Viscoelastic Properties of Lupin Proteins Produced by Ultrafiltration-Diafiltration

Jingyuan Xu*, Abdellatif A. Mohamed, Mila P. Hojilla-Evangelista, and David J. Sessa

National Center for Agricultural Utilization Research, ARS, USDA, Peoria, Illinois 61604

ABSTRACT: The linear and nonlinear rheological properties of defatted lupin proteins produced by ultrafiltration-diafiltration were investigated. Five concentrations ranging from 10 to 30% of the defatted ultrafiltered-diafiltered (DUD) lupin proteins were prepared. The viscoelastic properties strongly depended on concentrations. Below 12%, the DUD lupin proteins exhibited more fluid-like behavior. At 15%, lupin proteins became more viscoelastic, and above 20%, the viscoelastic solid-like properties became stronger. Below 12%, the high-frequency behaviors of moduli were proportional to $\omega^{3/4}$, as expected for a semiflexible coil. Above 20%, the high-frequency behaviors of moduli were proportional to $\omega^{1/2}$, indicating a flexible coil. The nonlinear steady shear rheological properties were also concentration-dependent and showed shear-thinning behavior, which could be described by a power law constitutive model. The trend of the power law exponent shift is very consistent with the linear viscoelastic behavior change with the lupin protein concentration. These results suggest DUD lupin proteins undergo a structural change between 12 and 20%.

Paper no. J11271 in *JAOCS 83*, 553–558 (June 2006).

KEY WORDS: Lupin proteins, rheology, ultrafiltration, viscoelastic properties.

Lupin is an important legume plant and is easy to grow and harvest. Its seeds contain more than 50% protein and up to 11% oil (1), values that are comparable with those of soybean (about 40% protein and 21% oil). Having a high concentration of protein makes lupin a good dietary element (2). Like many other plant proteins, lupin proteins are accompanied by various fibers. Fiber intake is known to improve glucose tolerance and consequently modify blood insulin and glucagon (2). Chango *et al*. (2) reported that lupin proteins have the function of lowering serum cholesterol level, similar to other proteins originating from plant origin. In addition, lupin proteins contain a good balance of essential amino acids (3). Therefore, lupin has great potential as a food or food ingredient. However, use of lupin is limited because of its high alkaloid level (4). Breeding programs in Australia, Chile, and the United States have reduced the alkaloid level, and there have been some applications for lupin in breadmaking (5), pasta products (5), meat substitutes (6), and egg and milk replacer (7). However, research on lupin and its products is much less than for soybean. Literature reports regarding lupin proteins and oils, especially their physical properties and structure/function relationships, are limited, therefore a better understanding of lupin is needed.

Lupin meal has strong viscoelastic solid behavior and a glass transition that is identical to that of wheat gluten, which offers new insight into the physical properties of the lupin meal (8). The thermal and rheological properties of lupin proteins produced by acid precipitation also have been investigated (Xu, J., A.A. Mohamed, and D.J. Sessa, unpublished data). In this work, we studied the viscoelastic properties of lupin proteins produced by ultrafiltration-diafiltration.

MATERIALS AND METHODS

Materials. Lupin seeds (*Lupinus albus* L.2043N) were donated by Resource Seeds Inc. (Gilroy, CA, courtesy of Gene Aksland). Lupin meal was prepared as described by Hojilla-Evangelista *et al*. (9). Seeds were dissected and dehulled manually. Ground whole lupin meal was then obtained by grinding the seeds at room temperature in a GlenMills (Clifton, NJ) Model S.500 disc mill, followed by passage of the material through a 30-mesh sieve. Grinding was repeated as needed for coarse particles retained on the 30-mesh sieve.

Ground whole lupin meal was defatted by using diethyl ether. The solvent was added to the meal (1.2 L/180 g meal) and the mixture was stirred with a magnetic bar for 1.5 h. The solvent was decanted into a funnel through Whatman no. 4 filter paper and discarded. Washing with diethyl ether was repeated two more times, each time using 800 mL of solvent with stirring for 30 min. The defatted lupin meal was air-dried in the hood until the ether smell was no longer detected and then stored in sealed polyethylene bags at room temperature until use.

Production of lupin protein isolate by ultrafiltration-diafiltration. Lupin protein isolate was produced by following the ultrafiltration-diafiltration protocol described by Hojilla-Evangelista *et al*. (9). Fifty grams of meal was homogenized with 1.7 L of water for 15 min at 5000 rpm by using the Ross Model HSM100LC mixer/emulsifier (Charles Ross and Son Co., Hauppauge, NY). The mixture was centrifuged (18°C, $15{,}344 \times g$ for 25 min) and then filtered through Whatman no. 5 paper. The filtrate was retained, while the solids were again homogenized with *ca*. 1.6 L water, centrifuged, and filtered as described above. The solids were discarded. Filtrates from the two extracts were pooled, and the volume was adjusted to

*To whom correspondence should be addressed at NCAUR, ARS, USDA, 1815 N. University St., Peoria, IL 61604. E-mail: xuj@ncaur.usda.gov

4 L with water. The pooled sample was ultrafiltered in a Pall Centramate system (East Hills, NY) using a polyether sulfone membrane (5 kDa M.W. cutoff). The unit was operated at a flux rate of 2.7 L/h and inlet and outlet pressure ranges of 21–25 and 27–34 psi, respectively. When *ca*. 3.4 L permeate had been collected, 2 L of water was added to the retentate sample, which was concentrated again to a final volume of about 600 mL. This concentrated retentate was freeze-dried to obtain the protein isolate, and the permeate was discarded. The M.W. distribution of defatted ultrafiltered-diafiltered (DUD) lupin proteins was in the range of 18–100 kDa, and the most concentrated parts were in the range of 31–66 kDa (9). The proteins were stable with biological activities at pH 5.5–10. The isoelectric point of DUD lupin proteins was around pH 4.

Preparation of solutions for rheological measurements. DUD lupin protein sample powder was suspended in a 10 mM imidazole buffer (pH 7.0 at 25°C) for the rheological measurements. Five concentrations of DUD lupin protein solutions were made: 10, 12, 15, 20, and 30 wt%). At least two solution samples were made per concentration for the measurements. The powder dispersed evenly in the buffer and produced a clear solution. No sedimentation was observed for 2 wk after the preparation.

Rheological measurements. A strain-controlled Rheometric ARES rheometer (TA Instruments, New Castle, DE) was used to perform the rheology studies. A 50-mm diameter cone-plate geometry was used. The temperature was controlled at 25 ± 0.1°C by a water circulation system. Linear viscoelastic measurements were conducted for the various concentrations of lupin protein solutions. To ensure that all the measurements were made within the linear viscoelastic range, a strain-sweep experiment was conducted initially. An applied shear strain in the linear range was adopted for the other viscoelastic property measurements for the same material; fresh samples were used for each experiment. Linear viscoelastic behavior indicates that the measured parameters are independent of applied shear strain. The linear range for all measured DUD lupin proteins was less than 2% of strain. One percent strain was adopted for the linear rheological measurements for all DUD lupin protein samples. Small-amplitude oscillatory shear experiments were conducted over a frequency (ω) range of 0.1–500 rad/s, yielding the oscillatory shear storage (G') and loss (G'') moduli. At low frequencies (0.1–1 rad/s), the measurement time was between 2 and 15 min, whereas at relatively high frequencies (1–500 rad/s), the measurement time was between a few seconds and 2 min. The storage modulus represents the nondissipative component of mechanical properties. Elastic or "rubber-like" behavior is suggested if the G' spectrum is independent of frequency and greater than the loss modulus over a certain range of frequency. The loss modulus represents the dissipative component of the mechanical properties and is characteristic of viscous flow. The phase shift or phase angle (δ) is defined by $\delta = \tan^{-1}(G''/G')$, and indicates whether a material is solid with perfect elasticity (δ = 0), liquid with pure viscosity (δ = 90°), or something in between. Stress relaxation experiments measured the stress relaxation with time after the material was subjected to a step increase in shear strain at the linear range of 1%. Nonlinear rheological studies were conducted on the same ARES instrument with the same geometry just described. The steady shear measurements were conducted with the shear rate increased stepwise in the range of shear rate of 1–700 s^{-1}. The delay time was 3 s.

RESULTS AND DISCUSSION

Five concentrations of DUD lupin protein solutions were made: 10, 12, 15, 20, and 30 wt%. In Figures 1–3, the linear dynamic frequency sweep results of storage (G') and loss (G'') moduli for five concentrations of DUD lupin proteins are displayed. The G' and G'' values for the DUD lupin proteins were found to be dependent on the oscillation frequency, especially at high frequencies. Both storage and loss moduli of the DUD lupin proteins depended strongly on concentration. For relatively low concentrations (10 and 12%), DUD lupin proteins exhibited weak viscoelastic fluid or liquid behavior, according to linear viscoelastic theory (10) (Fig. 1). The values of G'' were higher than those of G' at whole measured frequencies (Fig. 1). From 10 to 12%, both G' and G'' of the DUD lupin proteins increased with the concentration. At a frequency of 1 rad/s, G' of the 10% DUD lupin proteins was 5.5×10^{-3} Pa, whereas G' of the 12% DUD lupin proteins increased to 2.6×10^{-2} Pa. The phase shifts for both 10 and 12% of the DUD lupin proteins were in the same range of 46–73°, indicating that the fluid-like behavior was unchanged (Fig. 4). However, at 15%, G' and G'' increased and the values of G' were higher than those of G'' over parts of the measured frequency range, which suggested the viscoelastic behavior shift (Fig. 2). At a frequency of 1 rad/s, G' of the 15% DUD lupin proteins was 2.7×10^{-1} Pa, which was an increase of one order of magnitude over that for the concentration of 12%. The phase shifts for 15% DUD lupin proteins were dramatically decreased in the range of 23–45 degrees, thus explaining the property shift from liquid-like behavior for concentrations of 10 and 12% to viscoelastic solid-like behavior at 15% (Fig. 4). For the 20 and 30% concentrations of DUD lupin proteins, both G' and G'' increased further. At a frequency of 1 rad/s, G' of the 20 and 30% DUD lupin proteins were 0.48 and 10.56 Pa, respectively. The phase shifts further decreased into the range of 19–42 degrees and became the lowest for the 30% lupin proteins, which indicated that the viscoelastic solid-like behavior became stronger at higher concentrations.

To investigate the viscoelastic properties for the DUD lupin proteins further, we observed the high-frequency behavior of the moduli. The high-frequency moduli behavior was determined by log-log complex moduli $|G^*|$ ($|G^*| = G'^2 + G''^2)^{1/2}$ curve slope at frequencies higher than 50 rad/s. High-frequency moduli for 10, 12, and 15% DUD lupin proteins were proportional to $\omega^{3/4}$ (Figs. 1, 2), indicative of a semiflexible polymer (11–13). However, high-frequency moduli for 20 and 30%

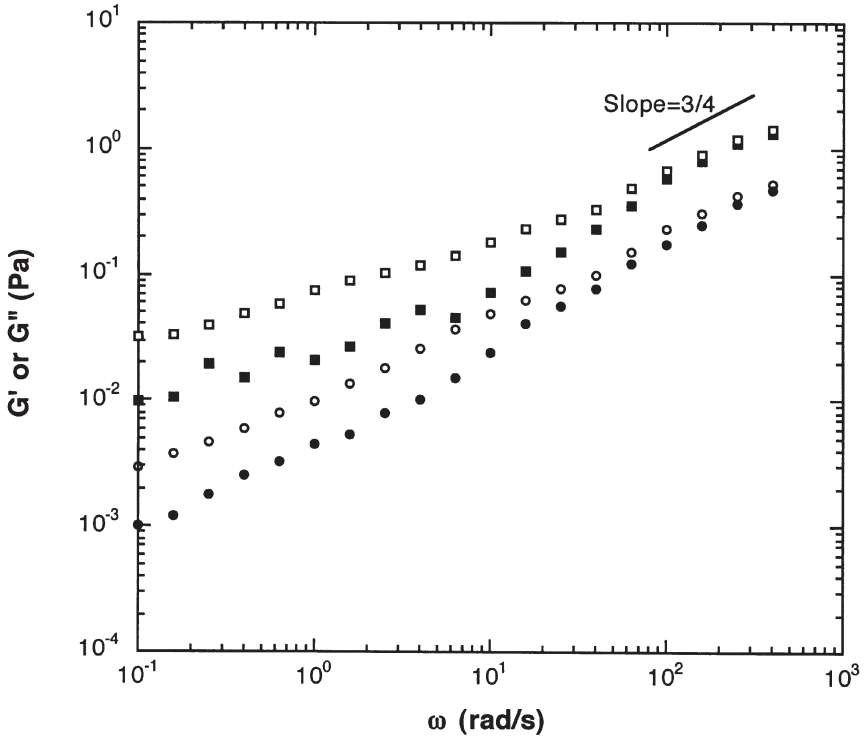

FIG. 1. The linear viscoelastic properties of defatted ultrafiltered-diafiltered (DUD) lupin proteins at 25°C. (●, ○) 10 wt%, (■, □) 12 wt%; (●, ■) *G′*, (○, □) *G″*.

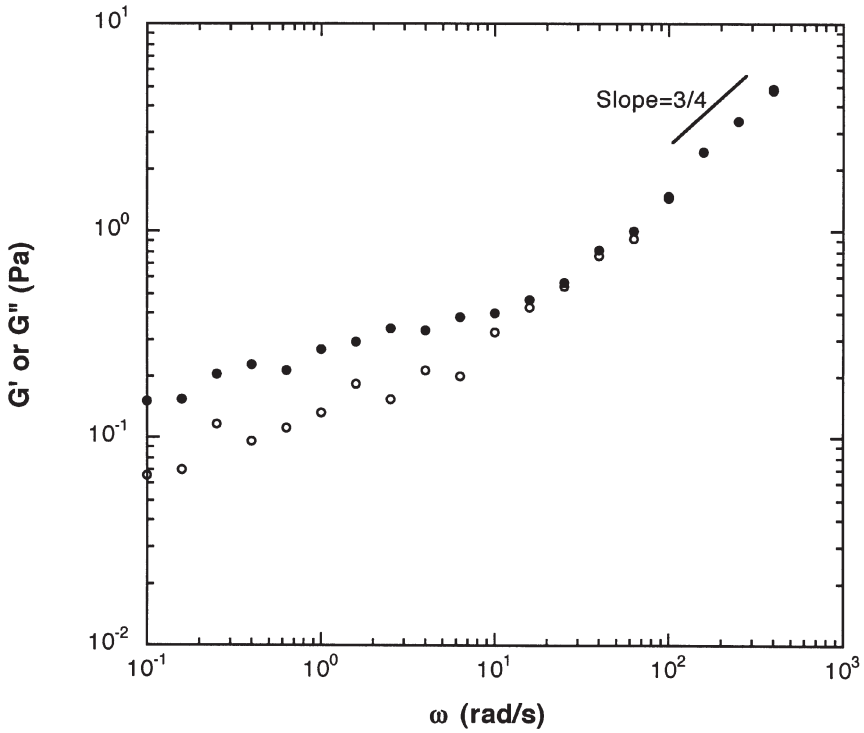

FIG. 2. The linear viscoelastic properties of 15 wt% DUD lupin proteins at 25°C. (●, ■) *G′*, (○, □) *G″*. For abbreviation see Figure 1.

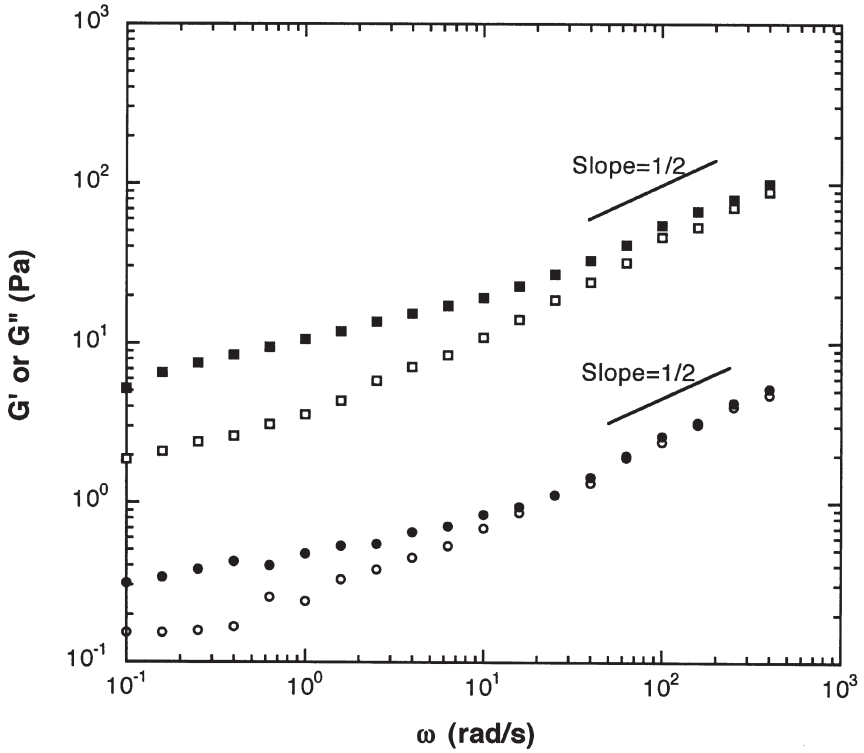

FIG. 3. The linear viscoelastic properties of the DUD lupin proteins at 25°C. (●, ○) 20 wt%, (■, □) 30 wt%; (●, ■) G', (○, □) G''. For abbreviation see Figure 1.

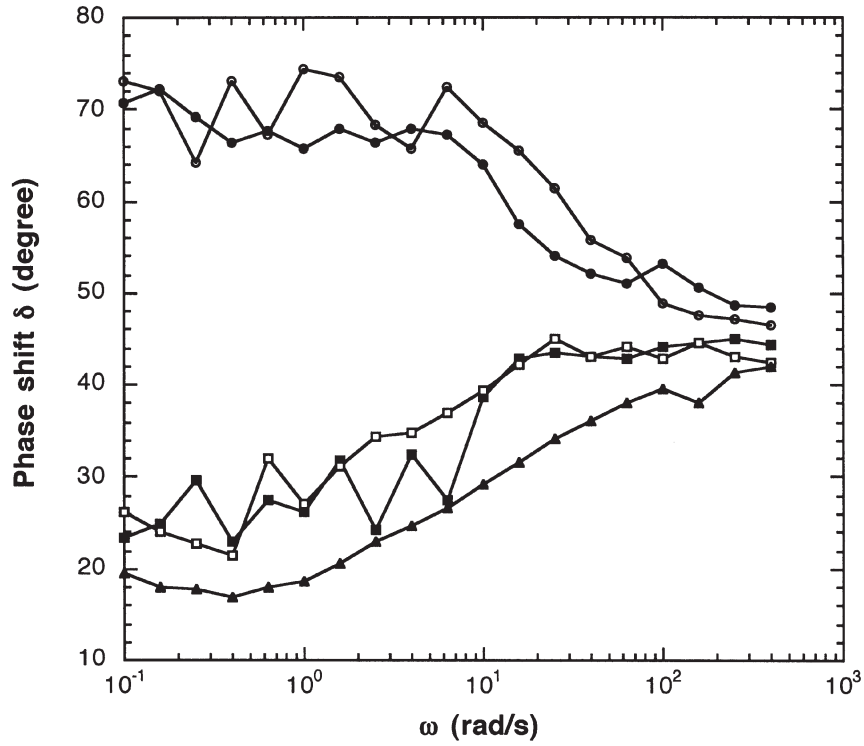

FIG. 4. Frequency-dependence phase shift of different concentrations of DUD lupin proteins. (●) 10 wt%, (○) 12 wt%, (■) 15 wt%, (□) 20 wt%, and (▲) 30 wt%. For abbreviation see Figure 1.

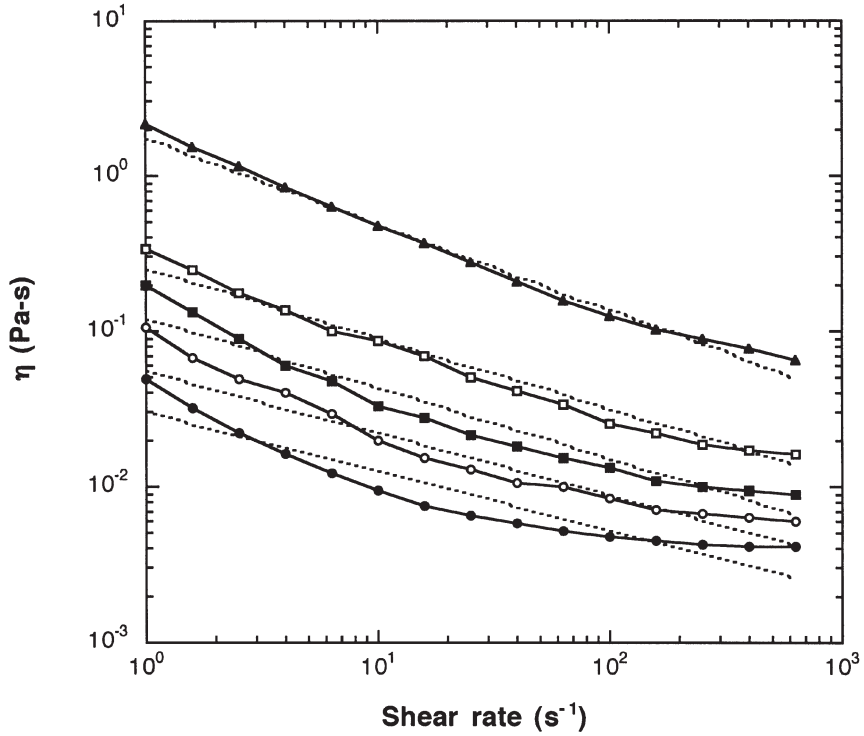

FIG. 5. The nonlinear steady shear viscosity vs. shear rate for the DUD lupin proteins. Symbols and solid lines are experimental results. Dashed lines are fitted with power law model. (●) 10 wt%, (○) 12 wt%, (■) 15 wt%, (□) 20 wt%, and (▲) 30 wt%. For abbreviation see Figure 1.

lupin proteins were proportional to $\omega^{1/2}$ (Fig. 3), indicating that the material would exhibit flexible coil-like behavior. For lupin protein concentrations ≤15%, log-log moduli curve slopes at high frequencies (exponents) were all close to 0.75 (Figs. 1, 2), but for suspensions ≥20%, exponents were close to 0.5 (Fig. 3). The above hypothesis of semiflexible or flexible behavior was based on the Doi–Edwards' theory, which is applicable to monodisperse solutions (14). This model may not apply directly to the DUD lupin proteins; however, information regarding the high-frequency moduli difference implicates a difference in polymer chain behavior. Thus, when the concentration of lupin protein increased from 12 to 15%, its viscoelastic properties shifted sharply from a viscoelastic fluid to a viscoelastic solid, but the high-frequency moduli did not change. When the concentration was further increased, from 15 to 20%, the high-frequency moduli behavior shift implied that the flexibility of the material changed. And within the concentration range from 10 to 30%, a dramatic increase of both the moduli (G' and G'') was observed. These results suggest that DUD lupin proteins undergo a major structural change between 12 and 20%.

To better understand the processing behavior, the nonlinear steady shear viscoelastic properties of DUD lupin proteins were studied. Figure 5 displays shear viscosity vs. shear rate for the five lupin protein concentrations. All five concentrations showed shear-thinning behavior over the entire measured shear rates (Fig. 5). Viscosities were higher at higher concentrations, as expected. These shear-thinning rheological behaviors can be characterized by a power law constitutive equation (15), which may be written as

$$\eta = K\dot{\gamma}^{n-1} \quad [1]$$

where η is the shear viscosity, K is the front factor, $\dot{\gamma}$ is the shear rate, and n is the power law exponent. Equation 1 was fit to shear-thinning viscosity for lupin proteins (Fig. 5). The results of the fits are summarized in Table 1. Higher concentrations of lupin proteins possess lower power law exponents (Table 1). The exponents for both 10 and 12% DUD lupin proteins are 0.60, the exponent for 15% proteins is 0.55, and the exponent for both 20 and 30% protein samples is 0.45 (Table 1). The trend of this shift in exponents is consistent with the linear viscoelastic behavior change with concentration discussed earlier.

The viscoelastic nature of DUD lupin proteins is totally different for samples <12% and >20%. Within this small concentration change, the values of moduli, moduli curve shape, phase shift, and high frequency moduli performance all become different. These results indicate variation in structure. This structure change may be due to such parameters as increased chain–chain and molecular interactions, entanglements, or cross-linking. It is difficult to observe these structure differences directly. The molecular interpretation of rheological properties

TABLE 1
Power Law Model Fitted Parameters[a]

Concentration of DUD lupin proteins	K (Pa-s^n)	n	R^2
10%	0.03	0.60	0.81
12%	0.06	0.60	0.87
15%	0.12	0.55	0.92
20%	0.33	0.45	0.93
30%	1.70	0.45	0.97

[a]DUD, defatted ultrafiltered-diafiltered; *K*, front factor; *n*, power law exponent.

is not as well understood as other modern biochemical and molecular biology techniques. The curve shapes of moduli do not show the evidence of rubber-like behavior of cross-linking. In addition, our relaxation experiments exhibit relative quick relaxation after the material is subject to a step increase in 1% strain, which also suggests that there is no cross-linking (data not shown). So, there must be more physical chain–chain than chemical interactions in the network. Therefore, the more reasonable explanation for the structural change is that a greater number of entanglements occur at DUD lupin protein concentrations of >20%. The high-frequency moduli performances indicate that the >20% lupin proteins are flexible, whereas <12% proteins are semiflexible polymers. DUD lupin proteins >30% probably have more bundles and parallel chains because of the higher likelihood of chain–chain entanglements. These bundles and parallel chains might allow the network to exhibit flexible coil behavior, further supporting the conclusion of a greater concentration of entanglements in more concentrated DUD lupin proteins.

ACKNOWLEDGMENT

This work was financially supported by he United States Department of Agriculture, Agricultural Research Service.

REFERENCES

1. Hill, G.D., The Composition and Nutritive Value of Lupin Seeds, *Nutr. Abstr. Rev. B47*:511–529 (1977).
2. Chango, A., C. Villaume, H.M. Bau, A. Schwertz, J. Nicolas, and L. Mejean, Effects of Casein, Sweet White Lupin and Sweet Yellow Lupin Diet on Cholesterol Metabolism in Rats, *J. Sci. Food Agric. 76*:301–309 (1998).
3. Hove, E.L., Composition and Protein Quality of Sweet Lupin Seed, *Ibid. 25*:851–859 (1974).
4. Mohamed, A.A., and P. Rayas-Duarte, Composition of *Lupinus albus*, *Cereal Chem. 72*:643–647 (1995).
5. Mohamed, A.A., Characterization of Carbohydrates in *Lupinus albus*, M.S. Thesis, North Dakota State University, Fargo, ND, 1994.
6. Alamanou, S., J.G. Bloukas, E.D. Paneras, and G. Doxastakis, Influence of Protein Isolate from Lupine Seeds (*Lupinus albus* ssp. Graecus) on Processing and Quality Characteristics of Frankfurters, *Meat Sci. 42*:79–93 (1996).
7. Antonio, C., and A. Infante, *Lupin Use in Nutrition Programs,* The 6th International Lupin Conference, Chile, 1990, pp. 168–174.
8. Xu, J., and A.A. Mohamed, Thermal and Rheological Properties of *Lupinus albus* Flour, *J. Am. Oil Chem. Soc. 80*:763–766 (2003).
9. Hojilla-Evangelista, M.P., D.J. Sessa, and A. Mohamed, Functional Properties of Soybean and Lupin Protein Concentrates Produced by Ultrafiltration-Diafiltration, *Ibid. 81*:1153–1157 (2004).
10. Ferry, J.D., *Viscoelastic Properties of Polymers,* 3rd edn. John Wiley & Sons, New York, 1980.
11. Morse, D.C., Viscoelasticity of Tightly Entangled Solutions of Semiflexible Polymers, *Phys. Rev. E. 58*:R1237–R1240 (1998).
12. Morse, D.C., Viscoelasticity of Concentrated Isotropic Solutions of Semiflexible Polymers. 1. Model and Stress Tensor, *Macromolecules 31*:7030–7043 (1998).
13. Morse, D.C., Viscoelasticity of Concentrated Isotropic Solutions of Semiflexible Polymers. 2. Linear Response, *Ibid. 31*:7044–7067 (1998).
14. Doi, M., and S.F. Edwards, *The Theory of Polymer Dynamics,* Oxford University Press, London, 1986.
15. Bird, R.B., R.C. Armstrong, and O. Hassager, *Dynamics of Polymeric Liquids,* Wiley, New York, 1977, Vol. 1, pp. 208–209.

[Received November 7, 2005; accepted April 1, 2006]