Rheology of Defatted Ultrafiltration-Diafiltration Soy Proteins

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Abstract: The linear and non-linear rheological properties of defatted soy proteins produced by ultrafiltration-diafiltration were investigated at three temperatures. Five concentrations ranging from 10 to 30% of the defatted Ultrafiltered-Diafiltered (UD) soy proteins were prepared. The properties of defatted UD soy proteins depended on concentration and temperature. At 10%, defatted UD soy proteins exhibited almost viscous fluid behavior. When concentration increased or temperature decreased, the properties of soy proteins shifted into viscoelastic. The higher the concentration or the lower the temperature, the stronger of the viscoelasticity was for the soy proteins. The non-linear rheological properties were also concentration and temperature dependent. The non-linear steady shear measurements for the defatted UD soy proteins exhibited shear-thinning behavior, which can 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 soy proteins concentration and temperature. The results of this study can be used to direct further food and non-food applications for defatted UD soy proteins.

Key words: Rheology, soy proteins, ultrafiltration, viscoelastic properties

INTRODUCTION

Vegetable proteins have been good resources for human diet. Among them, soybean proteins are the most important and widely used. They can be added to formulated bakery products, dairy products and meat substitutes. People love the food products produced from soy and soy proteins such as tofu and soymilk due to their great nutritional properties. FDA reviewed research from 27 studies that showed soy protein's value in lowering levels of total cholesterol and low-density lipoprotein (LDL, or bad cholesterol) and approved a health claim of Diets low in saturated fat and cholesterol that include 25 g of soy protein a day may reduce the risk of heart disease, which can be used on labels of soy-based foods (Henkel, 2000). Soybean proteins have been widely used in food industry not only due to their nutrition properties, but also due to their functional properties. Soybean proteins are being used as functional ingredients in many food products. In addition, soybean proteins also have extensively applications in industry such as in many types of paints and inks, in fire fighting foams and in paper coatings etc. Conventional methods of producing soy proteins include treatment of soy flour with acid or alcohol to separate proteins followed by centrifugation and/or filtrations. These methods generally can damage the protein functional properties. Purifying soy proteins with the method of UD can preserve the functionality of the proteins (Hojilla-Evangelista *et al.*, 2004).

Because of above reasons, soy proteins produced by ultrafiltration-diafiltration have great potential in both food and industrial applications. However, literature reports regarding UD soy protein physical properties and processing behaviors are limited. A better understanding of soy

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proteins and products should enhance wider usage of them. The objective of this study is to characterize the linear and non-linear rheological properties of the defatted Ultrafiltered-Diafiltered (UD) soy proteins so that we can have some insight for their properties and function relationships, which can be used to guide more food and non-food applications for defatted UD soy proteins. In this study, the effects of concentration and temperature on linear and non-linear viscoelastic properties of defatted UD soy proteins were studied. Power law model was used to describe the soy protein shearing behaviors.

MATERIALS AND METHODS

Materials

The starting material was an extruder-expelled, partially defatted soybean meal with a 66.5% protein dispersibility index provided by Nutriant (Cedar Falls, IA). Proximate analysis, on a dry weight basis, was 48.9% crude protein (Dumas N x 6.25), 11.1% crude fat, 2.2% crude fiber and 6.4% ash where the starting material had 6.1% moisture. The soy flour was defatted batch-wise with diethyl ether. The first batch extraction was performed at room temperature with a solvent to solids ratio of 6:1 in a beaker. After stirring occasionally for 1.5 h, the sample was decanted and filtered on a Buchner funnel equipped with a Whatman #4 filter. This process was repeated twice more at a solvent to meal ratio of 4:1 and 30 min extraction each batch. The collected defatted soy solids on the Buchner funnel were then air dried to remove volatile diethyl ether residues.

Production of Soy Protein Isolate by Ultrafiltration-Diafiltration

Soy protein isolate was produced by following the ultrafiltration-diafiltration protocol described in the study by Hojilla-Evangelista *et al.* (2004). Fifty-gram of defatted soy meal was homogenized with 1.7 L 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 x g for 25 min) and then filtered through Whatman No. 5 paper. The filtrate was saved, while the solids were again homogenized with ca. 1.6 L water, centrifuged and filtered as were done previously. The solids were discarded. Filtrates from the two extractions were pooled and volume was adjusted to 4 L with water. The filtrate was ultrafiltered in a Pall Centramate system using polyether sulfone membrane (5 kDa MWCO). The unit was operated at a flux rate of 2.7 L h^{-1} and inlet and outlet pressure ranges of 21-25 and 27-34 psi, respectively at room temperature. When ca. 3.4 L permeate was collected, 2 L 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 a protein isolate with a protein content of 85.2% (Dumas N x 6.25) and a moisture of 6.2%, while the permeate was discarded.

Preparation of Solutions for Rheological Measurements

The defatted Ultrafiltered-Diafiltered (UD) soy proteins sample powder was suspended in a 10 mM imidazole buffer (pH 7.0 at 25°C) for the rheological measurements. Five concentrations of defatted UD soy protein solutions were made: 10, 15, 20, 25 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 two weeks after the preparation.

Rheological Measurements

The methods of rheological measurements were similar to those described by Xu *et al.* (2001, 2007). 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 desired temperature ±0.1°C by a water circulation system. Linear viscoelastic measurements were conducted for the various concentrations of soy 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 defatted UD soy proteins was less than 10% of strain. Five percent of strain was adopted for the linear rheological measurements for all defatted UD soy protein samples in this study. Small-amplitude oscillatory shear experiments were conducted over a frequency (ω) range of 0.1-500 rad sec^{-1}, yielding the oscillatory shear storage (G') and loss (G'') moduli. At low frequencies (0.1-1 rad sec^{-1}), the measurement time was between 2 to 15 min, while at relative high frequencies (1-500 rad sec^{-1}), the measurement time was between few seconds to 2 min. The storage modulus represents the non-dissipative 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 ($\delta = 0$), or liquid with pure viscosity ($\delta = 90°$), or something in between. Non-linear rheological studies were conducted at the same ARES instrument with the same geometry described above. The steady shear measurements were conducted with the shear rate increased step-wise in the range of shear rate of 0.3-300 sec^{-1}. The delay time was 10 sec.

RESULTS AND DISCUSSION

Five concentrations of defatted UD soy protein solutions were made, 10, 15, 20, 25 and 30% (wt%). In Fig. 1, the linear dynamic frequency sweep results of storage (G') and loss (G'') moduli for five concentrations of defatted UD soy proteins are displayed. The G' and G'' values for the defatted UD soy proteins were found to be dependent on the oscillation frequency. Both storage and loss moduli of the defatted UD soy proteins depended on concentration. At 10%, the storage moduli (G') for defatted UD soy proteins were hardly detected at the measured frequency range; the loss moduli (G'') curve exhibited a straight line with a slope of one that is the phenomenon of a viscous liquid material (Fig. 1A). The phase shifts were all around 90 degrees (Fig. 2). This result indicated that 10% defatted UD soy proteins showed nearly perfect viscous liquid behavior. The concentration of 10% was relatively low and the protein molecules in the solution barely had interactions. The protein molecules were diluted and dispersed in the solution and there was no polymer network. So it behaved as viscous fluid. As the concentration increased, the properties of defatted UD soy protein solutions changed (Fig. 1). At 15%, both G' and G'' were measurable indicating the viscoelastic properties of the material. The values of G'' were higher than those of G' at whole measured frequencies; and the curve of the loss moduli (G'') still was a straight line with a slope of unity (Fig. 1B). The phase shifts were in the range of 64-73 degrees. At frequency of 1 rad sec^{-1}, G' of the 15% defatted UD soy proteins was 0.02 Pa. The above results suggested that 15% defatted UD soy proteins exhibited weak viscoelastic fluid or liquid behavior, according to linear viscoelastic theory (Ferry, 1980). Apparently, from 10 to 15%, with the concentration increased, there were more molecule-molecule interactions and a polymer network was formed. With the further increase of the concentration, both values of G' and G'' for defatted UD soy proteins increased. At 20%, defatted UD soy proteins exhibited viscoelastic liquid behavior; the loss moduli (G'') were greater than storage moduli (G') (Fig. 1A). The phase shifts were in the range of 64-72 degrees, which were similar as those for 15% defatted UD soy proteins (Fig. 2). At frequency of 1 rad sec^{-1}, G' of the 20% defatted UD soy proteins became 0.04 Pa. At
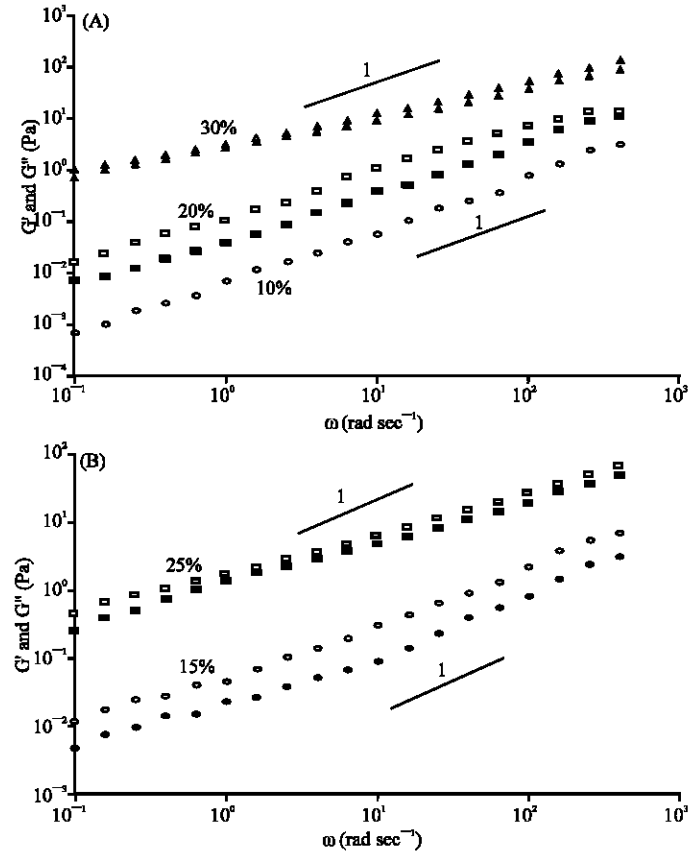
Fig. 1: The linear viscoelastic properties of the defatted UD soy proteins at the temperature of 25°C. Filled symbols G', opened symbols G. 1A: Circle 10% (wt%), square 20% (wt%), triangle 30% (wt%). 1B: Circle 15% (wt%), square 25% (wt%)
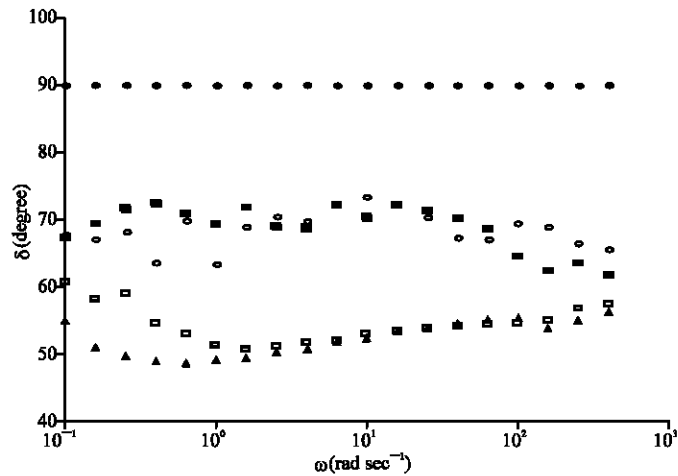

Fig. 2: Frequency-dependence phase shift of five different concentrations of defatted UD soy proteins at 25°C. Filled circle 10% (wt%), opened circle 15% (wt%), filled square 20% (wt%), opened square 25% (wt%) and filled triangle 30% (wt%)

25 and 30%, defatted UD soy proteins still showed viscoelastic liquid behavior; the loss moduli (G')
were higher than storage moduli (G') (Fig. 1). However, the phase shifts decreased into the range of 50-
60 degrees and 48-56 degrees, respectively for 25 and 30% defatted UD soy proteins (Fig. 2). At
frequency of 1 rad sec^{-1}, the values of G' for the 25 and 30% defatted UD soy proteins were 1.4 Pa
and 2.7 Pa, respectively. The shapes of moduli curves were further deviated from the line with a slope
of one (Fig. 1). Figure 2 clearly displayed the property shift for the defatted UD soy proteins. At
10%, defatted UD soy proteins exhibited almost viscous behavior with phase shift of 90 degrees.
However, defatted UD soy protein property changed from viscous at 10% into viscoelastic liquid at
15. 15 and 20% defatted UD soy proteins showed similar phase shifts. The phase shifts was further
decreased as the concentration increased into 25 and 30% (Fig. 2). These results suggested that
viscoelastic properties for defatted UD soy proteins become stronger with the increasing
concentration. The property shift from viscous behaviors of lower concentration into viscoelastic
properties of higher concentrations might be due to the more inter-molecular interactions with the
increasing concentration.

The linear viscoelastic properties of defatted UD soy proteins were also dependent on
temperature (Fig. 3). At lower temperature, the viscoelastic properties were stronger because the
mobility of molecules was confined (6). For example, at 25°C, G' nearly was zero for the 10% soy
proteins at the measured frequency range. However, at 5°C, G' was measurable and it was 0.01 Pa at
frequency of 1 rad sec^{-1}, which indicated that the 10% defatted UD soy proteins became viscoelastic
liquid at this lower temperature. At frequency of 1 rad sec^{-1}, 15 and 30% defatted UD soy proteins
had G' of 0.02 Pa and 2.7 Pa, respectively at 25°C. However, they became 0.09 Pa and 12.3 Pa,
respectively at 5°C. The phase shifts were in the range of 64-73 degrees and 48-56 degrees,
respectively for 15 and 30% at 25°C (Fig. 2). But at 5°C, the phase shifts decreased into 55-67 degrees
and 42-55 degrees, respectively for 15 and 30% defatted UD soy proteins (Fig. 4). At 5°C, 30%
defatted UD soy proteins even showed a little plateau and G' was slightly greater than G'' at low
frequencies (Fig. 3B), which indicated a transition from flow region to rubber-like region or from
viscoelastic fluid into viscoelastic solid (Bagley, 1992). The molecular interpretation of rheological
properties is not as well understood as other modern biochemical and molecular biology techniques.
The curve shapes of moduli did not show the evidence of cross-linking. In addition, our relaxation
experiments exhibited relative quick relaxation after the material was subject to a step increase in 3%
strain, which also suggested that there should be no cross-linking (data not shown).

To better understand the processing behavior, the non-linear steady shear viscoelastic properties
of defatted UD soy proteins were studied. The non-linear viscoelastic behaviors for defatted UD soy
proteins were also dependent on concentration and temperature. Viscosities were higher at higher
concentrations and at lower temperatures, as expected. Figure 5 displays the shear viscosity
versus the shear rate for the five soy protein concentrations at three temperatures. They all showed
shear-thinning behavior over the entire measured shear rates (Fig. 5). At 25°C, 10% defatted UD soy
proteins exhibited the behavior close to the Newtonian fluid and very little shear-thinning property
(Fig. 5A), which is consistent with the viscous behavior found at linear viscoelastic measurements. At
lower temperatures (Fig. 5B, C) or at higher concentrations (Fig. 5), soy proteins showed greater
shear-thinning behaviors. 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

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

(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 used to fit shear-thinning viscosity for soy proteins (Fig. 5). The
experimental data were very well fitted by the power law constitutive equation (Fig. 5). The results
of the fits are summarized in Table 1. At higher concentrations or at lower temperatures, defatted UD
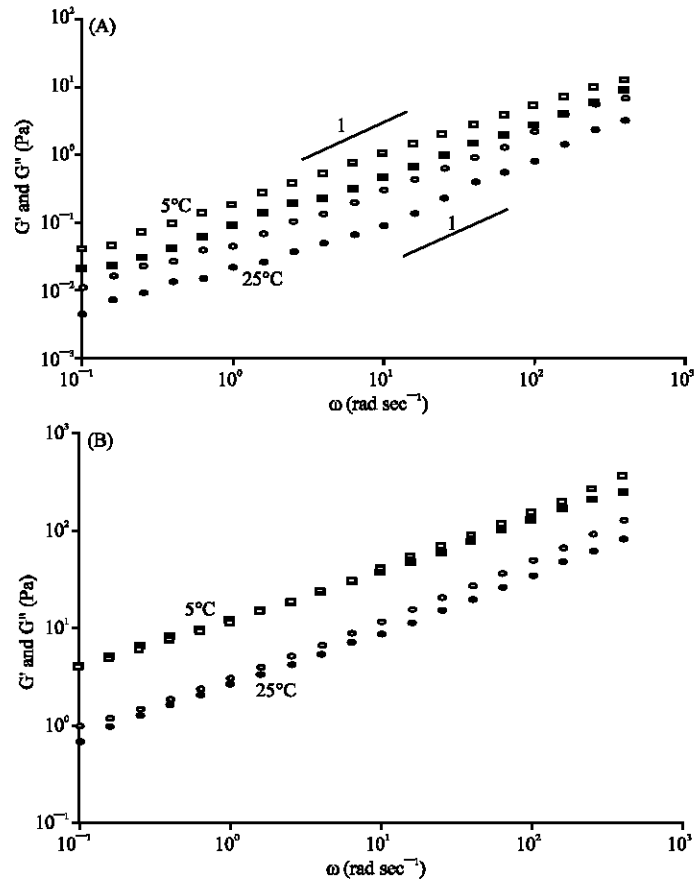soy proteins possess lower power law exponents (Table 1), which represented greater shear-thinning

Fig. 3: The linear viscoelastic properties of two different concentrations defatted UD soy proteins at the temperature of 25°C and 5°C. Filled symbol sG', opened symbols G. 3A: 15% (wt%) defatted UD soy proteins; circle 25°C, square 5°C. 3B: 30% (wt%) defatted UD soy proteins; circle 25°C, square 5°C
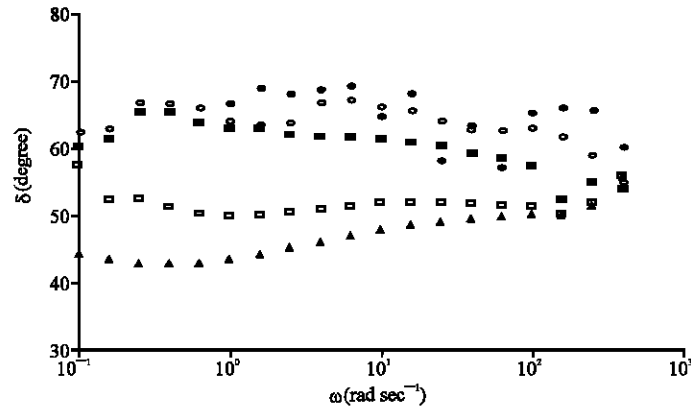

Fig. 4: Frequency-dependence phase shift of five different concentrations of defatted UD soy proteins at 5°C. Filled circle 10% (wt%), opened circle 15% (wt%), filled square 20% (wt%), opened square 25% (wt%) and filled triangle 30% (wt%)
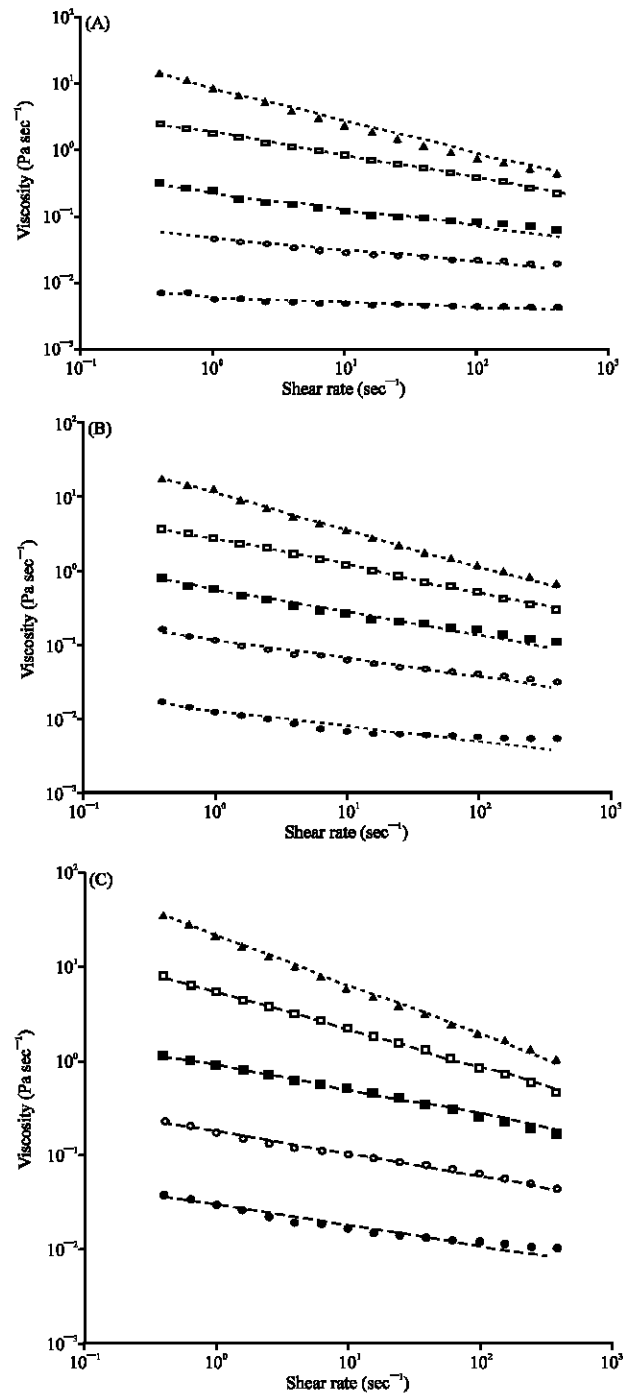
Fig. 5: The non-linear steady shear viscosity vs. shear rate for the defatted UD soy proteins at three different temperatures. Symbols are experiment results. Dashed lines are fitted with power law model. Filled circle 10% (wt%), opened circle 15% (wt%), filled square 20% (wt%), opened square 25% (wt%) and filled triangle 30% (wt%). (A), 25°C. (B), 15°C and (C), 5°C

Table 1: Power law model fitted parameters

Concentration of defatted UD soy proteins	K (Pa-s^n)	n	R^2
10% (25°C)	0.0061	0.92	0.80
10% (15°C)	0.0130	0.80	0.92
10% (5°C)	0.0300	0.78	0.97
15% (25°C)	0.0470	0.82	0.96
15% (15°C)	0.1200	0.77	0.98
15% (5°C)	0.1800	0.75	0.99
20% (25°C)	0.2300	0.74	0.96
20% (15°C)	0.5400	0.73	0.99
20% (5°C)	0.8900	0.72	0.99
25% (25°C)	1.7700	0.66	0.99
25% (15°C)	2.6200	0.64	0.99
25% (5°C)	5.5400	0.60	0.99
30% (25°C)	8.2500	0.52	0.98
30% (15°C)	10.6600	0.50	0.99
30% (5°C)	21.5900	0.48	0.99

extent. If the exponent was equal to one, it meant that the material was an Newtonian fluid. The exponent for 10% defatted UD soy proteins at 25°C was 0.92, which was very close to the behavior of a Newtonian viscous liquid. However, the exponent for 30% soy proteins at 25 and 5°C decreased into 0.52 and 0.48, respectively. Table 1 showed clearly the trend of these exponents shift that represented the material property change, which was consistent with the linear viscoelastic behavior change with the concentration and temperature stated above.

CONCLUSIONS

In summary, defatted soy proteins produced by ultrafiltration-diafiltration are water-soluble and should have their functionalities. Defatted UD soy proteins exhibit properties that depend on concentration and temperature. At 10%, which is a dilute concentration, defatted UD soy proteins displayed almost viscous liquid behavior. However, when concentration increased or temperature decreased, the properties of defatted UD soy proteins shifted into viscoelastic behaviors. The higher of the concentration or the lower of the temperature was, the stronger of the viscoelasticities for soy proteins would be. There was no evidence to show that defatted UD soy proteins formed a cross-linking network. Additional biochemical and biophysical studies are needed to explore the network structure. A non-linear steady shear study of the defatted UD soy proteins show shear-thinning properties for all measured concentrations and temperatures. The power law constitutive equation fitted the experiments very well. Therefore, the power law constitutive equation can be used to describe the defatted UD soy proteins shearing behavior within the range of shear rate of 0.3-300 sec^{-1}. Because the conditions that we adopted in this research are similar to the actual processing situations, all these discoveries from this study should be much helpful and useful for developing and processing new food and non-food applications of defatted UD soy proteins.

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