Rheological Characterization of Egg Yolk Processed by Spray-Drying and Lipid-Cholesterol Extraction with Carbon Dioxide

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ABSTRACT: The present work studies the rheological properties of avian egg yolks processed by spray-drying and by lipid-cholesterol extraction with CO_2 under near-critical conditions. The results have been compared with those obtained from native egg products in order to analyze the effect of processing on the microstructure and rheological properties of egg yolks. The rheological study included linear viscoelasticity measurements by means of dynamic oscillatory shear and creep tests, which provided information about the unperturbed structure of the material, as well as steady-state flow measurements. The thermal denaturation of proteins, which was produced during the spray-drying of egg yolk, was responsible for the dramatic change from fluid to gel-like behavior. The thermal denaturation of proteins promotes the formation of a gel-like network, which may be attributed to exposure of internal hydrophobic groups that favor protein aggregation. Lipid (and cholesterol) extraction from yolk increased the viscoelasticity functions and flow properties due to the increased protein concentration.

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KEYWORDS: Egg yolk, linear viscoelasticity, lipid and cholesterol extraction, rheology.

Egg yolk is a complex association of lipids and proteins, which represent about 33 and 17% (w/w), respectively, dispersed or dissolved in aqueous phase. The yolk consists of a dispersion of 20% (w/w) granules in the plasma, which contains the soluble protein fraction of which 85% is LDL and 15% is livetin. The granules are formed by association of three types of proteins: 70% lipovitellins, also called HDL; 16% phosvitin; and 12% LDL (1,2). The lipid fraction contains approximately 66% TAG, 28% phospholipids (lecithin and cephalin), and 5% cholesterol as well as minor amounts of other lipids (3). As a consequence, egg yolk is rich in essential amino acids and constitutes an excellent source of highly nutritional and functional ingredients in a wide variety of food products.

The major applications for egg yolk in the food industry are the manufacture and stabilization of emulsions, such as mayonnaise and salad dressings, foams involved in the preparation of a variety of bakery products, and gels formed in different yolk-containing desserts such as custard or caramel custard. The process of emulsification, foaming, and gelation as well as the quality of the final products is highly influenced by the rheological properties of the initial yolk-containing phase (4). Therefore, knowledge of the rheological properties and microstructure of yolk products is important for its commercial applications.

The main objective of this study was to characterize the rheological properties of egg yolk products processed by spray-drying and near-critical extraction with CO_2. Furthermore, the results of this study have been compared with those obtained from native egg products in order to analyze the effects of processing on the microstructure and rheological properties of egg. The results obtained may be related in future investigations to modifying functional properties, such as emulsification and gelation, of egg products and therefore may contribute to the development of food products formulated with low-fat, low-cholesterol yolk. The rheological study performed has included linear viscoelasticity measurements by means of dynamic oscillatory shear and creep tests, which provide information about the unperturbed structure of the material, as well as steady-state flow measurements.

EXPERIMENTAL PROCEDURES

Materials. The egg products used were technical yolk (NY) containing 10 wt% salt and 45 wt% total solids, supplied by Hijos de Ybarra, S.A. (Sevilla, Spain), spray-dried yolk (DY) from Ovosec, S.A. (Valladolid, Spain), and low-fat, low-cholesterol yolk extracted by a near-critical CO_2 process at lab scale (SCY) (5). Commercial spray-dried egg white (DW) from SBI, S.A. (Barcelona, Spain) was also used in the preparation of samples.

Solids concentrations of egg products ranged from 25 to 50%. DY was diluted with water up to 50 and 45 wt% solids, which corresponds to NY, and with DW/water (12 wt% solids) up to 25%, which represents the concentration of whole egg.

LCY samples, diluted with water up to 45 wt% solids, with different percentages of cholesterol and lipid extraction were used (Table 1). As shown in this table, the increase in lipid extraction corresponds to an increase in protein concentration.

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TABLE 1
Protein Concentration as a Function of Cholesterol and Lipid Extraction Percentage for Reconstituted Yolk (45% solids, T = 20°C)[a]

Solids (45%)	DY	SCY-27	SCY-42	SCY-73	SCY-80
Cholesterol extracted (%)	0	26.5	42.5	73	80
Lipids extracted (%)	0	34.5	57	72.2	86.5
Protein (%)	34.3	42.3	50	56.9	65.5
Lipid/protein ratio	1.7	1.4	40.4	0.06	0.01

[a] DY, spray-dried yolk from Ovosec, S.A., Valladolid, Spain; SCY, low-fat, low-cholesterol yolk extracted by a near-critical process at laboratory scale.

Rheological measurements. Steady flow and oscillatory measurements were performed by using a Control Stress RS100 rheometer from Haake (Karlsrühe, Germany) equipped with cone-plate (20 or 35 mm ϕ, 4°), plate-plate (20 or 60 mm ϕ, 1 mm gap), and serrated plate-plate (20 mm ϕ, 1 mm gap) sensor systems, depending on the sample tested. The temperature ranged from 5 to 50°C.

A low-viscosity silicone oil (0.15 Pa·s) from Panreac (Barcelona, Spain) was chosen as a sealing fluid for all the measurements in order to avoid coagulation of protein at the yolk surface, which would produce an increase in the registered rheological data. The contribution of the sealing fluid to the rheological properties of the samples was found to be negligible.

RESULTS AND DISCUSSION

Concentration of solids. (i) Linear viscoelastic behavior: linear viscoelastic range. The experimental region of linear viscoelasticity was determined by performing stress sweep tests at constant frequency (1 Hz) for each concentration (Fig. 1), in which the complex modulus is plotted vs. shear stress. The values of τ_c and γ_c were estimated from the stress sweep test by computing the point at which the γ-τ plot deviates from a straight line (in other words, the point at which R becomes lower than 0.999, where R is the linear regression coefficient). The viscoelasticity functions remained constant until a critical stress, τ_c, or strain, γ_c, was reached. As seen in Figure 2, τ_c and γ_c behaved completely differently with solid concentration. Thus, τ_c increased exponentially, while γ_c exhibited a minimum at 35% solids. However, the values of τ_c or γ_c must be carefully considered since some wall-slip effects might have taken place at low concentrations of solids. This effect may lead to underestimating τ_c or γ_c, but it does not affect the measurements under linear conditions (4,6). In any case, all the oscillatory measurements were performed in the region below the critical stress line (Fig. 2B).

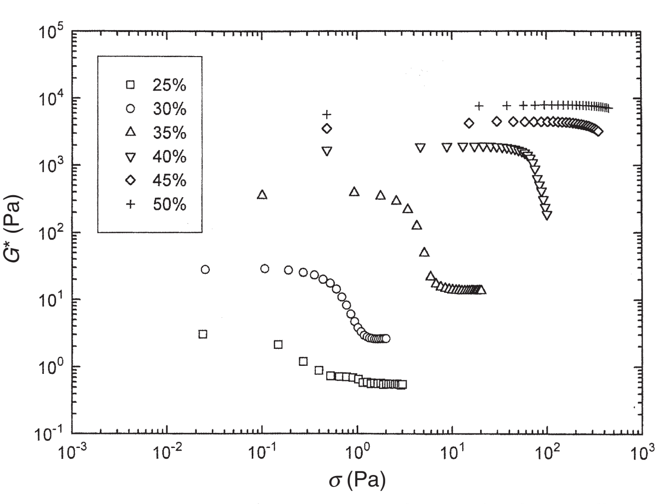

FIG. 1. Stress sweep tests for reconstituted spray-dried egg yolk as a function of solids concentration (T = 20°C).

(ii) Linear viscoelastic behavior: dynamic measurements. Figure 3 shows the values obtained for the storage and loss moduli (G' and G'') as a function of frequency. The mechanical spectrum obtained with reconstituted dried yolk, within the experimental frequency range, was similar to that found for many food gel or gel-like products such as protein gels (8), polysaccharide gels (9–12), or highly concentrated emulsions (13–17). This was particularly true for solid concentrations higher than 30% at which $G' > G''$, and with both moduli showing a slight dependence on frequency over the breadth of the experimental window. This was evidenced by a slope of 0.1 for the G'-ω power-law relationship. This fact indicated a high degree of structure, which may be attributed to thermal denaturation of proteins produced as a consequence of spray-drying. As reported by Rivas and Sherman (18), the unfolding of proteins produced by temperature promotes the formation of hydrophobic bonds that can participate in the formation of a gel-like network. However, it is recognized that only partial disruption of protein takes place by thermal denaturation (10,19). Thus, only a slight perturbation of the size and shape of ovalbumin proteins due to heat denaturation was reported by Matsumoto and Inoue (20). As stated by Ross-Murphy, thermal denaturation causes the exposure of pre-existing internal hydrophobic groups to the aqueous environment. This seems to be responsible for promoting aggregation and network formation. Moreover, since the values obtained for γ_c are lower than 5% at high concentration of solids (Fig. 2A), the yolk products studied were weak gels according to Ross-Murphy (10), who stated that in such cases the system behaves more like a colloidal particle network than a proper gel network.

A decrease in concentration produces a decrease in the storage and loss moduli, which is more important below 35% (Fig. 3). Weakening of the structure was also noted, since a remarkable increase in loss tangent was obtained for the lowest concentrations (i.e., loss tangent showed a minimum value of 0.2 at 35% and above, while the minimum shifted to 0.35 and 0.4 for concentrations of 30 and 25%, respectively).

The dynamic linear viscoelasticity behavior of these systems may be described by means of the Generalized Maxwell model with a high number of relaxation times, which leads to a relaxation spectrum of the material. Then the linear relaxation spectrum, $H(\lambda)$, may be obtained from G' and G'' by inverting the following equations:

$$G'(\omega) = G_e + \int_{-\infty}^{\infty} H(\lambda) \frac{\omega^2\lambda^2}{1+\omega^2\lambda^2} d(\ln \lambda) \qquad [1]$$

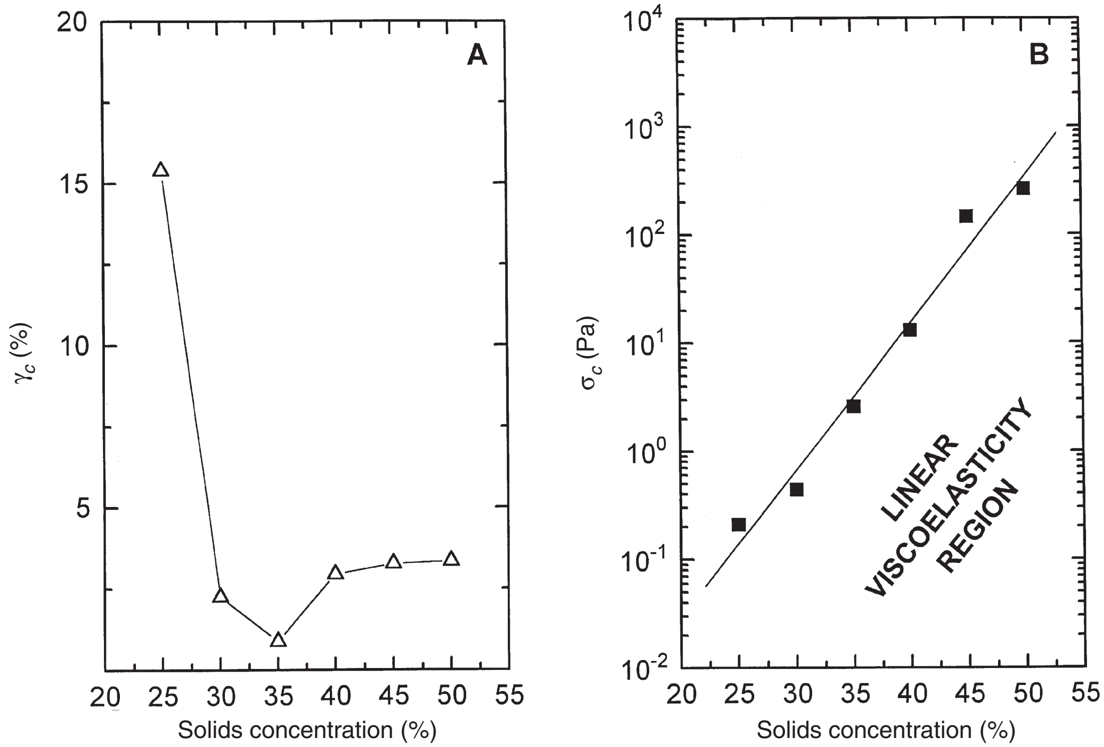

FIG. 2. Critical stress (A) and critical strain (B) values for reconstituted spray-dried egg yolk obtained from stress sweep tests.

$$G''(\omega) = \int_{-\infty}^{\infty} H(\lambda)\frac{\omega\lambda}{1+\omega^2\lambda^2}\, d(\ln \lambda) \qquad [2]$$

using regularization techniques (21). Its calculation was performed with the commercial software RheoSpec (22). Figure 3 shows the good agreement between the experimental values of the storage, $G'(\omega)$, and loss, $G''(\omega)$, moduli and those values predicted from the relaxation spectrum.

The relaxation spectrum obtained for each product was then modeled according to an empirical equation that was able to reproduce the transition, plateau, and terminal regions (22):

$$H(\lambda) = \frac{\alpha\lambda^m + \beta\lambda^n}{1+(\lambda/\lambda_t)^p} \qquad [3]$$

where m, n, and $(n - p)$ are the slopes of the transition, plateau, and pseudo-terminal zones, respectively; and λ_t is a characteristic relaxation time related to the boundary of the plateau region. Parameters α and β have the following expressions:

$$\alpha = \frac{mH_o}{(m-n)\lambda_o^m} \qquad [4]$$

$$\beta = \frac{nH_o}{\lambda_o^n(n-m)} \qquad [5]$$

where H_o is the value of the spectrum corresponding to the minimum in the plateau region and λ_o is a characteristic relaxation time at this minimum. The egg products studied showed only the plateau and the beginning of the transition region; consequently, Equation 3 could be simplified as follows:

$$H(\lambda) = \alpha\lambda^m + \beta\lambda^n \qquad [6]$$

Under such conditions, Equation 6 resembles the BSW model (23), in which parameter H_o equals the plateau modulus, $G_N^{\,o}$, times n, as previously pointed out for flocculated emulsions (14). In this work, the values of $G_N^{\,o}$ were obtained from the approximation reported by Wu (24) as the value of the storage modulus corresponding to the minimum in the loss tangent. Figure 4 shows the evolution of H_o and λ_o with concentration of solids. The evolution of parameter λ_o is similar to that found for γ_c since both of them pass through a minimum at

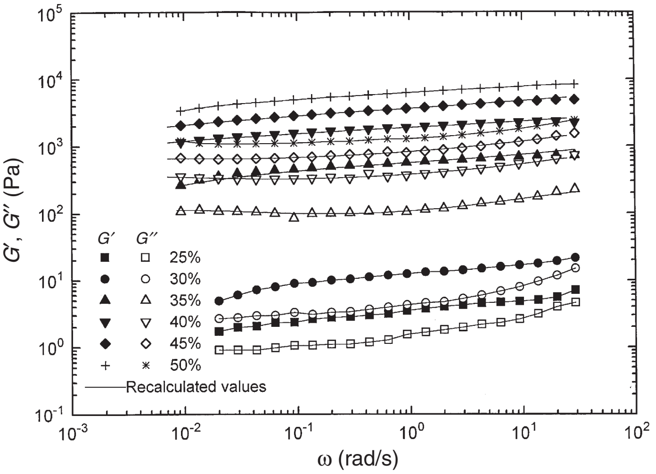

FIG. 3. Experimental and recalculated values from Equations 1 and 2 of the storage and loss moduli (G', G'') for reconstituted spray-dried egg yolk as a function of solids concentration (T = 20°C).

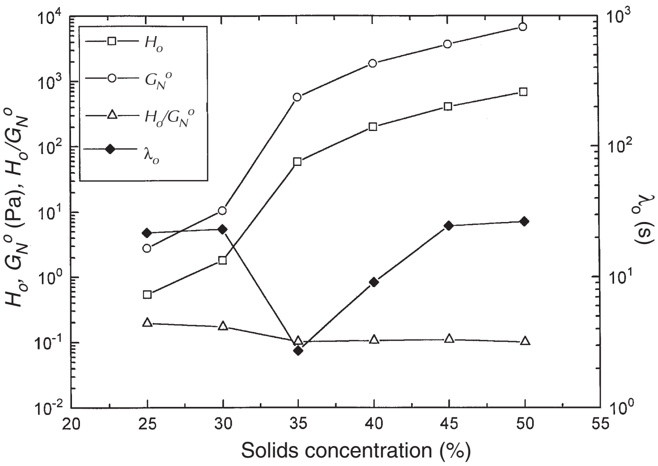

FIG. 4. Evolution of rheological parameters obtained from mechanical and relaxation spectra with solids concentration (T = 20°C).

35% solids. This behavior suggested a microstructural change at such a critical concentration. On the other hand, concentration exerts a similar effect on parameters H_o and $G_N{}^o$. In fact, there was an almost constant relationship between both parameters with a value of 0.13. Moreover, solids concentration did not exert any significant influence on the slope of the plateau region, since n remained constant with a value of 0.12 ± 0.03, which confirm the above-mentioned relationship between H_o and $G_N{}^o$.

A normalization of the spectrum, which provides useful information on the material microstructure, was carried out by using $n \cdot G_o$ and λ_o. Thus, Figure 5A clearly showed an extension of the plateau region with concentration. This effect may be attributed to an enhancement of the gel-like structures of the egg product. However, superposition failed at the lowest concentrations, which confirmed the change in the viscoelastic behavior below 35%. Similarly, the plateau modulus may be used to normalize both the storage and loss moduli (Fig. 5B). Only concentrations between 35 and 50% were plotted for the sake of clarity.

(iii) Linear viscoelastic behavior: creep compliance. The influence of concentration above 35% was also investigated by creep measurements under linear conditions. Figure 6 shows a decrease in the creep compliance curves as concentration of solids is raised. A generalized Kelvin-Voigt model with two elements describes fairly well the creep behavior at each concentration,

$$J(t) = J_e^o + \frac{t}{\eta_o} - \sum_{i=1}^{n} J_i e^{(-t/\tau_i)} \qquad [7]$$

where $n = 2$, η_o is the low-shear limiting viscosity, $J_e{}^o$ is the steady-state creep compliance, t is time, and τ_i is the retardation time for the ith element. An increase in concentration produced an increase in low-shear limiting viscosity as well as a decrease in $J_e{}^o$ (Table 2). This is consistent with the above-described results.

(iv) Flow behavior. Figure 7 shows the steady-state flow curves as a function of solids concentration. No slip effects were detected at the surface of the sensor system. Thus, no differences were found between the flow curves obtained using several geometries, including smooth and rough surfaces, as shown for the system containing 45% solids.

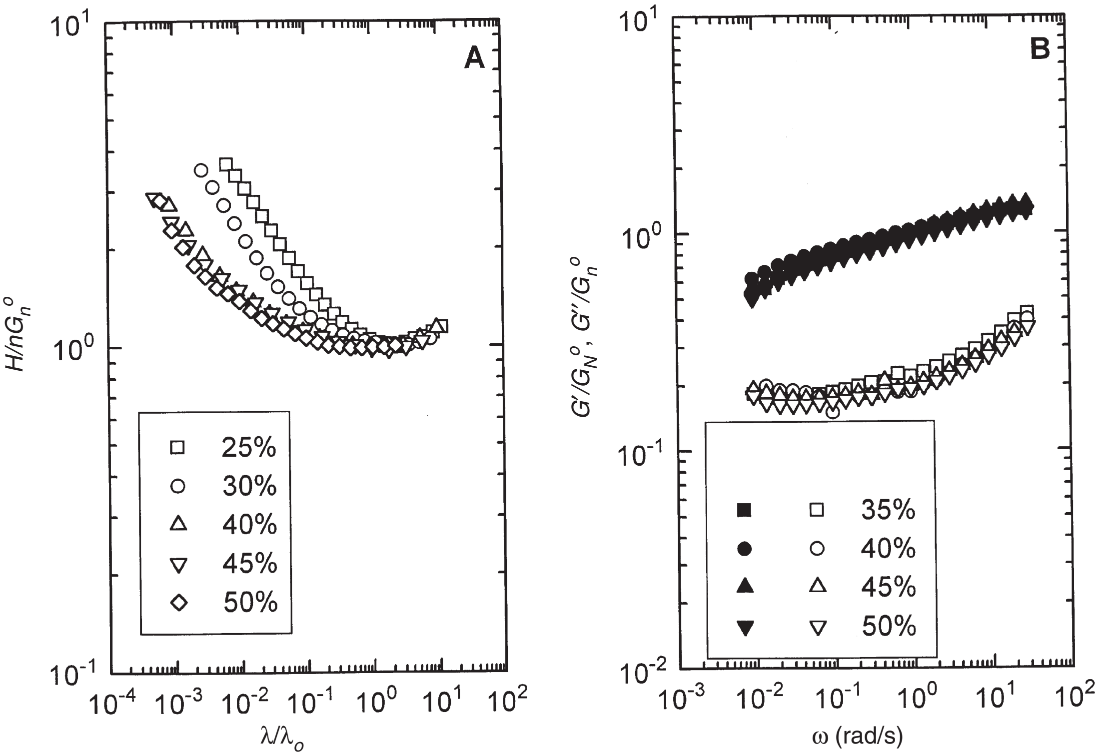

FIG. 5. Results obtained from the normalization procedure (A, normalized relaxation spectra; B, normalized mechanical spectra).

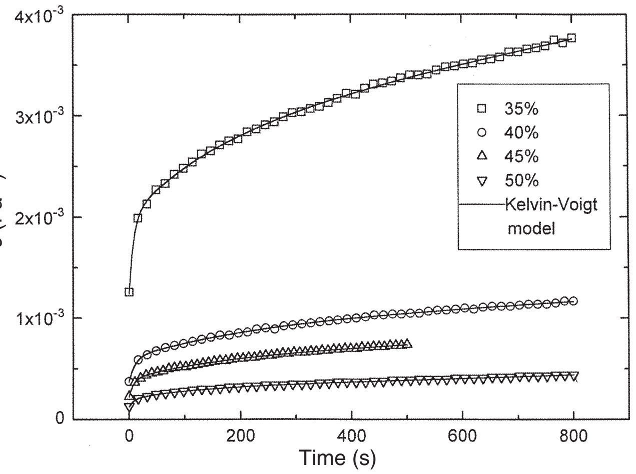

FIG. 6. Creep compliance measurements for reconstituted spray-dried egg yolk as a function of solids concentration ($T = 20°C$).

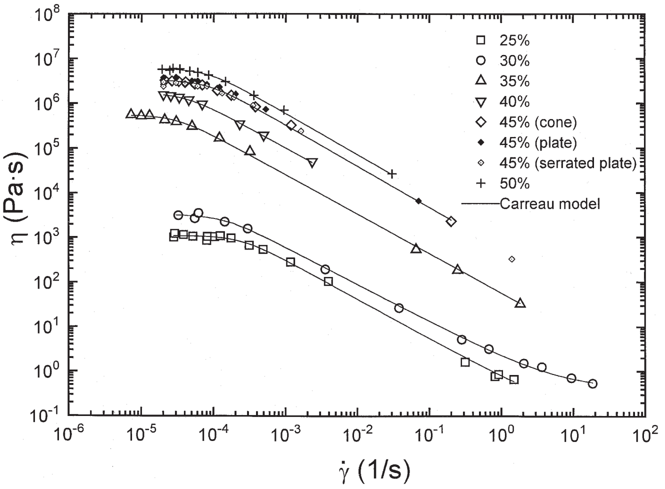

FIG. 7. Flow curves of the reconstituted egg yolk as a function of solids concentration ($T = 20°C$).

The flow behavior is properly described by the Carreau model:

$$\eta = \eta_\infty + \frac{\eta_o - \eta_\infty}{[1 + (\dot{\gamma}/\dot{\gamma}_c)^2]^s} \qquad [8]$$

where η_o and η_∞ are the low- and high-shear limiting viscosities, respectively, $\dot{\gamma}_c$ is the critical shear rate for the onset of the shear-thinning region, and s is a parameter related to the slope of the power-law region. Parameters η_o and $\dot{\gamma}_c$ were plotted in Figure 8 vs. solid concentration. The values of low-shear limiting viscosity calculated from flow measurements were in good agreement with those values estimated from creep measurements.

The enhancement of the network structure above 30% was again apparent, since a remarkable increase in the flow properties (Figs. 7 and 8) took place. A change in the evolution of the critical shear rate was also observed above 30% (Fig. 8). Nevertheless, parameter s remained constant ($s = 0.445 \pm 0.009$), which suggests that the shear-induced breakdown process is similar for all the concentrations studied.

Apparent and complex viscosities were compared (Fig. 9A). The Cox-Merz rule was not held for these materials, since apparent viscosity showed much lower values when frequency equaled shear rate. This behavior resulted from the structural breakdown caused by an intense shear rate. However, a similar slope of the power-law region was observed for both sets of curves. As a consequence, a modification of the Cox-Merz rule may be used to superpose apparent and complex viscosities (Fig. 9B), using an empirical shift factor (a_c*):

$$\eta = \eta^*\big|_{\lambda = a_c^*\omega} \qquad [9]$$

As a second step, a time-concentration superposition can be performed to obtain a master curve for all the flow and complex viscosity data above 30% of solids. Figure 10 shows the reduced viscosities η/η_o and η^*/η_o vs. the shifted shear rate or frequency. The values for both empirical shift factors, a_c* and a_c, are presented in Table 2. The first parameter is inversely related to the shear-induced structural breakdown, and the second one is in inverse proportion to the critical shear rate, $\dot{\gamma}_c$.

A remarkable result of the success of this superposition method was that a single Carreau model reproduced both lin-

TABLE 2
Different Rheological Parameters Obtained as a Function of Solids Concentration ($T = 20°C$)

	35%	40%	45%	50%
η_o (Pa·s)	5.5×10^5	1.6×10^6	3.2×10^6	6×10^6
J_e^o (Pa^{-1})	2.8×10^{-3}	9.5×10^{-4}	6×10^{-4}	3×10^{-4}
a_c*	0.07	0.08	0.14	0.15
a_c	2.2	1.6	1	1

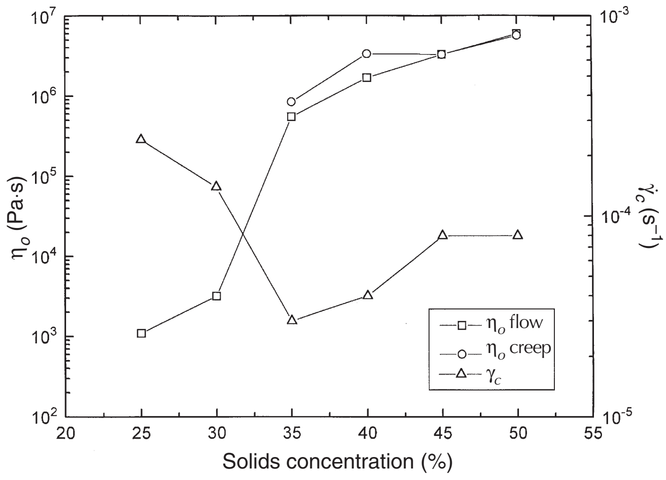

FIG. 8. Evolution of low-shear Newtonian viscosity and critical shear rate with solids concentration for reconstituted spray-dried egg yolk.

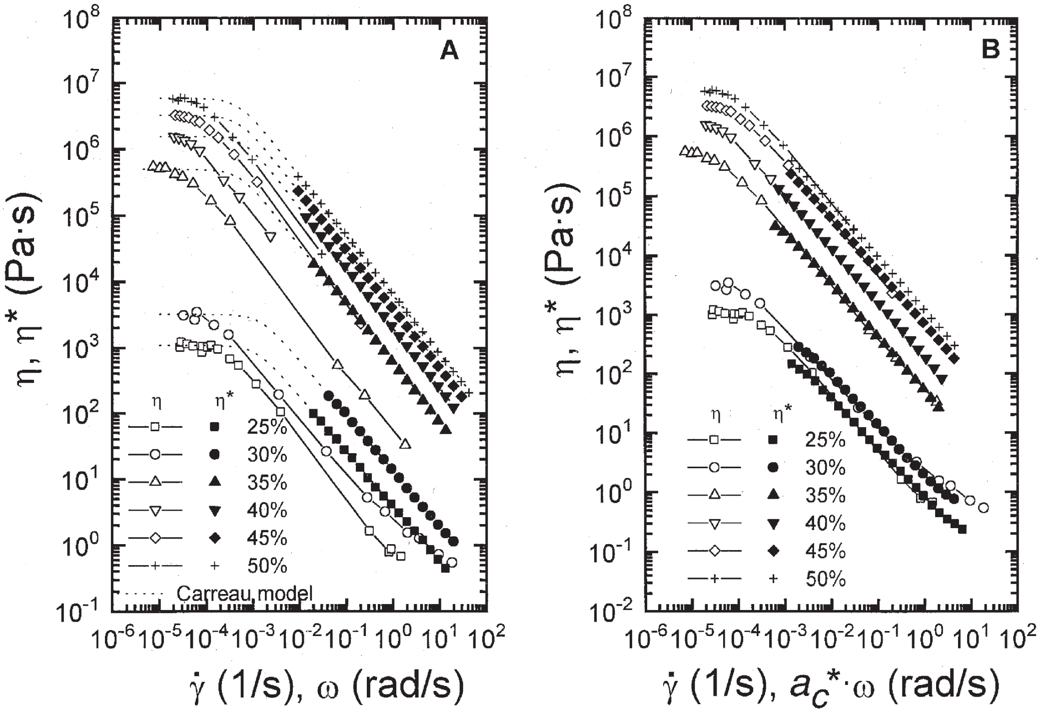

FIG. 9. Comparison between complex and apparent viscosities (A, for $\gamma = \omega$; B, for $\gamma = a_c^* \cdot \omega$).

ear viscoelasticity and flow behavior. Thus, apparent viscosity is represented as follows:

$$\frac{\eta\,(\dot{\gamma},C)}{\eta_o(C)}=\left[1+\left(a_c\,\frac{\dot{\gamma}}{\dot{\gamma}_c(45)}\right)^2\right]^{-s} \qquad [10]$$

where $\dot{\gamma}_c$ (45) is the critical shear rate for the concentration of reference (45% solids). As a result, for any other concentration higher than 30%,

$$\dot{\gamma}_c(C)=\frac{\dot{\gamma}_c(45)}{a_c} \qquad [11]$$

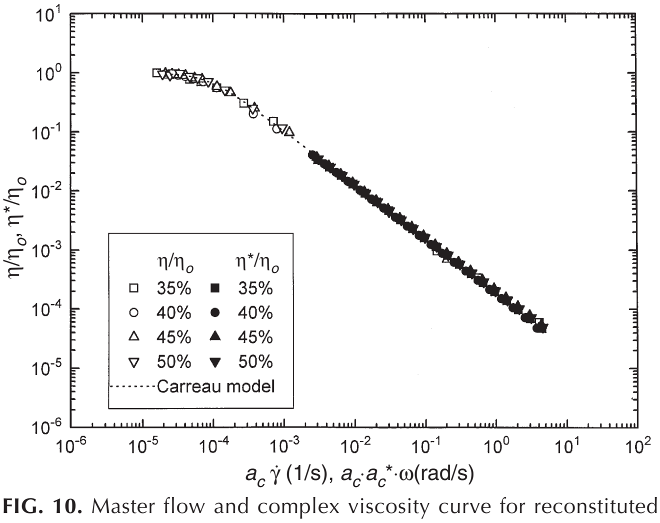

FIG. 10. Master flow and complex viscosity curve for reconstituted spray-dried egg yolk (T = 20°C).

According to the modified Cox-Merz rule, the complex viscosity may be obtained replacing $\dot{\gamma}_c$ by a_c*·ω:

$$\frac{\eta^*\,(\omega,C)}{\eta_o(C)}=\left[1+\left(a_c^*\cdot a_c\,\frac{\omega}{\gamma_c(45)}\right)^2\right]^{-s} \qquad [12]$$

The failure in the superposition procedure below 35% for both steady flow and linear viscoelasticity data confirms the above-mentioned microstructural change at this concentration.

The complex viscosity was also calculated from the Kelvin-Voigt parameters by using exact interrelations among viscoelastic functions (25). This procedure was described in a previous paper (26). The above-mentioned superposition was also carried out on the complex viscosity obtained from creep measurements.

Influence of lipid extraction. As previously mentioned, an increase in the lipid and cholesterol extraction of egg yolk at constant solids concentration gave rise to increased protein concentration (Table 1). The values of both critical stress and strain for linear viscoelasticity are shown in Table 3 as a function of lipid extraction, at 45% solids concentration. The increased protein concentration produced a continuous in-

TABLE 3
Values of Both Critical Stress and Deformation as a Function of Cholesterol and Lipid Extraction (45% solids)[a]

	DY	SCY-27	SCY-42	SCY-73	SCY-80
τ_c (Pa)	146	306.3	340	1251	1830
γ_c (%)	3.5	6.9	5.9	7	6.9

[a] For abbreviations see Table 1.

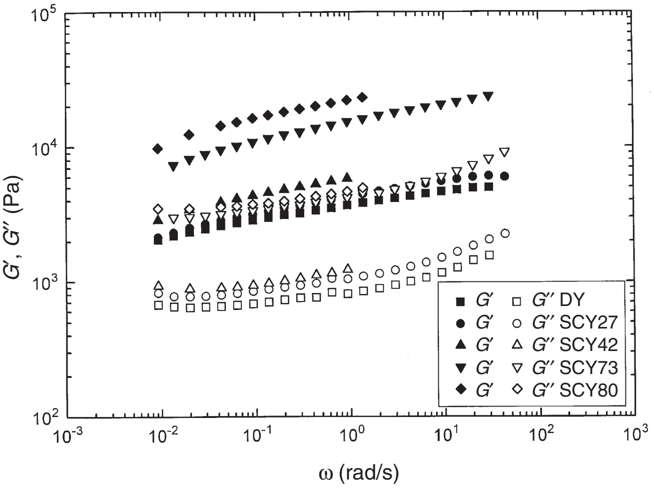

FIG. 11. Evolution of the storage and loss moduli with protein concentration (45% solids, $T = 20°C$). DY, spray-dried yolk from Ovosec S.A., Valladolid, Spain; SCY, low-fat, low-cholesterol yolk extracted by a near-critical CO_2 process at laboratory scale.

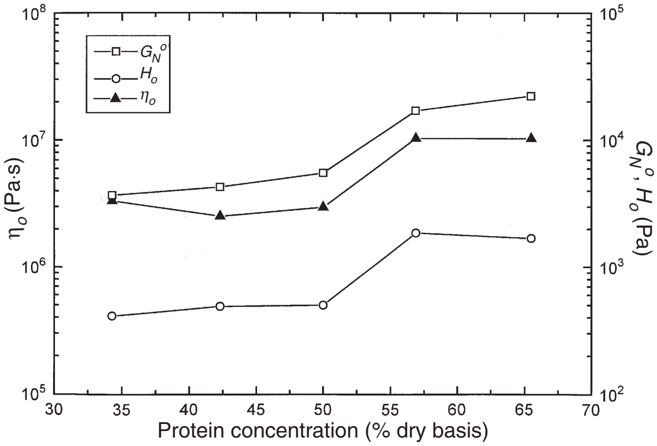

FIG. 13. Evolution of plateau modulus, parameter H_o and low-shear Newtonian viscosity with protein concentration (45% solids, $T = 20°C$).

crease in the values of τ_c. However, γ_c show a different behavior. A reduction of 27% lipids produced an initial growth of γ_c, which remained roughly constant with further extraction. Moreover, the above-mentioned increase in protein concentration seemed to displace the viscoelasticity functions toward higher values (Fig. 11). However, the shape of the mechanical spectrum remained unaltered by lipid extraction.

The increased protein concentration exerted a similar effect on the flow curves. Thus, the flow properties were successfully superposed into a master curve (Fig. 12), using a shift factor (a_e) that showed almost the same value for all the extracted yolk products. Therefore, the influence of protein concentration on the flow properties may be fully represented by the evolution of low-shear Newtonian viscosity (Fig. 13). It must be pointed out that the influence of parameters G_N^o and H_o obtained from the mechanical and relaxation spectra on protein concentration was similar to the evolution found for η_o obtained either by creep or flow measurements. Lipid extraction of lipids exerted the same effect on a_e and on critical shear rate, since both parameters are related. This is also consistent with the effect on critical strain mentioned before. Both parameters, $\dot{\gamma}_c$ and γ, provided information about the resistance of the microstructure of the elastic network to shear forces. All the extracted yolk samples showed the same structural resistance, even though the protein concentration was increasing. This effect took place when the lipid/protein ratio was close to 1 or below, which suggests that a major excess of lipids may distort the elastic network formed by proteins in the spray-dried egg yolk. Further investigations should be carried out to elucidate the contribution of lipids to the microstructure of the system, which, in any case, seems to be moderate.

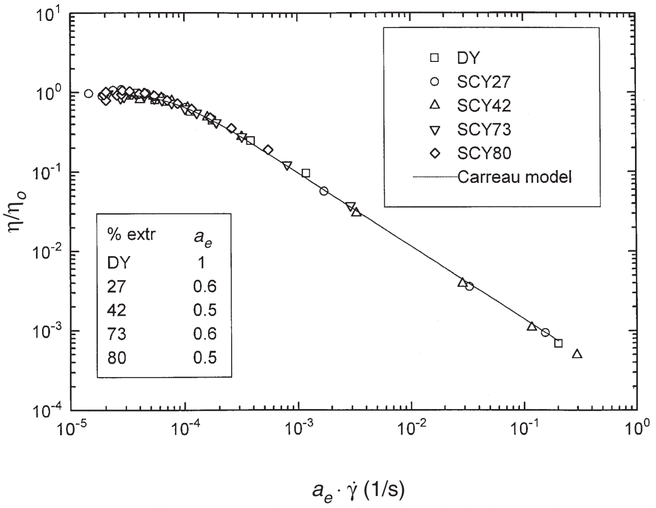

FIG. 12. Master flow curve obtained from lipid/cholesterol extracted egg yolk (45% solids, $T = 20°C$). For abbreviations see Figure 11.

The rheological properties of three different egg yolk products containing 45% solids are compared in Figure 14. As may be observed, the spray-drying process provided a dramatic increase in the viscoelastic and flow properties, as well as a shift from fluid to gel-like behavior. Thus, native yolk showed the terminal region of the mechanical spectrum and an almost Newtonian viscosity, while the spray-dried egg yolk showed the plateau region of the mechanical spectrum and a shear-thinning flow behavior. This change in rheological properties was due to the thermal denaturation of proteins during spray-drying, which was performed at about 120°C. The only effect produced by the near-critical extraction with CO_2 was the above-mentioned increase in protein concentration. This suggests that there is no evidence of further denaturation by the supercritical carbon dioxide extraction process, since the frequency and shear-rate dependence remained essentially the same.

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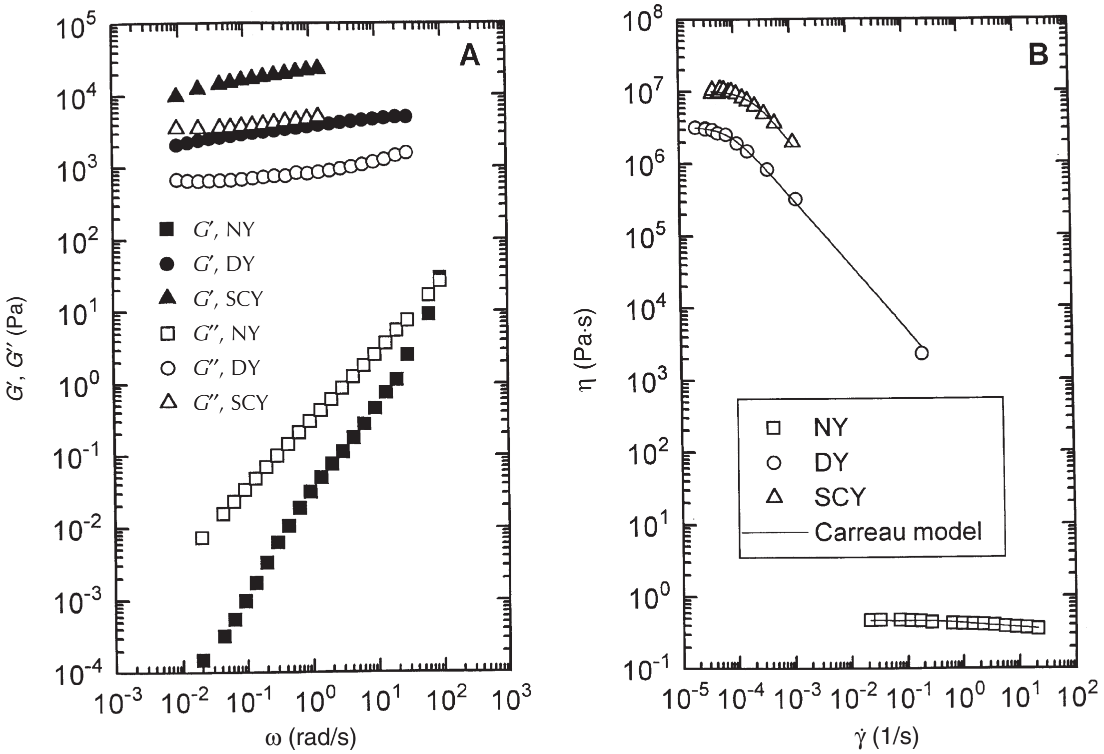

FIG. 14. Influence of processing on the rheological properties of egg yolk (A: viscoelasticity functions, B: flow properties) (45% solids, $T = 20°C$). NY, technical yolk, containing 10 wt% salt and 45 wt% total solids (Hijos de Ybarra, S.A., Sevilla, Spain); for other abbreviations see Figure 11.

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