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Rheological Properties of Lupin Protein Suspensions

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Abstract: The linear and non-linear rheological properties of whole (non-defatted) and defatted lupin proteins as well as industrial soy proteins were investigated. By measuring the linear rheological properties of a series of concentrations of whole and defatted lupin proteins as well as soy proteins in suspension, we found that all three concentrations of protein suspensions exhibited similar viscoelastic solid properties. Both storage (G') and loss (G") moduli increased with increase in concentration. The linear range of the rheological properties of all protein suspensions was very small. The stress relaxation studies showed that the three concentrations of protein suspensions relaxed quickly indicating that the molecules of these three kinds of proteins have neither cross-linking nor strong physical interactions. The non-linear rheological properties of the suspensions for whole and defatted lupin proteins as well as industrial soy proteins all displayed similar shear-thinning behavior and viscosities, which can be well described by a power law constitutive model. According to this study, in many food and non-food applications, there should be a high potential to replace soy proteins with lupin proteins or vice versa. More food applications using lupin proteins are expected.

Key words: Lupin proteins, rheology, soy proteins, viscoelastic properties

INTRODUCTION

As an important legume plant (Petterson, 2000; Ayaz *et al*., 2004), lupin seeds contain high concentrations of proteins (more than 50%) and oil (up to 11%). These protein and oil contents are comparable to those of soybean, which possess about 40% proteins and 21% oil. Possessing nutritional substances and having a high concentration of proteins make lupin to be a good element of diet. Like many other plant proteins, lupin proteins are accompanied by various fibers. People know that fiber intake could improve glucose tolerance and consequently modify blood insulin and glucagon. In addition, lupin proteins also have the function of lowering serum cholesterol level as do some other plant proteins (Chango *et al*., 1998). There have been many human consumption applications for lupin, such as breadmaking and pasta products (Mohamed, 1994), meat substituents (Alamanou *et al*., 1996) and egg and milk replacer (Antonio and Infante, 1990). Lupin appears to be a widely utilized commodity in both food and industrial applications because of the above reasons and its ease of growing and harvesting. However, there is very little published research concerning lupin and its products such as lupin proteins and oils especially their physical properties and structure/function relationships, both of which have limited the usage of lupin in the United States (Sousa *et al*., 1995, 1996).

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Previously, we reported that lupin meal had strong viscoelastic solid behavior, which offer the new insight into the physical properties of the lupin meal (Xu and Mohamed, 2003). This study investigated the rheological properties of the lupin proteins produced by acid precipitation and compare with the soy proteins viscoelastic properties.

MATERIALS AND METHODS

Materials

Lupinus albus (flour) was provided by the Agricultural Research Station, Virginia State University. Industrial soy protein isolate (EDIPro A) was provided by Protein Technology International (St. Louis, MO), a food grade soy protein isolate with 87.3% protein content (N **X** 6.25) and 5.3% moisture content, which was conducted by Technology International at St. Louis, MO.

Protein Extraction

Lupin flour whole and defatted meal (30 mesh) was suspended in 0.1 N NaOH (10:1, 100 g flour in 1 L 0.1 N NaOH). The pH of the suspension was adjusted to 9.9 and allowed to stir for 30 min, then the pH was adjusted to 9.0 using 0.1 N HCl. The suspension was then centrifuged at 10,000 X g for 30 min at 10°C. The extraction of precipitated residue was repeated twice. The pH of the collected supernatants was adjusted to 4.6 using 1.0 N HCl. The acidified dispersion was centrifuged at 10,000 X g and the precipitate was freeze-dried. The extracted lupin proteins contained 94% protein and 4.3% moisture content.

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 suspensions. 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 (0.1%) for the other viscoelastic property measurements for the same material; fresh samples were used for each experiment. Linear viscoelasticity indicates that the measured parameters are independent of applied shear strain. Small-amplitude oscillatory shear experiments were conducted over a frequency (ω) range of 0.1-100 rad sec^{-1}, yielding the oscillatory shear storage (G') and loss (G") moduli. The storage modulus represents the non-dissipative component of mechanical properties. The 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 ($\delta = 0$), or liquid with pure viscosity ($\delta = 90°$), or something in between. Stress relaxation experiments were conducted and they measured the decrease in the modulus, G(t), with the time after the material is subject to a step increase in shear strain. Non-linear rheological measurements were conducted as steady shear in the range of shear rate of 0.1-500 sec^{-1}.

The protein sample powder was suspended at various concentrations in a 0.05 M sodium phosphate buffer (pH 7.0 at 25°C) by mixing with a homogenizer for the rheological measurements. The powder was well dispersed in the buffer and no sedimentation was observed for two weeks after the preparation. At least two suspension samples were made for each concentration for the measurements.

RESULTS AND DISCUSSION

The linear rheological studies showed that lupin protein suspensions had strong viscoelastic properties. The dynamic frequency sweep results for three concentrations of whole (non-defatted) lupin protein suspensions are shown in Fig. 1. The shapes of the G' and G" curves were parallel and G' were much greater than G". The values of G' and G" were slightly dependent on the frequency. The phase shifts were in the range of 20 to 33°. This implied that the lupin proteins exhibited viscoelastic solid properties. At 150 mg mL^{-1}, the storage modulus (G') at the frequency of 1 rad sec^{-1} for whole lupin protein suspension was 162 Pa. The phase shift was 28° at this frequency. At 250 mg mL^{-1}, the storage modulus of 1 rad sec^{-1} for whole lupin proteins reached 365 Pa and the phase shift decreased to 23°. Within this small concentration range, the storage modulus was more than doubled. The moduli for lupin suspensions increased dramatically with the increased concentrations. The phase shifts were not changed much with the different concentrations, but became smaller at higher concentrations of lupin suspensions. This indicated that the lupin protein suspensions were more solid-like at higher concentrations. The measurements for the defatted lupin protein suspensions displayed identical behavior as the whole lupin protein suspensions. And the G' and G" of the defatted lupin proteins were almost identical to those of the whole lupin protein suspensions (data not shown). This result suggested that the small amount of fat, which was less than 1% in this case, affect the viscoelastic properties of the lupin proteins very little. The linear dynamic frequency sweep measurements for soy protein suspensions exhibited very similar behavior as lupin protein suspensions (Fig. 2). The shapes of the G' and G" curves for soy proteins were identical to those for lupin proteins suggesting the same viscoelastic solid properties. The phase shifts were in the range of 20 to 30°, which were also pretty close to those for lupin proteins. At 150 mg mL^{-1}, the storage modulus (G') at the frequency of 1 rad sec^{-1} for soy protein suspension was 171 Pa. The phase shift was 27° at this frequency. At 250 mg mL^{-1}, the storage modulus of 1 rad sec^{-1} for whole lupin proteins reached 367 Pa and the phase shift became 23°. It is noticed that the values of moduli and

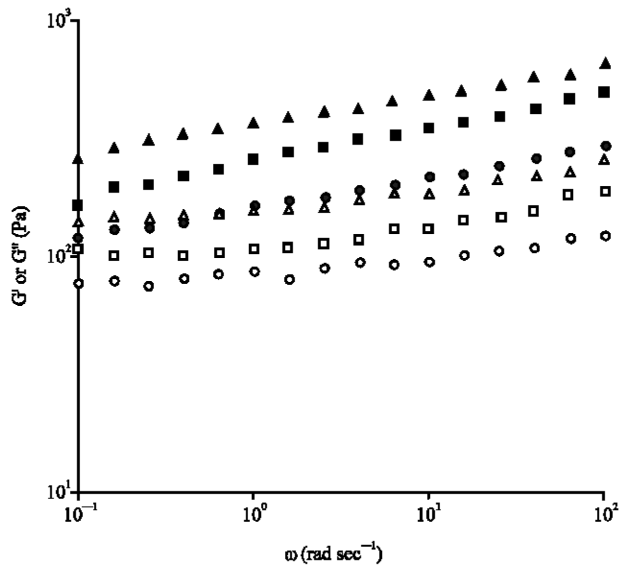

Fig. 1: The linear viscoelastic properties of the whole (non-defatted) lupin protein suspensions at shear strain of 0.1% and at the temperature of 25°C. Circle 150 mg mL^{-1}, square 200 mg mL^{-1}, triangle 250 mg mL^{-1}; filled symbols G', opened symbols G"

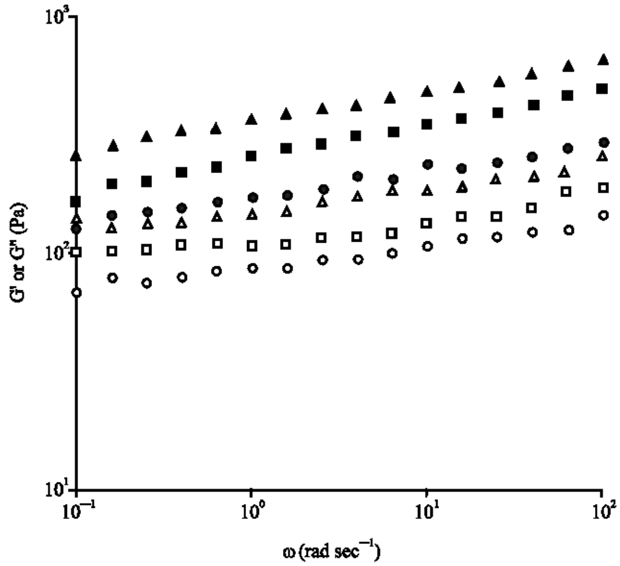

Fig. 2: The linear viscoelastic properties of the industrial soy protein suspensions at shear strain of 0.1% and at the temperature of 25°C. Circle 150 mg mL^{-1}, square 200 mg mL^{-1}, triangle 250 mg mL^{-1}; filled symbols G', opened symbols G"

phase shifts for both lupin and soy protein suspensions are very close to each other. The linear ranges of the rheological properties for both lupin and soy protein suspensions were all similar and small, less than 5% (Fig. 3 shows the non-defatted lupin proteins result only). In addition, the stress relaxation measurements for lupin and soy proteins displayed the same behavior of fast relaxation, which was less than 10 sec at 0.1% initial shear strain (Fig. 4 shows the industrial soy proteins result only). This quick relaxation behavior suggested that the molecules of lupin and soy proteins should not be chemical cross-linked. The reasonable explanation for the structures of both lupin and soy proteins networks is that they form networks with chain-chain physical interactions. Apparently, the physical chain-chain interactions for both lupin and soy proteins networks were stronger with the increasing concentration so that their suspensions exhibited stronger viscoelastic solid behaviors at higher concentrations. The results above indicate that lupin and soy proteins exhibit similar linear viscoelastic behaviors and they should displayed similar baking qualities.

To better understand the processing behavior, the non-linear steady shear viscoelastic properties of lupin and soy proteins were studied. The non-linear viscoelastic properties of whole lupin protein and soy protein suspensions are shown in Fig. 5 and 6, respectively. The defatted lupin protein suspensions showed identical non-linear shear behavior as the whole lupin proteins (data not shown). This result supported the conclusion from the linear viscoelastic properties measurements above, which implied that the small amount of fat would not affect the viscoelastic behavior of lupin proteins. Both defatted lupin protein suspension and industrial soy protein suspension exhibited shear-thinning behavior over the entire measured shear rates (Fig. 5, 6). Viscosities were higher at higher concentrations, as expected. Shear-thinning rheological behavior can be characterized by a power law constitutive equation (Bird *et al.*, 1977). The power law equation may be written as:

$$\eta = K\dot{\gamma}^{n-1} \quad (1)$$

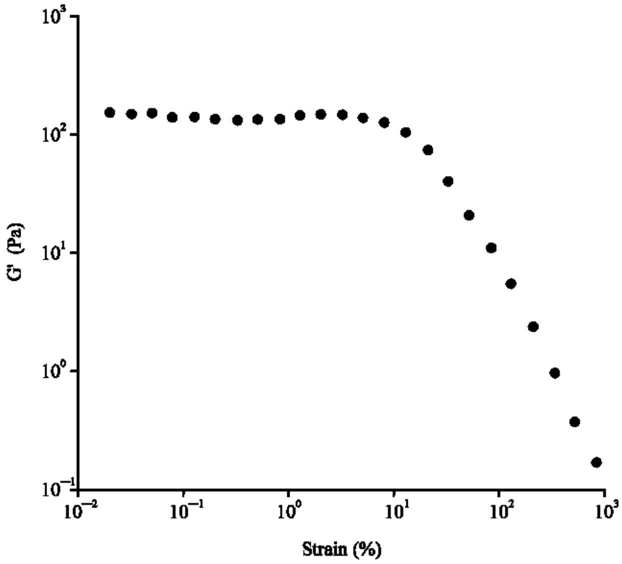

Fig. 3: Strain sweep experiment of the 150 mg mL^{-1} the whole (non-defatted) lupin protein suspension at 25°C

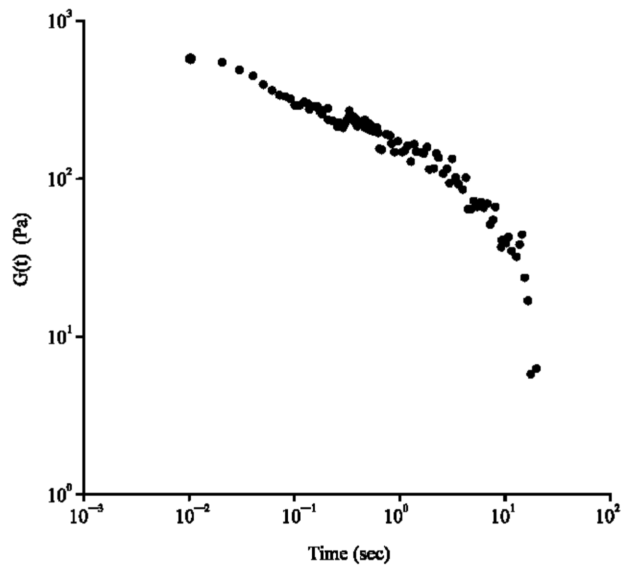

Fig. 4: Stress relaxation measurement of the 150 mg mL^{-1} industrial soy protein suspension at 25°C. The initial step strain was 0.1%

where, η is the shear viscosity, K is the front factor, $\dot{\gamma}$ is the shear rate and n is the power law exponent. The value of n is less than one for material shear-thinning behavior. Equation 1 was used to fit shear-thinning viscosity for both lupin and soy proteins (Fig. 5, 6). The experimental data were very well fitted by the power law constitutive equation (Fig. 5, 6). The results of the fits are summarized in Table 1. From Table 1, we can get the impression that non-defatted lupin proteins and industrial soy

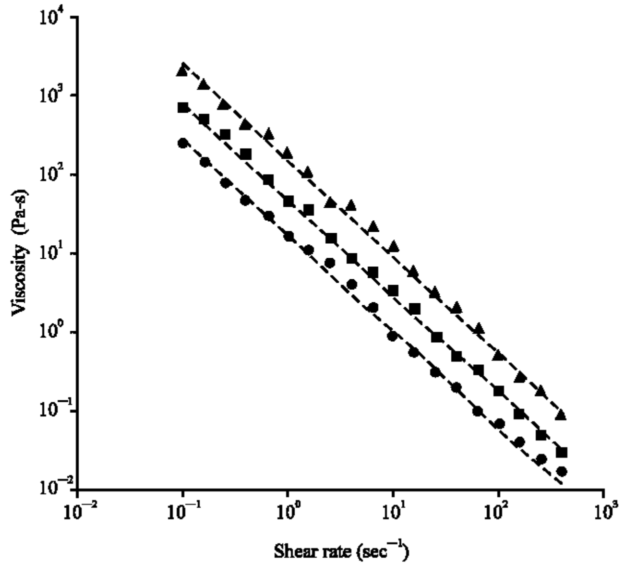

Fig. 5: The non-linear viscoelastic steady shear measurements of the whole (non-defatted) lupin protein suspensions at the temperature of 25°C. Symbols are experiment results. Dashed lines are fitted with power law model. Circle 150 mg mL^{-1}, square 200 mg mL^{-1}, triangle 250 mg mL^{-1}

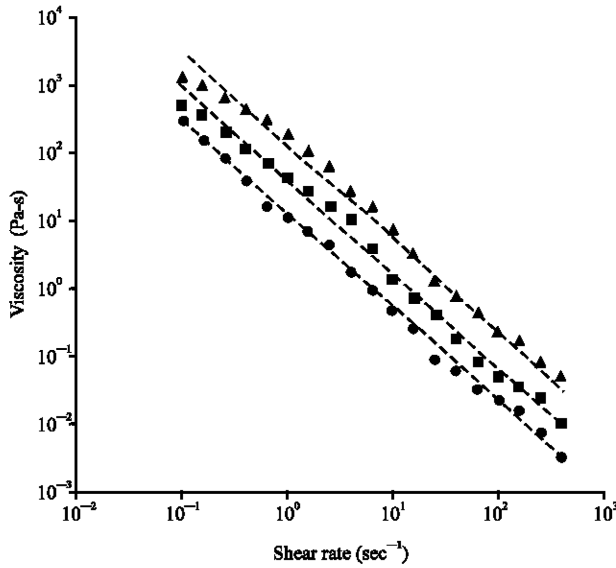

Fig. 6: The non-linear viscoelastic steady shear measurements of the industrial soy protein suspensions at the temperature of 25°C. Symbols are experiment results. Dashed lines are fitted with power law model. Circle 150 mg mL^{-1}, square 200 mg mL^{-1}, triangle 250 mg mL^{-1}

proteins possess the similar power law exponents, -0.22 and -0.38, respectively. Thus lupin proteins and soy proteins have close to each other shear-thinning extent. Most of the food processing is in the

Table 1: Power law model fitted parameters for whole (non-defatted) lupin protein and industrial soy protein suspensions

Concentration of protein suspensions	K (Pa-s^n)	n	R^2
150 mg mL^{-1} lupin	17.77	-0.22	0.98
200 mg mL^{-1} lupin	49.18	-0.22	0.93
250 mg mL^{-1} lupin	153.71	-0.22	0.90
150 mg mL^{-1} soy	13.79	-0.38	0.93
200 mg mL^{-1} soy	39.21	-0.38	0.87
250 mg mL^{-1} soy	131.88	-0.38	0.82

range of 1-100 sec^{-1} shear rate. It is evident that lupin and soy protein suspensions exhibit the similar shear behavior and viscosity values at this shear rate range (Fig. 5, 6). Therefore, it can be predicted that lupin proteins have the similar behavior as soy proteins during processing.

CONCLUSION

Lupin and soybean both are legume plants. Lupin contains more than 50% proteins and up to 11% oil, while soybeans contain about 40% proteins and 21% oil. Lupin's unique property makes it have potential usage in many food and non-food applications. However, in the literature, reports are rarely found about the physical properties studies for lupin. The results of current work on the physical properties of lupin proteins, which provide new insight of lupin proteins, are promising. Lupin proteins exhibit strong viscoelastic properties similar to those of soy proteins. Its shear thinning behavior is the same as soy proteins. Thus they perform very similar behavior during processing. Most lupin proteins are globular, as well as the soy proteins. The extraction procedure and isolation schematic for industrial soy proteins use an acid precipitation process similar to lupin protein extraction and isolation in current work. Therefore according to this study, in many food and non-food applications, there should be a high potential to replace soy proteins with lupin proteins or vice versa. Further research works need to be done for non-denatured lupin proteins, such as molecular weight, molecular structure and function relationship and material behavior etc.

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