Temperature and Shear Rate Affecting the Viscosity and Secondary Structural Changes of Soy 11S Globulin Measured by a Cone-Plate Viscometer and Fourier Transform Infrared Spectroscopy

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The effects of temperature, shear rate, shear time, and concentration on the rheological properties of 11S globulin in ionic strength 0.5, pH 7.6 phosphate buffer were studied by a cone and plate viscometer at shear rates up to 384 sec^{-1} . The secondary structure changes caused by elevated temperature and shearing were also monitored by Fourier transform infrared spectroscopy (FTIR). The effects of concentration on apparent viscosity were pronounced. A 3-percent solution behaved as a Newtonian solution. The solutions become pseudoplastic when the concentrations were higher than 5%. The effects of shearing time up to 30 min on apparent viscosity also depended on the concentration. Shear thinning first become nearly time-independent, later was observed in 5 or 10% solution, while 3% and 20% solutions behaved as time independent. Increasing temperature at $1.4^\circ\text{C}/\text{min}$ with shearing time up to 40 min result in the 3, 5, and 10% solutions behaving with shear thinning earlier and with shear thickening later. The reason that elevation temperature and longer shear time caused shear thickening in the solution was a change of secondary structure seen in the FTIR spectrum. The amount of α -helix of 11S globulin increased from 7.8% to 19.6 or 28.1% as the temperature increased from 25°C to 80 or 90°C , respectively.

Viscosity is an important functional property in fluid foods. The study of viscosity has practical significance in relation to process engineering, new product development, and quality control.^{1,2)} Molecular volume, mass, the distance between the molecules, and attractive forces between the molecules are factors affecting the viscosity. Therefore, changes in viscosity can be used to monitor the changes in physico-chemical interactions between proteins or conformational changes of component in solution or dispersion.³⁻⁴⁾ Soy proteins with their excellent functional properties have been used as an important ingredient in various food systems. Viscosity studies on soy proteins were mainly on extracted soy proteins, *e.g.*, acid-precipitated protein, soy isolate, or concentrates. The effects of protein concentration,⁵⁻⁷⁾ shearing time,^{5,8,9)} process,^{5,7)} NaCl concentration,^{5,7,9,10)} pH,^{5,7,11)} sonication,⁶⁾ and temperature,¹¹⁾ *etc.* on viscosity have been reported. However, few studies on soy

11S globulin have been done. Umeya *et al.*⁸⁾ studied the effects of ionic strength on the apparent viscosity of soy glycinin. Catsimpoolas and Meyer,¹²⁾ Ishino and Okamoto,¹³⁾ and Ishino and Kudo¹⁴⁾ investigated the changes of viscosity of sol/gels induced by heating or alcohol.

The objectives of this study are to elucidate the effects of concentration, shear time, shear rate, and temperature on the viscosity of soy 11S globulin in ionic strength 0.5, pH 7.6 phosphate buffer and to study the effects of temperature and shearing on the secondary structure of soy 11S globulin by FTIR.

Materials and Methods

Preparation of soybean 11S globulin. Purified soybean 11S globulin, prepared by selective thermal denaturation and solubility in a high ionic strength envelope,¹⁵⁾ was used as the raw material. The purity observed by gel

Table I. PROTEIN CONCENTRATION, SHEAR RATE, SHEAR TIME, TEMPERATURE INCREASING PROGRAM, AND TEMPERATURE USED IN RHEOLOGICAL PROPERTIES STUDIES OF 11S GLOBULIN

Protein conc. (% w/v)	Shear dependency study ^a	Time dependency study ^b	Temperature increasing and time dependency study ^c	Elevated temperature and time dependency study ^d
	Shear rate (sec ⁻¹)	Shear time (min)	Temp. increasing program (25–80°C)	Temperature (°C)
3	9.6–384	30	1.4°C/min	
5	9.6–384	30	1.4°C/min	80, 90
10	9.6–384	30	1.4°C/min	25, 50, 80, 90
20		30	1.4°C/min	80, 90

^a Temperature was 25°C and the hysteresis loop was completed within 8 min.

^b Shear rate was 9.6–384 sec⁻¹ and temperature was 25°C.

^c Shear rate and shear time at this study were 384 sec⁻¹ and 30 min, respectively.

^d Same as c.

electrophoresis was greater than 95%. It was stored in a desiccator until use.

Rheological properties. A Brookfield cone and plate viscometer (Brookfield, DV-II, U.S.A.) was used with a 2.4 cm diameter and 1.565° angle cone spindle. The concentration of sample was adjusted to 3, 5, 10, and 20% (from lyophilized 11S globulin (weight) with ionic strength 0.5, pH 7.6 phosphate buffer (volume)). About 1 ml of sample was carefully spread to cover all the plate surface. The distance between the cone and plate was also adjusted such that the cone apex just touched the surface of the plate. (Cone/plate temperature was controlled by a Firstek programmed isothermer (Firste, KB403, Taipei) (Accuracy ± 0.01°C), while the cone/plate was sealed with a tape on the outside to avoid water vaporization during the experiment, which might cause error due to the change of concentration for those runs at temperatures higher than 50°C or the long running time of the time dependency study. The corresponding viscosity (or shear stress) was recorded by a recorder (Watanabe, SR6311, Japan) automatically. Table I lists the protein concentration, shear rate, shear time, temperature increasing program, and temperature used in the rheological properties studies of 11S globulin in this experiment.

Fourier transform infrared (FTIR) analysis. A Boman DA3.002 FT-IR spectrometer (Boman, DA3.002, Canada) with a Hg–Cd–Te detector was used for the experiment. The spectral resolution is 2 cm⁻¹. Fourier self-deconvolution was done using the technique of Kauppinen *et al.*¹⁶⁾ The *k*, a resolution enhancement of 2.4 and the *s*, the half width at half height of the chosen line shape function of 6.5 cm⁻¹ were used. The *k* value of 2.4 was justified from the log(*S/N*) of approximately larger than 500. After self-deconvolution and a second derivative of deconvolution, the original overlapped peaks can be sepa-

rated, the second derivative of deconvolution peak position and its absorbance can then be picked and printed out using a peak picking program (interval spectra was 1.0 cm⁻¹ and tolerance was 1.0), which was used to calculate the quantity of secondary structure of the protein.

The spectra of the sheared 11S globulin under different heating temperatures of 25, 50, 80, and 90°C were obtained by the following procedure: the sample was cooled down to room temperature immediately after the shearing and thermal treatment. The shearing and thermal treatments were restricted to a shear rate of 384 sec⁻¹ and heating times and temperatures of 30 min at 50°C, 16 min at 80°C, and 8 min at 90°C, due to the limitation of the viscometer of 64 cPs. The samples were collected and then lyophilized and made into KBr pellets by mixing the lyophilized 11S globulin (2 mg) with KBr (10 mg) and pressing to form the pellet. The pellet was placed in the sample cell and scanning 200 times.

Results and Discussion

Effects of shear rate on apparent viscosity of 11S globulin

Figure 1 shows the relationships between shear rate and shear stress and the hysteresis curves resulted of soybean 11S globulin at 3, 5, and 10% in ionic strength 0.5, pH 7.6 phosphate buffer. The flow behavior index (*n*) and fluid consistency index (*b*) (dyn·s/cm²) calculated from the power law equation were 0.68 (*n*), 4.4 (*b*), and 0.50 (*n*), 2.4 (*b*) for 5% and 10% respectively. The flow behavior index (*n*) was less than one and caused a hysteresis loop

indicated the fluids have pseudoplastic flow, which means either a continuous breakdown or realignment of the molecules of 11S globulin along the streamline of flow or both, thus there was less resistance to flow while the shear

Fig. 1. Effects of Shear Rate on Shear Stress of 3, 5 and 10% 11S Globulin Solutions at 25°C, and on the Hysteresis Behavior.

rate was increasing.¹⁷⁾ However, the 3% solution was shear-independent and had Newtonian flow. The results indicated that the effect of concentration on apparent viscosity was pronounced. The 3% solution behaved as a Newtonian solution and became pseudoplastic when the concentration was higher than 5%.

Effects of shearing time on apparent viscosity of 11S globulin

The relationship between apparent viscosity and shearing time from 0 to 30 min of 3% and 20%, 5%, and 10% solutions at 25°C and a shear rate 9.6 to 384 sec⁻¹ is shown in Figs. 2, 3, and 4, respectively. For 3% 11S globulin, the apparent viscosity remained constant during the entire shearing period from 9.6 to 384 sec⁻¹. The fluid was time-independent

Fig. 2. Effects of Shearing Time on the Apparent Viscosity of 3% 11S Globulin Solution at a Shear Rate of 9.6–384 sec⁻¹ and 25°C, and of 20% 11S Globulin at a Shear Rate of 76.8, 384 sec⁻¹ and 25°C.

Fig. 3. Effects of Shearing Time on the Apparent Viscosity of 5% 11S Globulin Solution at a Shear Rate of 9.6–384 sec^{-1} and 25°C.

during the whole shearing period of 30 min as a Newtonian fluid at such low concentrations. For the 20% solution, shear thinning occurred in a very short time, less than 1 and 1.5 min for 384 sec^{-1} and 76.8 sec^{-1} shear rate respectively, then remained constant during the rest of the shear period. The cause for shear thinning at the beginning was that the particle size distribution was large and the associated particles had to break apart to a uniform dispersion right after the start of shearing thus decreasing the apparent viscosity. The time-independence may due to hydration of 11S globulin making system too concentrated to flow freely and rendering the apparent viscosity constant, as shown in

Fig. 2.

The 5% and 10% solutions showed similar flow behavior such as 1) Shear thinning at the beginning of shearing, comparing the same concentration solutions. 2) The lower the shear rate, the larger the apparent viscosity difference was observed. The apparent viscosity difference was the difference between the beginning and at the time of equilibrium apparent viscosity (EAV), which is a hydrodynamic equilibrium state. 3) The lower the shear rate, the longer the shearing time needed to reach the EAV at the same shearing rate. 4) The higher the concentration, the longer the shearing time needed to reach the EAV. 5) The higher the concentration, the higher the ap-

Fig. 4. Effects of Shearing Time on the Apparent Viscosity of 10% 11S Globulin Solution at a Shear Rate of 9.6–384 sec^{-1} and 25°C.

parent viscosity difference was observed. Hermansson⁵⁾ reported that the dispersion of promine-D (12%) was thixotropic; there was no difference between the flow curve before and after shearing and resetting was observed. This indicated that the structure of promine-D, broken down during shearing, was reversibly reformed. But Pradipasena and Rha¹⁸⁾ re-

ported that the relationship between viscosity and shearing time of β -lactoglobulin at protein concentrations of 10–30%, at a shear rate of 6856–17140 sec^{-1} , showed rheopectic properties. The rheopectic properties were explained by the fact that in shearing, protein molecules could be unfolded or uncoiled, thus changing or increasing the shape, size, and effective

Fig. 5. Effects of Shearing Time and Heating from 25°C at (1.4°C/min) to 85°C on Apparent Viscosity of 3, 5, 10, and 20% 11S Globulin Solutions at a Shear Rate of 384 sec^{-1} .

volume of the solute in solution. However, in this study, the fluid showed shear-thinning at the beginning of shearing. It can be explained because under shearing the hydrodynamic forces were large enough to break apart the linking force that originally maintained the associated molecules or particles. The reduction in size of the associated structural units by hydrodynamic force corresponds to a smaller resistance unit against the flow, thus decreasing the apparent viscosity with time.¹⁹⁾ In addition, the lower the shear rate, the smaller the shear energy to be dissipated. In other words, the magnitude of breaking the associated particles apart was small, therefore it needed a longer time to break apart the associated particles that is, it needed a longer time to reach the EAV state. Assuming the extent of hydrodynamic force was nearly equal to breaking apart the associated particles, the lower the shear rate, the larger the EAV was observed; the higher the concentration, the larger the numbers of linking forces, therefore it need more time to break apart or to reach the EAV, and a relative high apparent viscosity difference. The reasons for the time-independent flow properties after the shear thinning period may be inferred as (1) the soybean 11S globulin was more compact and stable, higher molecular weight molecule than β -lactoglobulin, (2) the shear rate and temperature used in this experiment were too low to cause any conformational change of 11S globulin, and (3) the equilibrium hydrodynamics state was obtained.³⁾

Effects of shearing time and temperature on apparent viscosity

Figure 5 shows the changes of apparent viscosity of 11S globulin with shearing time and temperature. The increasing in temperature was controlled by a constant heating rate of 1.4°C/min up to 85°C. For the 3% solution, the apparent viscosity didn't change during the first 30 min of shearing or temperature up to 70°C, then increased with the increase of temperature and shearing time although at a small magnitude. The increase may be attributed to

the change of 11S globulin due to the syneresis effects of hydration, shearing, and higher temperature, which induced the unfolding of 11S globulin or increased its axial ratio.^{15,20)} For the 10% solution, before 20 min or before the solution temperature reached 54.5°C, the apparent viscosity decreased with shearing time. The decrease in the apparent viscosity may due to: 1) the solvent viscosity decreasing with the increased temperature, 2) the tendency of 11S globulins to align themselves along the streamlines of flow with shearing time that resulting in a reduced shear stress with shearing time, or 3) the shearing force was large enough to break apart the associated molecules or particles resulting in a smaller resistance unit against the flow. However, after 20 min or after the solution temperature went above 54.5°C, the apparent viscosity significantly increased along with the elevation of temperature up to 82°C. The reasons for this significant increase were thought to be the syneresis effects described above plus the molecule entanglement factor, the interaction of shearing, high temperature, and concentration increasing the effective volume of 11S globulin to a point above the coil overlap concentration.²¹⁾ The 5% solution showed trends similar to the 10% solution but different in magnitude in the decrease or increase in the apparent viscosity, and different in the shearing time at which the shear thinning changed to shear thickening. The difference may due to the difference in effective volume in solution. However, for the 20% solution, the apparent viscosity was independent of shearing time and temperature. It might due to aggregates formed from highly concentrated swelled molecules at 25°C and later gel formed by the thermal activation of the protein sol to a gel as the temperature increased; the shear stress applied at this shear rate had no effect upon the system.

The apparent viscosity changes with increasing temperature and shearing time were most pronounced for the 10% solution among those shown in Fig. 5. Therefore 10% 11S globulin was further studied at constant temperatures of 25°C, 50°C, 80°C, and 90°C, shown in Fig.

Fig. 6. Effects of Shearing Time and Temperatures on the Apparent Viscosity of 10% 11S Globulin Solution at a Shear Rate of 384 sec^{-1} .

6. At 25°C , it was shear thinning during the first 4 min of shearing, then the change remained constant. At 50°C , the 11S globulin showed shear thinning during the first 8 min of shearing. After that, nevertheless, the increase in apparent viscosity as the shearing time increased was within 3 cPs. However, at 80°C and 90°C , the time to reach the limit of this viscometer, 64 cPs, was 16 and 8 min, respectively. The reasons for the sharp increase of apparent viscosity were the same as described

above.

Since the apparent viscosity of the 10% solution increased sharply at temperatures higher than 80°C , the apparent viscosity of 5% and 20% solutions at temperatures higher than 80°C and a shear rate of 384 sec^{-1} were studied for comparison. For the 5% solution, the way the apparent viscosity decreased and then increased was similar to the 10% solution. The reflection time occurred around 3 min and 1.5 min for 80 and 90°C , respectively,

Fig. 7. The Amide I and Amide II Spectra of Sheared 11S Globulin at a Shear Rate of 384 sec^{-1} and 25°C , Undeconvoluted Spectra (Bottom), Deconvoluted Spectra (Middle), and Second Derivative of Deconvoluted Spectra (top).

($k=2.4$ and $s=6.5 \text{ cm}^{-1}$ were used for the deconvolution.)

compared to 2 min and right after shearing for 10% in the same order. It reaffirmed the combined effects of temperature, shearing, and concentration on the conformational changes. Compared to progress heating in our previous study of 5% solutions, the reflection time occurred at shearing for more than 25 min. Because 11S globulin dissociating into subunits or unfolding shortened the reflection time as the temperature increased above 80°C .²²⁾ However, for the 20% solution, the apparent viscosity remained constant. It may due to gel forming at high concentrations and temperatures and the low shearing force used in this study could not affect the gel system.

Changes of secondary structure of protein molecules caused by the thermal and shearing effects

The reasons for the sharp increase in the apparent viscosity as the temperature increased to 80°C and 90°C as shown in Fig. 6 may be due to the unfolding of soy 11S globulin molecules. To elucidate the attributes of temperature and shear on the conformation, especial on the secondary structure of 11S globulin, shearing at 384 sec^{-1} and different temperatures was studied. Figure 7 shows the undeconvoluted (bottom), self-deconvoluted (middle), and second derivative of deconvoluted spectra of 11S globulin obtained from shear at 25°C and shear rate of 384 sec^{-1} . The pairs wavenumber of Amide I and Amide II

Table II. CHANGES OF INFRARED BANDS OF IIS GLOBULIN WITH DIFFERENT TEMPERATURE AND SHEAR AT A SHEAR RATE OF 384 sec^{-1} .

Conformation	Mode	Native		25		50		80		90	
		Amide I	Amide II	Amide I	Amide II	Amide I	Amide II	Amide I	Amide II	Amide I	Amide II
α -Helix	$v_{\parallel}(0)$					1649.3	1515.4	1650.3	1514.5	1649.8	1517.2
	$v_{\perp}(2\pi/n)$	1647.6	1540.7	1647.2	1540.2	1647.0	1538.7	1645.7 1648.5	1540.3	1645.2 1647.0 1648.3	1539.5
Antiparallel β -Sheet	$v_{\parallel}(0, \pi)$	1685.9	1531.1	1683.7	1530.1 1532.2	1683.5	1530.4	1684.0	1530.1 1531.2	1684.2 1686.3	1530.6
	$v_{\perp}(\pi, \pi)$	1667.0	1550.2	1668.0	1548.7	1667.8	1549.1	1667.2	1548.3	1666.4	1548.0
Parallel β -Sheet	$v_{\perp}(\pi, 0)$	1630.3	1551.7	1630.5		1633.2	1551.7	1632.7		1631.4	1548.2
		1633.6		1633.1						1633.8	1551.2
Random coil		1634.6	1518.7	1636.2	1518.6	1636.7	1525.5	1635.6	1519.2	1639.3	1521.4
		1639.8	1524.2	1639.2	1519.2	1640.2	1535.1	1638.2	1521.6	1653.4	1523.3
		1644.2	1525.3	1642.9	1525.8	1644.3	1536.5	1640.5	1524.8	1655.4	1534.5
		1651.8	1534.3	1643.3	1533.6	1654.9	1544.2	1657.5	1533.6	1658.9	1543.9
		1653.2	1537.2	1657.2	1544.5	1659.3		1661.5	1545.4	1660.4	
		1657.5	1545.8	1659.6		1662.4		1663.2		1665.6	
		1659.4		1660.8		1665.4		1671.1		1679.7	
		1675.0		1665.3		1679.8		1674.9		1681.3	
		1679.7		1672.3		1681.9		1679.9			
		1682.8		1678.1							
				1682.1							

^a The positions of wavenumber are verified by self-deconvolution spectra and second derivative of deconvolution spectra, which are printed out directly from the computer program.

^b The selecting of the coupling wavenumber combination are referred to in Miyazawa and Blout (1961) in ± 2 wavenumber.

^c 25, 50, 80, 90 are shearing temperature ($^{\circ}\text{C}$) at a shear rate of 384 sec^{-1} .

Table III. CHANGES OF SECONDARY STRUCTURE OF 11S GLOBULIN IN PHOSPHATE BUFFER WITH TEMPERATURE AT A SHEAR RATE OF 384 sec^{-1}

Temperature (°C)	Native	25	50	80	90
α -Helix	7.9	7.8	13.8	19.6	28.1
β -Sheet	28.3	26.6	27.6	25.4	29.4
Order structure	36.2	34.4	41.4	45.0	57.5
Random coil (unorder structure)	63.8	65.6	58.6	55.0	42.5

used to calculate the quantitative changes of secondary structure were also labelled on the figure. The other spectra of shearing at 384 sec^{-1} and heating at 50, 80, and 90°C were omitted, but pairs wavenumbers were all listed in Table II. The percentages were 7.8, 13.8, 19.6, and 28.1% of α -helix; 26.6, 27.6, 25.4, and 29.4% of β -sheet and 65.6, 58.6, 55.0, and 42.5% of random coil for heating at 25, 50, 80, and 90°C respectively (Table III). It indicated that the higher the temperature that 11S globulin was subjected to, the more α -helix and the less random coil conformation of the molecules were formed. Especially, for the 90°C one, the percentage of α -helix was 28.1% which is 3.5 times larger than the value of the native one, but the percentage of β -sheet changes was not significant. The increase of α -helix should be at the expense of random coil conformation. The change of α -helix was mainly the result of the vibration mode $\nu_{\parallel}$ (0) indicated by the stronger 1650 , 1516 cm^{-1} and 1647 , 1540 cm^{-1} peaks shown in the spectra at higher temperature. The data was consistency with the result of Damodaran²³⁾ who reported CD spectrum of 11S globulin supernatant from the gel preparation had a shoulder at 222 nm and a trough at 210 nm , characteristic of α -helix structure. It indicated that the heated unfolded 11S globulin in 0.5 M NaCl solution partially refolds to an ordered structure containing more α -helix than β -sheet conformation during the cooling phase. Suresh Chandra²⁴⁾ also reported that the α -helix structure was increased by increasing temperature. Therefore, the apparent viscosity increasing drastically was the increase of the flow re-

sistance that was induced by increasing axial ratio and/or molecular entanglements due to the more extended and stretched molecules result from the increase of α -helix at the expense of random coil structure at 90°C . Koshiyama *et al.*²⁵⁾ reported the denaturation temperature of soy 11S globulin in ionic strength 0.5, pH 7.6 buffer to be 89.5°C , considering that the time to reach the limit of the viscometer was 8 and 16 min for 90 and 80 respectively. The difference mainly reflected the syneresis effect of shear/the thermal denaturation (unfolding, dissociation) and shear/the thermal unfolding respectively. Therefore, increasing the temperature to higher than 89.6°C will cause a more significantly effect on increasing the apparent viscosity, gelling formation capacity, axial ratio, *etc.* On the other hand, comparing the secondary structure changes of native 11S globulin and that sheared at 25°C in Table III, the percentage of the random coil structure of the sheared one was larger than the native one, the result implying that the hydration and shearing at 25°C , 384 sec^{-1} may cause a quaternary or tertiary structure change and result in some slight relative increase or decrease change in order and random coil structure changes.

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