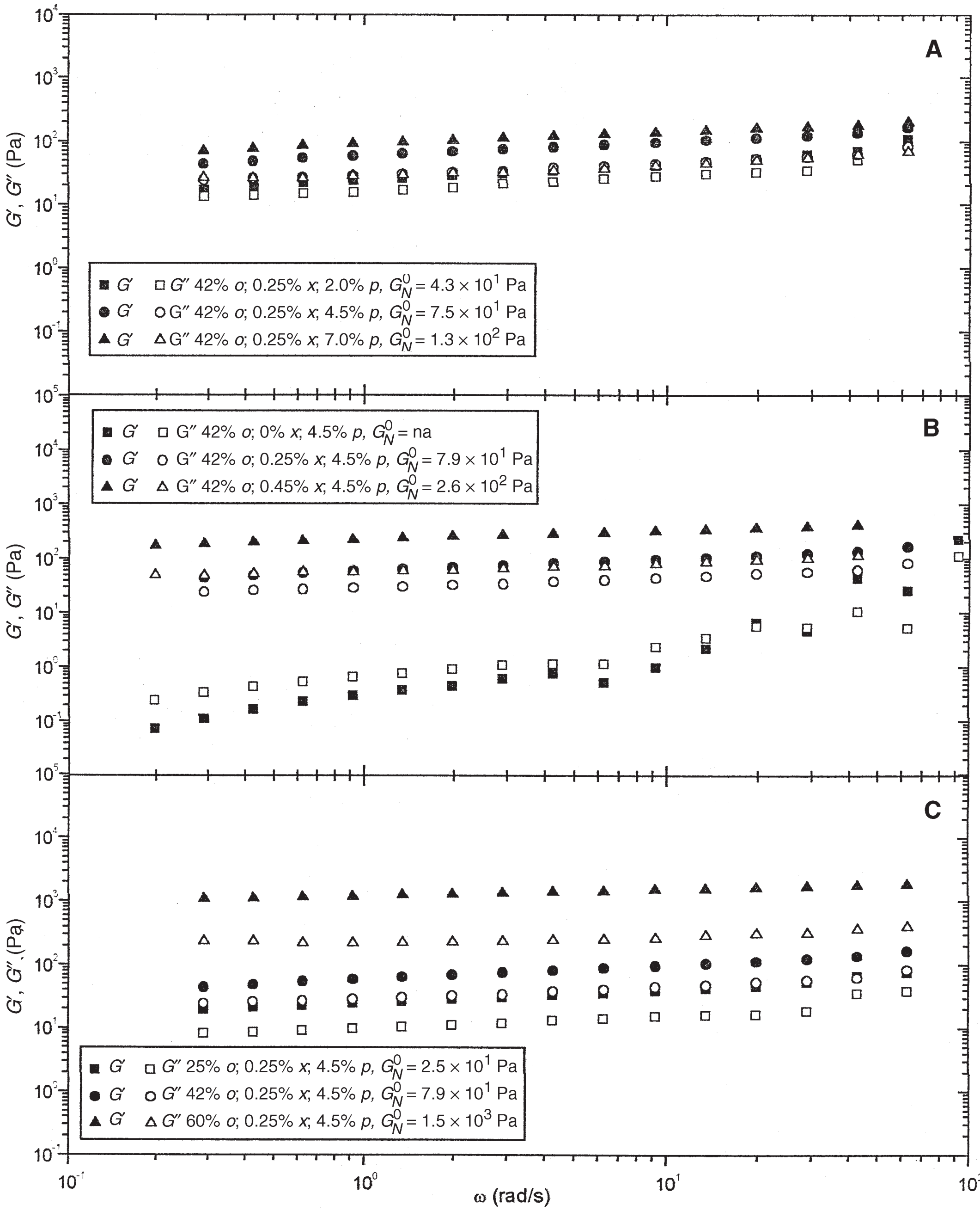

FIG. 2. Evolution of the storage and loss moduli with changes in protein (A), xanthan gum (B) and oil concentrations (C) for lupin protein-stabilized emulsions. *G′*, storage modulus; *G″*, loss modulus; G_N^0, plateau modulus; *p*, protein; *x*, xanthan gum; *o*, oil.

emulsions (0.40 ± 0.03) and not statistically influenced by emulsion composition.

Textural parameters. The mathematical models explaining how firmness and adhesiveness of emulsions behave for different compositions in terms of protein, xanthan gum, and oil contents, according to the design matrix given in Table 1, are

$$\begin{aligned}\text{Firmness} &= 3.2\times10^{2^{**}} - 2.1\times10^{1^{*}}p - 1.7\times10^{2^{*}}x \\ &\quad - 1.4\times10^{1^{***}}o + 6.1\times10^{0^{**}}xo + 6.4\times10^{-1^{**}}po \\ &\quad + 1.4\times10^{-1^{***}}o^2 \end{aligned} \qquad [8]$$

with $R^2 = 0.91$ a mean absolute error of 6.30 and significances of $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

$$\begin{aligned}\text{Adhesiveness} &= 4.1\times10^{2^{**}} - 2.3\times10^{1^{*}}p - 2.8\times10^{2^{*}}x \\ &\quad - 2.0\times10^{1^{***}}o + 7.3\times10^{-1^{*}}po + 9.3\times10^{0^{**}}xo \\ &\quad + 2.1\times10^{-1^{***}}o^2 \end{aligned} \qquad [9]$$

with $R^2 = 0.90$, a mean absolute error of 10.30 and significances of $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

Both textural parameters showed a similar trend with the

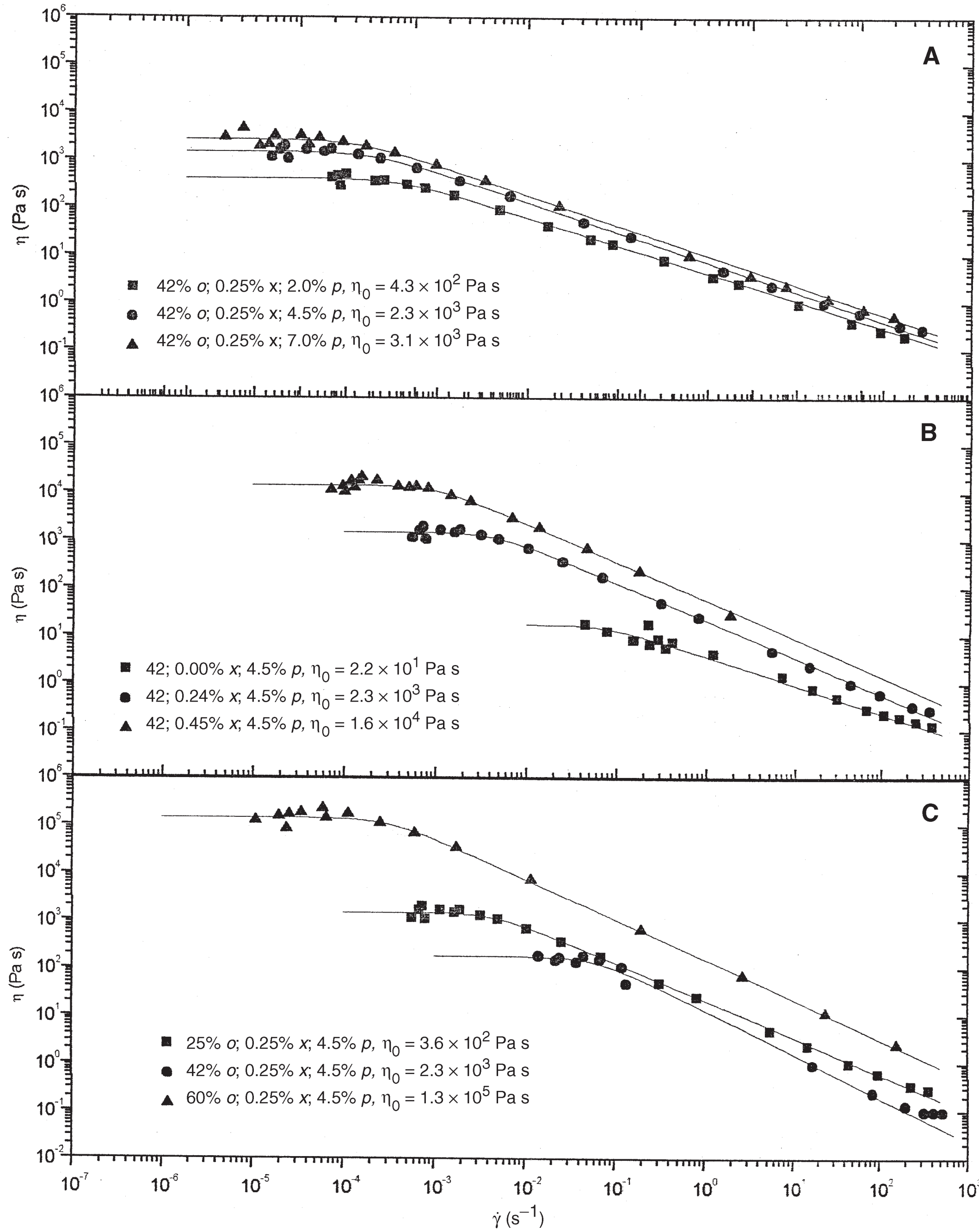

FIG. 3. Evolution of the steady-state flow curves with changes in protein (A), xanthan gum (B), and oil concentrations (C) for lupin protein-stabilized emulsions.η_0, zero-shear rate-limiting viscosity; *p,* protein; *x,* xanthan gum; *o,* oil.

independent variables, i.e., firmness and adhesiveness increased with protein, xanthan gum, and oil concentration. These results are consistent with the development of a gel-like structure or with a substantial increase in the viscosity of the continuous phase, imparting a more firm and adhesive structure. A similar effect was found for emulsions prepared under emulsifying conditions varying in speed and time of stirring (11).

Optimization. To optimize the lupine protein-emulsion composition, 10 commercial mayonnaises were tested. The average values of each response and the respective ranges of variation are summarized in Table 2. An optimal lupin protein-emulsion composition was obtained by solving the previous equations to yield the average values of each independent variable measured for these mayonnaises as a response. Using this approach, a set of combinations of protein, xanthan gum, and oil content was found (Table 2). All of these combinations, in terms of emulsion composition, had physical properties within the ranges of variation of the tested commercial mayonnaises. To define an optimal formulation, it is

TABLE 2
Emulsions with Different Contents of Lupin Protein, Xanthan Gum, and Oil with Respective Physical Parameter Values Forcast from the Mathematical Models Evolved[a]

x_1 Protein (%, w/w)	x_2 Xanthan (%,w/w)	x_3 Oil (%, w/w)	Firmness (g)	Adhesiveness (g s)	G_N^0 (Pa)	η_0 (Pa s)	d_{SV} (μm)
			Av: 113 Rv: 51–151	Av: 135 Rv: 64–182	Av: 427 Rv: 106–806	Av: 2×10^5 Rv: 3×10^4–4×10^5	Av: 5.5 Rv: 5.6–10.5
2.0	0.44	60	112	153	832	1.2×10^5	3.47
4.5	0.20	60	110	137	1238	9.7×10^4	2.11
6.0	0.25	56	113	135	1187	7.4×10^4	1.74
7.0	0.36	52	115	135	1250	5.7×10^4	1.59

[a]Average (Av) and range (Rv) values that can be used as target values of these properties, previously obtained for 10 commercial mayonnaises. See Table 1 for other abbreviations.

necessary to use an additional constraint. Therefore, if the main goal is to produce low-fat emulsions (less than 60% of oil), formulations with about 6 to 7% protein and 0.3 to 0.4% xanthan should be used. In these cases, high contents of emulsifier and gum are used to serve the role of fat mimetics. Alternatively, if the protein level is the limiting factor, as in a diet for people with gout, a composition with 2% protein and 0.4% of xanthan gum or 4.5% protein and 0.2% of this gum, both with 60% of sunflower oil, could be used. In addition, lupin protein-stabilized emulsions with rheological and textural parameters lower than those shown in Table 2 may be used as an alternative to other types of food emulsions, such as in more fluid salad dressings.

Thus, an increase of protein, xanthan gum, and oil concentrations yielded greater values for the plateau modulus, zero-shear rate-limiting viscosity, firmness, and adhesivness and smaller oil droplet size. These are typical data characteristic of emulsions with high stability (23). Depending on the desirable level of protein, xanthan gum, and oil concentration, creation of lupin protein emulsions with physical properties closely matching those of commercial mayonnaises is possible. A set of emulsion compositions can be formulated to suit many special diet requirements.

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