GELATION OF ALFALFA SOLUBLE LEAF PROTEINS

B. P. Lamsal, R. G. Koegel, S. Gunasekaran

ABSTRACT. Various alfalfa soluble leaf protein concentrates were prepared by freeze-drying acid-precipitated proteins (pH 3.5), resolubilized proteins (pH 7), and membrane-concentrated clarified alfalfa juice. Dilute leaf protein solutions were prepared by dissolving these concentrates in water. Storage modulus (G') of soluble leaf protein solutions as they gel was monitored with a cone-and-plate probe during temperature sweep from 25°C to 90°C and back to 25°C. G' values during the heating phase ranged from 5 to 120 Pa for 1.6% to 4% protein solutions. They increased to 3 kPa for 4% protein solution while cooling back to 25°C. Although resulting gels were weaker, they exhibited distinct gelation temperatures of 77°C, 68°C, 66°C, and 60°C for 1.6%, 2.7%, 3.6%, and 3.9% acid-precipitated SLP solutions, respectively. Apparent viscosities of solutions were also studied as a function of temperature and shear rate. Similar tests were conducted for soluble leaf protein and whey protein isolate biopolymer systems at different ratios. At 1:3 ratio, leaf proteins interacted with whey proteins to increase G' during heating and cooling. The compressive failure force of standing gels produced from soluble leaf proteins and whey protein mixtures correlated well with their G' values from solution rheology. Results indicated that stable soluble leaf protein gels could be made in combination with whey protein isolates, presenting the possibilities to incorporate them in food and non-food systems.

Keywords. Gelation, Rheology, Soluble leaf proteins, Steady shear, Whey protein isolate.

Leaf proteins are one of the promising sources of protein. They contain one of the purest and highly nutritive components, ribulose 1,5-bisphosphate carboxylase/oxygenase (rubisco), which is contained in the stroma of chloroplasts and is an enzymatic protein that plays a part in photosynthesis. Rubisco is one of the two main components, accounting for up to 70% of cytoplasmic soluble leaf proteins (SLP) (Douillard and de Mathan, 1994). It constitutes up to 25% of total protein in green leaves (Barbeau and Kinsella, 1988) and is an oligomeric protein having a molecular weight (MW) of about 550 kDa in its most prevalent L8S8 structural form. Rubisco is composed of two non-identical types of subunits: eight large subunits (MW about 55 kDa), and eight small subunits (MW about 14 kDa) (Johal and Chollet, 1980; Barbeau and Kinsella, 1988; Lamsal, 2004). In the L8S8 form, the protein molecule has a sedimentation coefficient ($S_{20,W}$) close to 18.5 and a diffusion coefficient ($D_{20,W}$) of 2.97 (Barbeau and Kinsella, 1988). SLP concentrates, consisting mostly of rubisco, have commonly been prepared by differential heating ($55^\circ C$ to $60^\circ C$) of alfalfa juice followed by centrifugation. The clear brown supernatant containing soluble proteins can be heat-coagulated to yield a light-colored bland protein concentrate, more than 90% protein on dry basis (Miller et al., 1975). The range of application in the food industry for such heat-coagulated concentrate is limited by its low solubility. Precipitation of protein through pH reduction (pH 3.5) at low temperature ($2^\circ C$) was investigated as an alternative to the heat-coagulation process (Miller et al., 1975). Raising the pH to 7.0 redissolved most of the protein precipitated at pH 3.5. The potential of SLP in general and rubisco in particular as a human food ingredient have been investigated and reviewed by various researchers (Douillard and de Mathan, 1994; Barbeau and Kinsella, 1988; Kohler and Knuckles, 1977). Besides being nutritionally competitive with other proteins, SLP was reported to possess an excellent array of functional properties like solubility, water and fat absorption, emulsification, foaming and whipping, and gelation (Wang and Kinsella, 1976; Knuckles and Kohler, 1982; Barbeau, 1990).

Gelation is one of the most important functional properties of food proteins, forming a three-dimensional matrix or peptide network that provides a structural basis for holding water, flavors, and sugars (Kinsella, 1976; Turgeon and Beaulieu, 2001). Many important foods have proteins, starches, or pectins as gelling agents, whey proteins being one of the most widely used ones. Such gel formation usually requires prior heating of proteins to cause at least partial denaturation or unfolding of its polypeptide chains (Renkema et al., 2002). In the early stages of thermal denaturation, most protein molecules begin to unfold due to weakened secondary and tertiary structures. If protein-protein interactions lead to formation of a three-dimensional network capable of entraining water molecules, a gel can form. Small-amplitude oscillatory shear (SAOS) testing has traditionally been used to characterize such gel formation, for example, interactions of $\beta$-lactoglobulin and bovine serum albumin with $\beta$-lactoglobulin (Hines and Foegeding, 1993), egg white proteins (Ould Eleya and Gunasekaran, 2002), and...
Materials and Methods

Preparation of SLP Concentrates

Clarified alfalfa juice (CAJ) was prepared as per Lamsal et al. (2003) and Lamsal (2004). The pH of CAJ was 5.8 to 6.0, and SLP were acid-precipitated (pH 3.5) with addition of 1 N HCl (Miller et al., 1975). Precipitate protein was then centrifuged at 12000 g and 4°C for 10 min (model J2-21, Beckman, Palo Alto, Cal.) and washed three times with pH-adjusted distilled water. During precipitation and washing, the juice and protein were kept in an ice-water bath to minimize proteolytic activities. One half of the washed precipitate at pH 3.5 was freeze-dried (Freezemobile 35ES, Virtis, Inc., Gardiner, N.Y.) and denoted AP1 (3 July 2002 preparation) or AP2 (10 July 2002 preparation). The other half of the precipitate was resolubilized by raising the pH to 7.0 with the addition of 0.1 N NaOH. This resolubilized protein slurry was then centrifuged at 37000 g for 20 min and decant freeze-dried. These concentrates were called RP1 (3 July 2002 preparation) or RP2 (10 July 2002 preparation).

Part of the 3 July 2002 CAJ preparation was concentrated in a custom-made rotary ultrafiltration device (Lamsal and Koegel, 2005) using a new 10 kDa molecular weight cut-off (MWCO) membrane (PBGC membrane, Millipore Corporation, Bedford, Mass.). The filter was operated at a feed pressure of 242 kPa (35 psi), membrane clearance of 5 mm, and rotor speed of 16.7 rev·s⁻¹. The retentate flow rate was maintained at one-fourth of permeate flow rate, and by the end of 10 h run, about 3 L of permeate and 750 mL of retentate was collected. The retentate was then freeze-dried and named UFR. Total nitrogen (TN) and non-protein nitrogen (NPN) in samples were measured in a combustion-type nitrogen analyzer (FP-2000, Leco Corp., St. Joseph, Mich.). NPN was taken as protein soluble in 20% trichloroacetic acid (TCA), and protein-nitrogen was obtained by difference (Eakin and Singh, 1978; Knuckles et al., 1975). Protein nitrogen multiplied by 6.25 (Knuckles et al., 1975; Knuckles et al., 1980) gave true protein content of the samples, which on dry basis were 72% for AP1, 64% for AP2, 63% for RP1, 52% for RP2, and 35% for UFR.

Rheological evaluations

Small amplitude oscillatory shear (SAOS) tests were performed with a dynamic rheometer (Bohlin CVO, Bohlin Rheologies Inc., Cranbury, N.J.) using cone-and-plate geometry (cone angle = 4°; plate diameter = 40 mm). G' and G" were monitored while protein samples were heated from 25°C to 90°C and subsequently cooled to 25°C with a temperature ramp of 5°C min⁻¹. The applied strain was 0.5% at 1 Hz frequency. Solutions at various SLP concentrations (1.6% to 3.95% true protein) were prepared at pH 7.0. Samples AP1 and AP2 were insoluble in water, as they were at pH 3.5 when prepared, so pH was raised to 7.0 with 0.1 N NaOH to solubilize them (Miller et al., 1975). All reconstituted samples were centrifuged at 37000g for 20 min, and decant was used for testing. True protein content of the sample decant was determined at this point, as described earlier.

Apparent viscosity of the samples was monitored against a shear rate sweep of up to 1000 s⁻¹ at 20°C with the same cone-and-plate probe. The flow curves were fitted with a power law model for non-Newtonian fluids: \( \tau = K\gamma^n \) where \( \tau \) is shear stress, \( K \) is consistency coefficient, \( \gamma \) is shear rate (s⁻¹), and \( n \) is flow behavior index. Apparent viscosity variation with temperature was measured at the constant shear rate of 1 s⁻¹ while heating the protein solution from 25°C to 90°C. Three to four measurements were performed.

The effect of salt addition to SLP gelation was studied with reconstituted solution from concentrates AP2 and UFR. UFR samples when dissolved in water had pH of about 6.0, which was raised to pH 7.0 with 0.1 N NaOH. The final true protein concentration obtained with the UFR solution for this experiment was 1.7%, to which 0.1 M NaCl was added. Similarly, the reconstituted AP2 protein solution was at 2.3% true proteins, to which 0.1 M and 0.2 M NaCl were added. G’ evolution with temperature and apparent viscosity with temperature and shear rate were recorded for these salt-added solutions.

Steady Shear Rheology of Mixtures of SLP with Other Biopolymers

A mixture of 11% whey protein isolate solution (WPI) (90% protein, Davisco Foods Intl., Eden Prairie, Minn.) and 1.9% SLP solution (referred to as mixture I) and a mixture of 13.2% WPI solution and 2.2% SLP solution (referred to as mixture II) were prepared at SLP:WPI ratios of 1:0, 3:1, 1:1, 1:3, and 0:1. Variation in G’ with temperature and variation in apparent viscosity with temperature and shear were monitored, as described earlier. Standing gels also were prepared from mixture I and II solutions at the above SLP:WPI ratios in stainless steel molds (10 mm dia × 40 mm long) by heating in a 90°C water bath for 1 h (Ould Eleya and Gunasekaran, 2002). They were then cooled in a 20°C water bath for 10 min and left overnight at 4°C. Uniaxial compression at 80% strain and crosshead speed of 1 mm s⁻¹ (Synergie 200, MTS Corp., Cary, N.C.) was carried out on 6 to 7 mm long gel specimens placed with their cross-section facing the probe. The maximum compressive force at failure
was considered the gel strength for comparison purposes under prevailing conditions (Lee et al., 1997). Before testing, the gels were equilibrated to ambient temperature for 1 h.

**Statistical Evaluations**

Means were compared with the GLM procedure in the SAS statistical software (SAS Institute, Inc., Cary, N.C.) at p < 0.05 where appropriate.

**Results and Discussions**

**Steady Shear of SLP Solutions**

Figure 1 shows a representative plot of $G'$ as a function of temperature for dilute SLP solutions during heating and cooling. Upon heating from 25°C to 90°C, storage modulus $G'$ remained small and almost constant until gelation temperature ($T_{gel}$) and then increased rapidly, signifying transition from liquid-like (sol) to solid-like state (inset graph). $T_{gel}$ was judged by the abrupt increase in the modulus or a sudden decrease in phase angle. Gelation temperature corresponds to the temperature at which $G'$ increases and becomes greater than background noise, which is one of the common methods of gel point detection in the absence of crossover between $G'$ and $G''$ (Ould Eleya and Gunasekaran, 2002; Matsumura and Mori, 1996; Ikeda and Nishinari, 2001). $G'$ of gels further increased during subsequent cooling from 90°C to 25°C. This increase in $G'$ during cooling is typical of protein gels and has also been observed for other protein systems like egg white proteins (Ould Eleya and Gunasekaran, 2002) and whey proteins (Cooney et al., 1993). This is generally attributed to the consolidation of attractive forces such as van der Waals and hydrogen bonding between proteins within the gel primary network that forms during gelation.

The $G'$ values at the end of heating (90°C) and cooling (25°C), and $T_{gel}$ for various SLP concentrations, among other things, are presented in Table 1. The $T_{gel}$ values obtained are within reported gelation temperatures for alfalfa SLP. Knuckles and Kohler (1982) obtained gels from purified alfalfa protein at different concentrations by heating at 72°C. The $T_{gel}$ of 77°C we observed for 1.6% SLP solution was just above the denaturation temperature (76.2°C; Tomimatsu, 1980) for alfalfa rubisco. Gelation temperature clearly was a function of protein concentration. $T_{gel}$ decreased as protein concentration increased. A similar trend for egg white proteins was reported by Ould Eleya and Gunasekaran (2002).

Availability of more protein in the solution for increased protein-protein interaction is thought to be responsible for this effect. For sample UF-VE, the true protein concentration was only 0.1%, but its $T_{gel}$ was a low 49°C and its $G'$ values were only 118 and 316 Pa during heating and cooling, respectively. The total crude protein for this sample (UF-VE) was 13.9%, but most of it (13.8%) was non-protein nitrogen; thus, only 0.1% of proteins were true protein that was able to gel. This sample was prepared by concentrating ultrafiltration retentate in a vacuum evaporator, and soluble sugars present in the sample also got concentrated during the process. Some interaction probably occurred between proteins and polysaccharides within the sample to start gelation earlier. It was generally observed that dilute SLP produced a weaker gel system. At relatively higher protein concentration of 7.1%, sample R-VE produced firm gel at the end of cooling ($G'$ about 36 kPa, Table 1).

The $G'$ for SLP solutions increased exponentially with protein concentration ($c$) following the relationship: $G' = k c^n$, where $n$ is the power law exponent and $k$ is a constant. The first five sample $G'$ values at 25°C in Table 1 and their protein concentrations were fitted with this relationship. The remaining two $G'$ values in Table 1 were not included as they were prepared differently (vacuum concentration). Figure 2 shows

![Figure 1. Typical storage modulus ($G'$, Pa) and temperature (°C) plots for SLP solutions during heating and cooling. Large graph shows replicate runs for 3.9% SLP solution. Inset shows dramatic rise in $G'$ at the onset of gelation.](image)

<table>
<thead>
<tr>
<th>SLP</th>
<th>SLP Concentration (% TP$^{[b]}$)</th>
<th>$T_{gel}$ ±SD$^{[c]}$ (°C)</th>
<th>Storage Modulus, $G'$ ±SD (Pa) at 90°C</th>
<th>Storage Modulus, $G'$ ±SD (Pa) at 25°C</th>
<th>Viscosity Transition$^{[d]}$ ±SD (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RP2</td>
<td>1.6</td>
<td>77 ±0.5 a</td>
<td>5 ±0.6 a</td>
<td>130 ±30 a</td>
<td>84 ±0.3 a</td>
</tr>
<tr>
<td>AP2</td>
<td>2.7</td>
<td>68 ±0.4 b</td>
<td>49 ±0.4 b</td>
<td>1224 ±331 bc</td>
<td>75 ±0.5 b</td>
</tr>
<tr>
<td>AP2</td>
<td>3.6</td>
<td>64 ±0.8 c</td>
<td>148 ±34 c</td>
<td>1881 ±276 bd</td>
<td>70 ±0.1 c</td>
</tr>
<tr>
<td>RP2</td>
<td>4.0</td>
<td>66 ±0.7 d</td>
<td>120 ±1 cd</td>
<td>2910 ±714 d</td>
<td>69 ±0.6 c</td>
</tr>
<tr>
<td>AP2</td>
<td>3.9</td>
<td>60 ±0.5 e</td>
<td>100 ±2 d</td>
<td>1523 ±185 bc</td>
<td>67 ±0.4 d</td>
</tr>
<tr>
<td>R-VE$^{[e]}$</td>
<td>7.1</td>
<td>49 ±0.2 f</td>
<td>2531 ±49 e</td>
<td>36199 ±1599 e</td>
<td>46, 54 ±0.4 e</td>
</tr>
<tr>
<td>UF-VE$^{[f]}$</td>
<td>0.1</td>
<td>52 ±0.8 g</td>
<td>118 ±13 cd</td>
<td>316 ±45 a</td>
<td>62 ±2 f</td>
</tr>
</tbody>
</table>

$^{[a]}$ Means within the same column followed by different letters are statistically different (p < 0.05, n = 3).

$^{[b]}$ TP = true protein, i.e., crude protein adjusted for non-protein nitrogen.

$^{[c]}$ SD = standard deviation of mean.

$^{[d]}$ Temperature at which solution viscosity increased rapidly.

$^{[e]}$ R-VE was prepared by vacuum evaporation of redissolved protein of different date.

$^{[f]}$ UF-VE was prepared by vacuum evaporation of ultrafiltration retentate of different date.
the plot; the value of exponent $n$ was 3. The coefficient of determination ($R^2$) of the fit was 0.92, which is good, given that the experiments were conducted on different samples on different dates. The value of exponent $n$ is a function of the gel structure and has been reported between 2.2 to 2.8 for most protein systems (Ould Eleya and Gunasekaran, 2002; Hagiwara et al., 1998). However, Weijers et al. (2002) explain that the value of $n$ varies depending on such factors as pH and salt concentration. Higher values of $n$ indicate a relatively short and compact gel structure needing a higher concentration of protein to form gels. Moderate values of $n$ (around 3, which is still higher than typical $n$ values for linear particles) suggest that the linear aggregates are either branched or organized in large clusters with a low density. Increase in both aggregate size and compactness is accompanied by a decrease in the exponent $n$ (Weijers et al., 2002). Based on the exponent value we obtained, we speculate that SLP gels could be considered to consist of moderate-sized aggregates that are not very compact or loose.

The observed rapid increase in solution apparent viscosity in the vicinity of gelation temperature was consistent with the increase in sample storage modulus, as seen in table 1. Even though $T_{gel}$ and the viscosity-transition temperatures were not identical, they followed the similar trend with respect to solution protein concentration. Dilute protein solution required higher temperatures to attain viscosity-transition. Two distinct viscosity-transition points (46°C and 54°C) were seen for sample R-VE, which was relatively higher in protein concentration (7.1%). This recognizable spike in viscosity at around 46°C, which could be due to early-precipitating proteins in alfalfa SLP, was not seen for comparatively dilute samples. Possible trace amount of early-precipitating proteins present in dilute samples might be one of the reasons.

Figure 3 shows the power law fit for apparent viscosity and shear rate data for some SLP samples. A very good fit was found for samples UF-VE (0.1% protein solution) ($R^2 = 0.97$) and API (1.9% protein solution) ($R^2 = 0.95$), whereas for samples UFR (2% protein solution) ($R^2 = 0.7$) and R-VE (7.1% protein solution) ($R^2 = 0.32$), the curve fit was rather poor. The solution preparation method might have played a role in the poor fit for these samples. The general trend showed shear-thinning behavior (consistency coefficient $K > 0$, $0 < n < 1$) as the shear rate increased from 1 to 1000 s$^{-1}$, i.e., viscosity decreased with increase in shear. The viscosity values ranged from 0.032 to 0.33 Pa·s at 1 s$^{-1}$, and from 0.009 to 0.15 Pa·s at 1000 s$^{-1}$ for the sample protein solutions tested.

Initial viscosities were similar irrespective of protein concentration, except for UF-VE. Even though sample UF-VE had lower protein concentration, it had higher viscosity values at all shear rates, which could have been affected by the sample preparation method. Sample UF-VE was prepared by vacuum evaporation of an ultrafiltration retentate. While the true protein content of the retentate was low, it also contained other soluble components of CAJ, notably sugars. Vacuum evaporation concentrated the sugars as well as the proteins, resulting in a thicker solution. On the other hand, even though the protein content of R-VE was 7.1% and showed very high $G'$ during heating and cooling (table 1), its apparent viscosities were similar to those of the other dilute protein samples. This is most likely because of the absence of sugar in sample, as it was a vacuum-concentrated redissolved protein after repeated washing of acid-precipitated protein.

**EFFECT OF SALT ON SLP GELATION**

The results of the salt experiments are summarized in table 2. Addition of 0.1 M NaCl slightly aided in gelation of the 1.7% UFR samples, increasing $G'$ at the end of the heating and cooling cycle (from 84 Pa to 625 Pa). In addition, it caused $T_{gel}$ to increase, but the viscosity-transition temperature remained same. The same was not true for the 2.3% AP2 samples. In fact, addition of salt to the AP2 samples at 0.1 M and 0.2 M caused the samples not to gel. This was supported by phase angle observations, which varied very little for the AP2 samples (Lamsal, 2004). Initial pH content may have been one of the reasons behind such an opposite effect of salt addition on SLP gelation. SLP concentrate AP2 was prepared by modifying the pH of CAJ to 3.5 and freeze-drying at that pH. Test samples were then prepared by raising the pH to 7.0 with 0.1 N NaOH. As the isoelectric point (pI) of SLP is about 4.5 (Barbeau and Kinsella, 1988), proteins would be negatively charged at neutral pH. Addition of salt would increase the electrostatic repulsion between the protein par-

![Figure 2](image-url) Figure 2. Power law relationship between SLP concentration (%) and storage modulus $G'$ at 25°C at the end of the heating and cooling cycle.

![Figure 3](image-url) Figure 3. Power law fit for viscosity-shear rate data at 25°C for various SLP samples: diamonds = sample UF-VE (ultrafiltered vacuum evaporated, 0.1% protein solution, $n = 0.85, R^2 = 0.97$); squares = sample R-VE (redissolved vacuum evaporated, 7.1% protein solution, $n = 0.92, R^2 = 0.32$); triangles = sample UFR (ultrafiltered retentate, 2% protein solution, $n = 0.62, R^2 = 0.70$); and open circles = sample AP (acid precipitated, 1.9% protein solution, $n = 0.41, R^2 = 0.95$).
Table 2. Gelling temperature (T_{gel}), storage modulus (G’), and viscosity-transition temperature for SLP solutions with and without salt added.[a]

<table>
<thead>
<tr>
<th>SLP</th>
<th>NaCl Concentration (M)</th>
<th>T_{gel} ±SD[b] (°C)</th>
<th>Storage Modulus, G’ ±SD (Pa) at 90°C</th>
<th>Viscosity Transition[c] ±SD (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.7% UFR</td>
<td>0</td>
<td>67 ±1 a</td>
<td>11 ±1 a</td>
<td>84 ±18 a</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>71 ±1 b</td>
<td>262 ±10 b</td>
<td>625 ±154 b</td>
</tr>
<tr>
<td>2.3% AP2</td>
<td>0.1</td>
<td>Not observed</td>
<td>No gelation</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>Not observed</td>
<td>No gelation</td>
<td>---</td>
</tr>
</tbody>
</table>

[a] Means within the same column followed by different letters are statistically different (p < 0.05, n = 3).
[b] SD = standard deviation of mean.
[c] Temperature at which solution viscosity increased rapidly.

ticles. This probably resulted in no gelation altogether for the AP2 samples. On the other hand, UFR was the freeze-dried ultrafiltration retentate at near-neutral pH (6.0), and not much base was needed to raise the pH to 7.0. Salt addition helped moderately in gelation in this situation.

**Mixed Biopolymer SLP/WPI Systems**

Table 3 shows T_{gel}, G’ during heating (90°C) and cooling (25°C), and viscosity-transition temperatures for mixture I and mixture II protein solutions at different SLP:WPI ratios. Soluble leaf proteins had gelation temperatures (78°C and 77°C for 1.9% and 2.2% protein solutions, respectively) compared to the whey proteins gelation temperature (83°C for 13.2% protein), even though the WPI solution had higher protein content. This is probably due to the fact that SLP have relatively lower denaturation temperatures of around 76°C (Tomimatsu, 1980), as opposed to whey proteins, gels of which have been prepared by heating protein solution beyond 80°C (Chantrapornchai and McClements, 2002; Verheul and Roefs, 1999b). The principal component of WPI, β-lactoglobulin, is reported to gel above 80°C (Zayas, 1997). As for the mixtures, T_{gel} was not clearly detected for mixture I at an SLP:WPI ratio of 1:1, probably because the protein concentration (6.4%) was too low for gelation to occur. Even then, at this ratio, the mixture became progressively solid-like during heating, as was indicated by the falling phase angle values, from about 10° at 25°C to about 1° at 90°C (Lamsal, 2004). During cooling to 25°C, the phase angle increased (to about 11°), as did the G’ value (from 2 Pa to 4186 Pa). Similar gelation behavior was seen for mixture I at the 1:3 ratio, which gelled at lower T_{gel} (71°C) compared to the pure SLP or WPI solutions at a given concentration. This is possibly indicative of protein-protein interaction and the synergistic effect of SLP addition to the WPI solution, which requires gelation temperatures above 80°C. The viscosity-transition temperature for mixture I was not observed except at the 1:0 ratio, again probably owing to mixture protein content. For mixture II, as the SLP:WPI ratio increased, the mixture showed progressive decrease in G’ during heating and cooling at all ratios. No synergistic effect between SLP and WPI proteins was observed at these ratios. WPI itself is capable of producing strong gels at this concentration, and SLP had a detrimental effect on G’ values, resulting in softer gels. The apparent viscosity for these mixtures showed a rapid increase in the vicinity of T_{gel} at all ratios. Mixtures I and II both showed typical shear-thinning behavior at all ratios, with viscosity approaching almost zero when they were subjected to shear rates up to 1000 s⁻¹ (Lamsal, 2004).

Figure 4 shows compressive failure forces of SLP/WPI mixed gels prepared in the mold at different ratios. WPI gel at 11% protein was very soft and almost sticky, which did not show failure, but the compressive force was much smaller (2.4 N) at 80% strain. Contrarily, the 13.2% protein WPI gel produced the strongest gel (compressive failure force 36 N), which also did not show failure up to 80% strain. The strength of the mixed gels decreased with the increase in SLP proportion in both mixtures, except for mixture I at an SLP:WPI ratio of 1:3, at which the gel offered more compressive force at failure (12.32 N), indicating synergistic effects of mixing the proteins. Mixed gels were crumbly for both mixtures and more susceptible to failure, as was indicated by lower failure strains. Mixture I gel failure strains were 0.77 and 0.61 at SLP:WPI ratios of 1:3 and 1:1, respectively, whereas mixture II gel failure strains were 0.73, 0.7 and 0.53 for 1:3, 1:1, and 3:1 ratios. This trend in gel strength for mixed protein system was consistent with the observed variation in storage modulus at different SLP:WPI ratios (table 3). In fact, when G’ values at 25°C from table 3 for mixture II were plotted against respective compressive failure forces for standing gels at respective protein ratios, a

Table 3. Gelling temperature (T_{gel}), storage modulus (G’), and viscosity-transition temperatures for SLP/WPI mixtures.[a]

<table>
<thead>
<tr>
<th>Sample</th>
<th>SLP/WPI Mixture[b]</th>
<th>T_{gel} ±SD[c] (°C)</th>
<th>Storage Modulus, G’ ±SD, (Pa) at 90°C</th>
<th>Viscosity Transition[d] ±SD (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VR</td>
<td>% TP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixture I</td>
<td>1.0</td>
<td>1.9</td>
<td>78 ±1 a</td>
<td>2 ±0 a</td>
</tr>
<tr>
<td>(1.9% SLP, 11% WPI)</td>
<td>1.1</td>
<td>6.4</td>
<td>ND[e]</td>
<td>4186 ±629 b</td>
</tr>
<tr>
<td></td>
<td>1.3</td>
<td>8.7</td>
<td>71 ±0.2 b</td>
<td>3 ±2 a</td>
</tr>
<tr>
<td>Mixture II</td>
<td>1.0</td>
<td>2.2</td>
<td>77 ±1 a</td>
<td>3 ±0.3 a</td>
</tr>
<tr>
<td>(2.2% SLP, 13.2% WPI)</td>
<td>1.1</td>
<td>7.7</td>
<td>87 ±0.2 c</td>
<td>52 ±2 b</td>
</tr>
<tr>
<td></td>
<td>1.3</td>
<td>10.4</td>
<td>84 ±0.4 d</td>
<td>351 ±2 c</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>13.2</td>
<td>83 ±0.4 d</td>
<td>454 ±5 d</td>
</tr>
</tbody>
</table>

[a] Means within the same column followed by different letters are statistically different (p < 0.05, n = 3).
[b] VR = volume ratio (SLP:WPI); TP = true protein, i.e., crude protein adjusted for non-protein nitrogen.
[c] SD = standard deviation of means.
[d] Temperature at which solution viscosity increased rapidly.
[e] ND = not detected.
linear trend was seen, with a correlation coefficient (R^2) of 0.95 (plot not shown).

SLP gels prepared in the mold at neutral pH were opaque and dark brown in color, whereas WPI gels were clear and transparent. Their mixed gels were opaque and cream colored at neutral pH. The appearance of globular protein gels depends on the conditions of gelation, such as pH, ionic species and strength, heating temperature, etc. Transparent or translucent gels are produced when the proteins form a network by connecting long molecules at points, like a string of beads, as a result of relatively strong electrostatic repulsion between molecules. When proteins are thought to assemble in this type of gel structure, as they only have a limited number of hydrophobic patches on their surface (Chantrapornchai and McClements, 2002). Particulate networks are formed when the electrostatic repulsion between the molecules is relatively weak, i.e., when the proteins have a low net charge near their isoelectric point, or when the solution's ionic strength is high enough to screen electrostatic interactions. In such cases, protein molecules connect randomly at junction zones and are opaque (Chantrapornchai and McClements, 2002; Matsumura and Mori, 1996). We speculate that such particulate gels may have formed with the SLP and SLP/WPI mixed systems, as they were opaque. Sensory analysis of the SLP and SLP/WPI gels, while an important property to characterize, was not the focus of this research.

**CONCLUSIONS**

Temperature sweep data showed that gelation temperature of dilute SLP solutions varied from 60°C to 77°C depending on protein concentration. Similar trends were seen with a rapid increase in viscosity of SLP solutions during heating. A synergistic effect of mixing SLP with WPI was seen for 1.9% SLP mixed with 11% WPI at a 1:3 ratio. For other protein mixtures at various ratios, storage modulus G’ progressively decreased with an increase in the SLP proportion in the mixture. SLP solutions and mixtures with WPI all showed shear-thinning behavior. Self-standing gels were also prepared in molds and tested for compressive failure, exhibiting a clear correlation with G’ values for the same solutions.

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**REFERENCES**


