

Rheological Characterization of a Novel Functional Food: Tomato Juice with Soy Germ

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The rheological properties of tomato juice containing 1.5% soy germ were compared to plain tomato juice with and without soy protein isolate. This novel product was developed to provide a delivery system of carotenoids, soy protein, and significant isoflavone content without compromising the perceived juice characteristics of tomato product. Rheological tests depicted physical gel characteristics for all three products. Dynamic tests as a function of temperature showed that the stability and the compatibility between tomato juice and soy germ were higher as compared to soy protein isolate. The hydrophobic and electrostatic interactions between pectin and protein in the tomato soy protein isolate system were weakened as the temperature was increased. In the case of tomato juice with soy germ, the viscosity did not change during heating. The addition of soy germ increased the viscosity of tomato juice reinforcing the entire system without major qualitative effects on the rheological properties of plain tomato juice.

KEYWORDS: Tomato juice; soy germ; dynamic and steady rheology; temperature

INTRODUCTION

Functional foods are defined as products providing health benefits beyond their contribution to nutrient requirements (1). Tomatoes and soy are two foods that possess biologically active components that may significantly impact health. In part, the health-promoting activity associated with soy and tomato consumption has focused upon isoflavones and carotenoids (especially lycopene), respectively, although other components may prove to be important (2, 3). At the present time, the potential health benefits of soy and tomato products are undergoing extensive investigation as individual foods (4, 5). However, the impact of combining these foods into new products and characterizing their physicochemical properties has not been adequately investigated.

The viscosity of plain tomato juice (TJ) is highly dependent on the high molecular weights of water soluble pectins and their degrees of esterification (6, 7). A high degree of esterification of pectin is favored by hot break thermal treatment and affects functional properties of TJ by forming a network where smaller particles are physically entrapped (8). Furthermore, highly esterified pectins in aqueous solutions form intermolecular associations governed by hydrogen bonds and hydrophobic interactions (9). Regardless of the degree of esterification of the pectin, TJ, like other tomato products, exhibits a shear thinning behavior and a slight thixotropy due to the breakdown of noncovalent bonds related to pectic links (10, 11).

In the selection of the appropriate soy ingredient used to manufacture new products, an important factor is the isoflavone

contribution of that ingredient (2, 12). The concentration of isoflavones found in soy protein isolate (SPI) is often compared with that found in soy germ (SG). SG is the hypocotyl of the soybean (13, 14), and on the basis of weight, it comprises only 2% of the total soybean, but it contains an isoflavone concentration approximately 10-fold higher than that found in other SPIs. From a product development point of view, the significant difference between these two natural soy isoflavones sources is the amount of protein and fat present (13). SPI has approximately double the protein concentration as that found in SG where proteins are replaced mainly by carbohydrates, such as sucrose and stachyose, soluble fiber, and fat (13).

The added protein present in the soy isolate makes it a difficult ingredient to incorporate in high concentrations into TJ due to the increased viscosity imparted by the interactions between protein and tomato particles present in juice (15, 16). Rheological properties were studied recently to compare plain TJ to TJ after addition of 1% SPI at ambient temperature (15). The addition of SPI, once denatured, increased the viscosity of TJ due to protein–pectin interactions leading to increased stability of the suspension, without major qualitative effects on the shear-thinning and gellike behavior of the starting TJ product. On the other hand, the rheological time-dependent behavior of TJ was significantly affected by soy protein. A dynamic system where protein–pectin aggregates influenced the tomato–soy system by a competition of breakdown and buildup phenomena was observed. The thixotropic and rheopectic behaviors were affected by conformational changes of protein with pectin and all other particles present in the TJ upon application of different shear rates. Previous studies on TJ reported that the maximum juice viscosity was reached between

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Table 1. Comparison of TSS, pH, and Density Values for Plain TJ, TJSG, and TJSPI

samples	TSS (°Brix)	pH	density (g cm ⁻³)
TJ	5.1	4.17	1.025
TJSPI	5.2	4.19	1.027
TJSG	6.3	4.17	1.026

pH 4 and pH 4.5, and this was attributed to pectin/protein interactions that resulted in the formation of a pH-dependent reversible electrostatic complex (17).

Currently, SG incorporation in products such as soy milk is limited, due to sedimentation of insoluble carbohydrates, such as cellulose, lignin, and hemicellulose (13, 14), resulting in an unacceptable product. A novel TJ containing soy could benefit from the high concentration of isoflavones found in SG without compromising the perceived juice characteristics of tomato products.

This work deals with the development of tomato juice with SG (TJSG) as a novel functional product that delivers carotenoids, soy proteins, and significant isoflavone contents. Rheological studies were performed to compare TJSG to plain TJ with and without SPI at ambient temperature. In addition, temperature dependence was studied for all three products to investigate possible protein/pectin/cosolute interactions in the tomato-soy system after the addition of two different soy ingredients.

MATERIALS AND METHODS

Materials. Locally grown fresh tomatoes were processed into juice at the Food Industries Center Pilot Plant located in Howlett Hall on the OSU campus in Columbus, OH, using a hot break treatment (18). Tomatoes were sorted, washed, chopped, blended, and filled in salt-added cans (white/pln, 300 × 407; Ball Corporation, Columbus, OH). One salt tablet (Canning Tablets for Tomatoes, Morton) was added to each can. Cans were then steam sealed and retorted (Food Machine and Chemical Corporation, Hoopston, IL) at 105 °C for 30 min.

The hot break TJ contained 5.6% solid content and 7.25% water soluble pectins on a dry solid basis (19). The degree of esterification of the pectins was found to be 76.9% (20).

FXPH0159 soy proteins isolate (PTI, St. Louis, MO) and SoyLife Complex SG (Acatris Inc., Edinborough, MN) were used as soy ingredients. According to the manufacturer's specifications, SPI and SG contained a minimum amount of protein: 84 (w/w dry basis) and 38% (w/w dry basis), respectively. The solubility of 1% soy protein was determined (21) in retorted aqueous solutions and acidified with 1 N HCl to pH 4.2. The protein solubility (22) was found to be ~70%.

The surface hydrophobicity of SPI was determined according to titration with 1-anilino-8-naphthalene-sulfonate and compared to bovine serum albumin (BSA) (23). The hydrophobicity of soluble protein isolate in retorted acidified aqueous solution was approximately 1000 times lower than that of BSA on a protein mass basis.

Preparation of TJ with Soy. The two tomato soy products were prepared by addition of 1% SPI and 1.5% SG to processed TJ, respectively. Both soy-tomato mixtures were homogenized at 60 °C for 10 min and then retorted at ~100 °C for 15 min. The cans were stored at room temperature prior to analysis. No endothermic peak was visible in a DSC (DSC 2920 TA Instruments, New Castle, DE) analysis of the processed TJ with soy indicating that the protein in the two products was denatured (24).

Tomato Products Characteristics. The pH (Orion Research, Beverly, MA), the density (determined using a picnometer), and the total percent soluble solids (TSS) (°Brix) (Abbe Refractometer, American Optical Corporation Scientific Instrument Division, Buffalo, NY) at ambient temperature of the final products are reported in **Table 1**. Protein, carbohydrate, ash, and lipid compositions of the final products were determined using AOAC (22) methods and are reported

Table 2. Comparison of Protein, Carbohydrate, Lipid, Ash, and Isoflavones Contents for Plain TJ, TJSG, and TJSPI

samples	% w ^a				isoflavone (mg/100 mL)
	protein	carbohydrate	lipid	ash	
TJ	0.7	4	<0.1	0.8	
TJSPI	1.5	4.5	<0.1	0.7	3.0
TJSG	1.3	6.2	1.4	1.1	22.7

^a w, g/100 g wet basis.

in **Table 2**. The total isoflavone content of each soy tomato product was determined using high-pressure liquid chromatography as specified elsewhere (25), and results are in **Table 2**.

Rheological Measurements. Rheological experiments were conducted using a strain-controlled rheometer (RFS II Rheometrics system, Rheometrics Inc., Piscataway, NJ). Couette geometry was used (diameter of cup, 34 mm; diameter of bob, 32 mm; length of bob, 33.3 mm) to minimize end effects (26). All isothermal experiments were conducted at ambient temperature (25 °C), using a thermostatic bath (Rheometrics Inc.). Temperature dependence studies were performed between 5 and 65 °C under thermostatic control; thermal losses were minimized by covering the sample with a solvent trap. Prior to the analysis, the product was agitated several times and then left to rest for 1 min at room temperature to limit serum-pulp separation. All samples were subjected to the same standard routine, and experiments were performed at least in triplicate.

Steady rate sweep measurements were applied across a range of steady strain rates (0.02–200 s⁻¹). Startup experiments, to record apparent viscosity, were performed using a range of shear rates between 50 and 1500 s⁻¹ until an equilibrium value was reached. Loops also called triangular procedures (26) were performed, to investigate the time dependence of viscosity, by applying two cycles: an increasing ramp of shear rate values from zero to selected different final shear rates (50–1500 s⁻¹) and a decreasing ramp of the final shear rate to zero. Loop tests were performed selecting different cycle times, and all cycle times tested (1–15 min) resulted in similar behavior. The step shear rate mode was also performed using stepwise sequences of different shear rate values (100–200–100 s⁻¹; 500–600–500 s⁻¹) for an overall time per each sequence of 1800 s.

Dynamic strain sweep and frequency sweep tests were performed in order to study the viscoelastic behavior of the tomato products. Dynamic strain sweep experiments were used at different frequencies (0.01–10 Hz). The phase shift values of tan δ (where $\tan \delta = G''/G'$), plotted vs percent strain (data not shown), indicated that at a frequency of 1 Hz, all products exhibited a linear response for strain values up to about 1%. Dynamic frequency sweep tests were carried out in a range of 0.1–10 Hz using a percent strain value of 0.5 at selected temperature in the range between 5 and 65 °C. Dynamic temperature ramps at 1 °C/min and 1 Hz were performed between 5 and 65 °C. From these dynamic tests, complex viscosity, loss, and storage moduli were obtained.

RESULTS AND DISCUSSION

Characteristics of Tomato Products. One and a half percent of SG was added to the plain TJ in order to obtain a product having an isoflavone content 10-fold higher than TJ with 1% SPI (**Table 2**). Adding a similar amount of isoflavones from SPI would compromise drastically the juice characteristics of the tomato product (16).

The TSS content of hot break TJ was approximately the same as that reported elsewhere (7) for TJ processed under the same thermal treatment conditions (**Table 1**). TJ with 1% SPI had about the same TSS amount as compared to plain TJ (**Table 1**). The addition of SG increased the TSS content of TJ by 8% (**Table 1**) mostly due to the presence of sucrose and oligosaccharides such as stachyose (13). The overall protein content found in TJ after addition of 1.5% SG was slightly less than

that found in the TJ with 1% SPI (Table 2). The amount of lipid in TJSG was found to be higher than in two other products (Table 2).

Temperature Dependence of the Complex Viscosity. Temperature has an important influence on the flow behavior of food hydrocolloid solutions (27, 28). The temperature dependence of complex viscosity was studied to understand the role of different soy ingredients in the TJ. Dynamic frequency sweep experiments are reported in Figure 1a–c for all three products. The trend of complex viscosity vs frequency, collected in isothermal conditions at different temperatures in the range from 5 to 65 °C, showed different behaviors for the three TJs.

The complex viscosity of plain TJ decreased slightly as the temperature increased (Figure 1a). This phenomenon can be attributed to the Brownian motion that facilitates the relaxation of pectin and other tomato particles in the serum medium. Because the physical gel behavior of TJ is mainly attributed to the presence of highly esterified pectins that form calcium-mediated bridges (29), it can be hypothesized that heating affects this gel system by weakening the hydrophilic and hydrophobic interactions between pectins and all other tomato particles (17). The contribution of pectins to the decrease of viscosity with increasing temperature was shown to be predominant in other juices as well (28, 30).

Figure 1b shows that the addition of 1% of soy protein not only increased the magnitude of the complex viscosity of TJ, due to aggregation between protein and pectin, but also affected its pattern as a function of temperature. In fact, the isothermal experiments (Figure 1b) show that heating weakened the soy–tomato interactions as compared to the behavior of plain TJ (Figure 1a). Because the difference between these two systems (Table 2) was exclusively the addition of soy protein, it can be hypothesized that a higher temperature decreased hydrophobic and electrostatic interactions of protein with pectins (17).

All three tomato products behave as weak “physical gels” since the storage moduli as a function of frequency were consistently greater than the loss moduli values (data not shown) (26, 31). However, the effects of the addition of SG on the overall rheological characteristics of TJ were different from the ones induced by the addition of SPI (15). In Figure 1c, logarithmic plots of complex viscosity vs frequency, collected in isothermal conditions at different temperatures, revealed that the viscoelastic response of TJSG was unaffected by temperature. This pattern observed in the dynamic frequency sweep experiments was confirmed by application of dynamic temperature tests (Figure 2, TJSG). As compared to plain TJ (Figure 2, TJ), the addition of SG increased the magnitude of the complex viscosity of juice in the temperature region studied, reinforcing the entire system.

The addition of SPI to TJ increased the magnitude of the complex viscosity at lower temperatures. However, the viscosity of this system decreased drastically as the temperature increased (Figure 2, TJSPI). At temperatures larger than 50 °C, the complex viscosity of TJ with soy protein was lower than TJSG. Higher temperatures seem to weaken the interactions between soy protein and tomato particles.

To better understand not only the general viscoelastic behavior of the products under analysis but also which component of the system was more affected by heating, storage G' (Pa) and loss G'' (Pa) moduli were plotted vs temperature (28) (Figures 3 and 4). The trend observed for G'' was almost the same for all three products (Figure 3). In fact, although their loss moduli had different magnitudes, their trend as a function of temperature, that is the slope of G'' vs T , was approximately the same

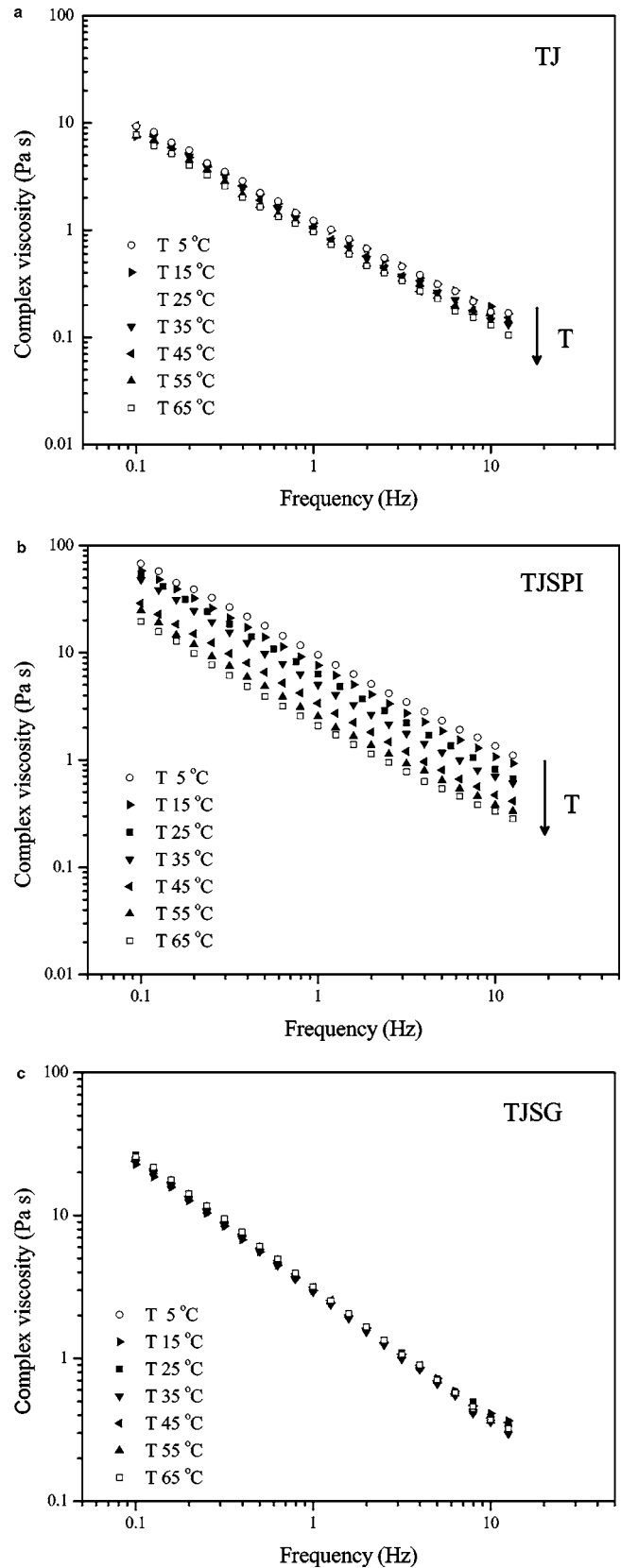


Figure 1. (a) Complex viscosity as a function of frequency for TJ performed in isothermal conditions at temperatures ranging from 5 to 65 °C [the arrow indicates the pattern of complex viscosity vs temperature (T)]. (b) Complex viscosity as a function of frequency for TJSPI performed in isothermal conditions at temperatures ranging from 5 to 65 °C [the arrow indicates the pattern of complex viscosity vs temperature (T)]. (c) Complex viscosity as a function of frequency for TJSG performed in isothermal conditions at temperatures ranging from 5 to 65 °C.

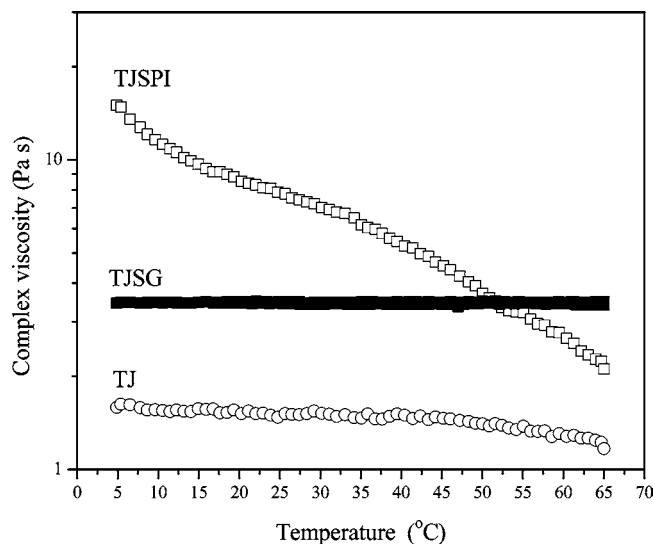


Figure 2. Complex viscosity as a function of temperature obtained by dynamic temperature ramp experiments run at 1 °C/min and at temperatures ranging from 5 to 65 °C for plain TJ, TJSG, and TJSPI.

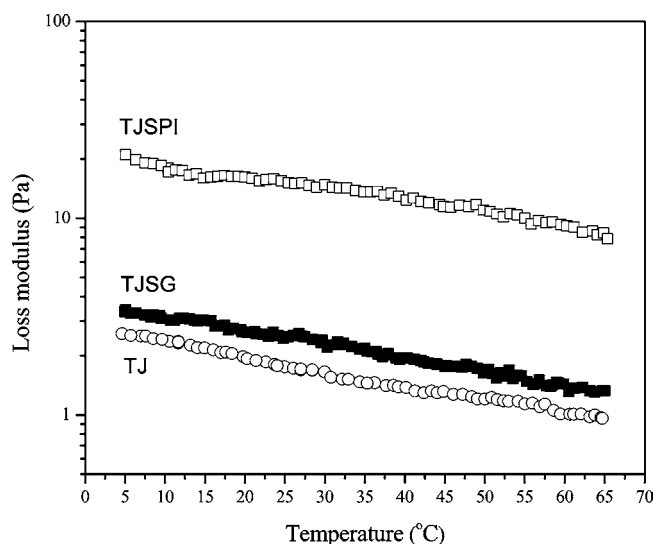


Figure 3. Loss modulus as a function of temperature obtained by dynamic temperature ramp experiments run at 1 °C/min and at temperatures ranging from 5 to 65 °C for plain TJ, TJSG, and TJSPI.

for all three products (**Figure 3**). Because the G'' modulus represents the viscous contributions to the viscoelastic behavior (28), it can be assumed that the viscous component of plain TJ was not qualitatively affected by the addition of the two soy ingredients except for an increase of its magnitude resulting from the addition of high molecular weight compounds. **Figure 4** depicts the pattern of the storage moduli vs temperature of the tomato systems. G' is usually associated with the ability of the system to store energy. The storage modulus G' of tomato juice with SPI (TJSPI) decreased significantly during heating and confirmed the trend of complex viscosity observed in **Figure 2**. The increasing temperature seems to weaken the tomato soy system most probably affecting interactions between soy protein and pectin. The addition of SG seemed to stabilize this elastic modulus pattern (no change during heating) as compared to plain TJ (slight decrease during heating). The storage modulus was found to be the component that was most affected qualitatively in the temperature region studied. Therefore, it was the component that qualitatively dictated the rheological response of the tomato systems studied (**Figure 4**).

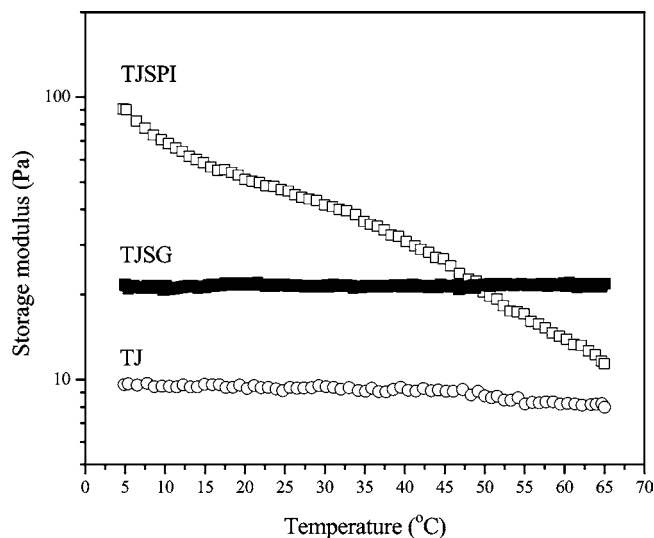


Figure 4. Storage modulus as a function of temperature obtained by dynamic temperature ramp experiments run at 1 °C/min and at temperatures ranging from 5 to 65 °C for plain TJ, TJSG, and TJSPI.

Table 3. Estimated Parameters of Activation Energy (E_a) with Respective Regression Coefficients (R^2) Obtained from the Arrhenius Type Eq 1 in Dynamic Frequency Sweep and Dynamic Temperature Tests for Plain TJ, TJSG, and TJSPI

samples	dynamic frequency sweep test		dynamic temperature ramp test	
	E_a (kJ/mol)	R^2	E_a (kJ/mol)	R^2
TJ	0.043	0.980	0.033	0.891
TJSPI	0.305	0.985	0.321	0.990
TJSG	0.026	0.383	0.001	0.280

Several empirical equations are proposed in order to correlate the effect of temperature on viscosity of Newtonian and non-Newtonian food fluids. An Arrhenius type model (26, 28) is frequently used to describe this relationship, and it is expressed in the following way:

$$\eta_T = f(T) = A \exp\left(\frac{E_a}{RT}\right) \quad (1)$$

where η_T (Pa s) is the viscosity of a fluid at the absolute temperature T (K), R ($J K^{-1} mol^{-1}$) is the universal gas constant, E_a ($J mol^{-1}$) is the energy of activation of flow process, and A is a characteristic constant. The regression coefficients of $\ln(\eta_T)$ vs inverse of absolute temperature were calculated by linearly fitting the complex viscosity data collected at 1 Hz and at different temperatures, using the least-squares method. The activation energy was calculated for all three tomato products. The values obtained by dynamic frequency sweep tests were compared to the dynamic temperature ramps after application of the same fitting procedure. The activation energy values of two dynamic tests with respective regression coefficients (R^2) are reported in **Table 3**.

In suspensions, when the temperature is increased, the Arrhenius type equation is not always obeyed due to the competition of two effects. On one hand, the viscosity can monotonically decrease, obeying Arrhenius behavior (32). On the other hand, as the continuous phase becomes more fluid and the dispersed particles acquire more energy, the rate of structure formation increases and consequently an increasing viscosity can be observed (32). When these two effects balance each other, no changes as a function of temperature are observed.

Data fitting of complex viscosity (obtained by dynamic frequency sweep experiments) of tomato products vs the inverse of absolute temperature was performed using the linear regression method, and results were reported in **Table 3**. The Arrhenius type equation was found to be suitable in describing the relationship between viscosity and temperature in TJ with and without soy protein. The activation energy values, extrapolated also by dynamic temperature ramp tests, were found to be similar to those obtained by dynamic frequency sweep experiments (**Table 3**). Because the TSS content was the same in the TJ with and without soy protein, the changes of viscosity observed in **Figure 2** with increased temperature between these products were most likely due to interactions of soy protein with tomato particles. The activation energy of TJ with soy protein was higher than in plain TJ (**Table 3**) indicating low particle–particle interactions (30). The increased viscosity of TJ with soy protein, attributed to hydrophobic bonds and electrostatic interactions between pectin and protein, is strongly weakened during heating, likely due to rearrangement of this globular protein (33) in the tomato system.

Deviation from the Arrhenius behavior was observed for TJSG for both dynamic tests since the complex viscosity did not change as a function of temperature (**Table 3**). In this system, lower protein and higher carbohydrate and lipid contents seem to play an important role on the physical stability of the juice as compared to the TJ with soy protein (**Table 2**). The amino acid profile of protein in SPI and in SG was similar (14) even though their total protein content was different (**Table 2**). Additionally, differential scanning calorimetry analysis of two native soy ingredients showed that the denaturation phenomenon occurs in the same temperature range (data not shown).

The higher lipid content of TJSG most likely affected the overall viscosity and its pattern vs temperature by interfering with the interactions between pectins and soy proteins. Additionally, esterified pectins were shown to form bridges between lipids and globular proteins under acidic conditions (34). In part, the presence of lipids in the SG could prevent the aggregation between polysaccharides and proteins in this juice system (35).

Steady Shear Flow Experiments. The shear flow properties of TJ with and without the addition of soy protein was non-Newtonian (15). On the basis of preliminary rheological studies (15), the addition of SPI to TJ increased the magnitude of the apparent viscosity without major qualitative effects on the shear-thinning behavior of the plain TJ. In this study, the same trend was observed after addition of SG to tomato product even though the magnitude of the viscosity was lower than that of TJ with soy protein (data not shown).

The time dependence of TJSG was studied comparing to the results obtained for TJ with and without SPI (15). Examples of loop, startup tests, and stepwise sequences were used to compare the time dependence of TJSG with the other two tomato products (15).

Figure 5 shows hysteresis loops for tomato SG as compared to the other two products by the application of a continuous shear from zero to a final shear rate value between 50 and 1500 s^{-1} (for example 750 s^{-1} ; **Figure 5**). The shear stress values in the increasing ramp were always greater than those in the decreasing ramp suggesting an incomplete structure recovery leading to a thixotropic behavior. The same pattern was observed for all of the different final shear rate values (data not shown). The addition of SG did not affect qualitatively the time dependence of plain TJ, while soy protein addition showed significant differences as was discussed previously (15). Hysteresis loops can depend on the ramp duration, selected shear

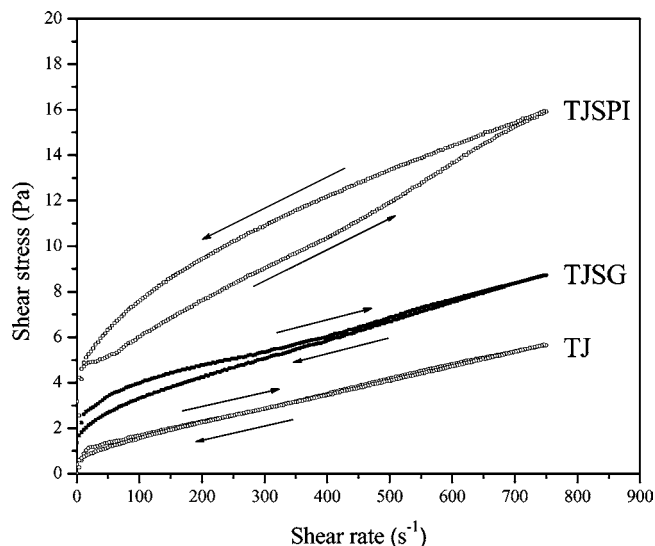


Figure 5. Shear stress as a function of shear rate using loop tests for plain TJ, TJSG, and TJSPI by application of ramp up (from 0 to 750 s^{-1}) and ramp down (from 750 to 0 s^{-1}).

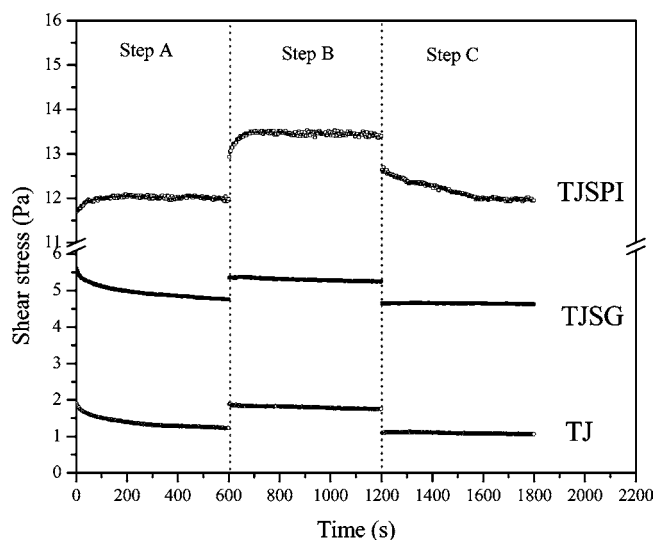


Figure 6. Shear stress as a function of time obtained using a shear rate stepwise procedure [500(step A) – 600(step B) – 500(step C) s^{-1}] for plain TJ, TJSG, and TJSPI.

rates, and inertial characteristics of the rheometric system (26); therefore, startup tests and stepwise sequences at different values of shear rate were applied. **Figure 6** shows the shear stress response of TJSG as compared to plain tomato with and without soy protein when a stepwise sequence of 500–600–500 s^{-1} (A–B–C regions, **Figure 6**) was applied for an overall time of 1800 s. For the TJSG, by application of the first step (step A), the shear stress decayed as a function of time. As the shear rate was increased (step B), again the shear stress slightly decayed indicating a breakdown of the network system with partial structure recovery. Finally, after application of the last step (step C), the shear stress was unchanged due to limited structure recovery resulting in an irreversible thixotropic behavior (**Figure 6**). For all of the stepwise sequences applied, the trend observed for TJSG was qualitatively the same as that found in plain TJ. These trends were confirmed by startup test where the time of the shearing was much longer (data not shown).

All three different experiments confirmed the thixotropic behavior of TJSG. The higher TSS content of SG does not seem

to affect qualitatively the rheological trend of plain TJ. Two counteracting phenomena of breaking the pulp network structure and, simultaneously, restoring interparticle interactions, reforming the network, was observed in the case of plain TJ (10) and enhanced after addition of 1% SPI (15, 36). The thixotropic and rheopectic behaviors were hypothesized to be affected by rearrangement of soy protein with pectin and all other particles present in the TJ upon application of different shear rates. The different chemical profile of the SG seemed to limit the rearrangements of this globular protein and improve the texture and the stability of the final product. The addition of SG increased the viscosity without affecting the shear and time dependence of the plain TJ.

From the dynamic and steady shear flow experiments, we conclude that the addition of SG to TJ did not affect qualitatively the rheological properties of TJ while addition of soy protein showed significant qualitative and quantitative differences. The addition of SG did not change the shear-thinning and time dependence behaviors of TJ contrary to the observed behavior after addition of SPI. Thus, the addition of SG is not expected to change the texture of plain TJ as compared to TJSPI while significantly enhancing its isoflavones content. Further studies comparing the sensory characteristics of the three products are planned to confirm these findings.

Dynamic tests depicted "physical gel" characteristics for all three products. The higher gel strength of the two soy-containing tomato systems reduced the serum separation and increased the water-holding capacity of TJ (16). However, oscillatory tests showed that the stability and the compatibility between the two different soy ingredients and TJ were different. Heating weakened the hydrophobic and electrostatic interactions between pectin and protein (17) in the tomato SPI system. The effect of temperature on the viscosity was confirmed by the activation energy values obtained by the Arrhenius type model. In the case of TJSG, the Arrhenius equation was not found suitable. The complex viscosity was unaffected by the increasing temperature. The overall stability of TJSG might be maintained by interactions between tomato particles and all the different components present in this soy ingredient.

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