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ORIGINAL ARTICLE

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Jet cooking dramatically improves the wet strength of soy adhesives

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Abstract

The impact of jet cooking on shear strength of soy-and-water adhesives was investigated to understand the higher shear strength of commercial soy protein isolates compared to soy flours. Soy flour-based wood adhesives are appealing because of their bio-based content, low formaldehyde emission, and low cost, but their commercial application is limited by low wet cohesive strength. Previous researchers proposed that the process of jet cooking (steam injection with high turbulence followed by rapid cooling) was responsible for the high (~3 MPa) wet shear strength of adhesives made with commercially produced soy protein isolate, using the ASTM D 7998 test. In this work, we show that jet cooking did dramatically increase the wet strength of laboratory-produced, native-state soy protein isolate from 0.6 to 3 MPa, a strength similar to many commercial isolates. Jet cooking was far less effective at developing wet strength of soy flours, but greatly increased the viscosity of virtually all our soy materials. We hypothesize that the benefits of jet cooking are primarily a result of nonequilibrium protein aggregation states because subsequent wet autoclaving of jet cooked soy proteins dramatically decreased wet strength. The dramatic differences in adhesive properties between commercial soy protein isolates and soy flours suggests that the common practice of using results obtained with commercial isolates to predict the performance of soy flour adhesives is inappropriate.

KEYWORDS
adhesives, hydrothermal, jet cooking, protein denaturation, soy, wet strength

INTRODUCTION

Soy adhesives for wood bonding have been extensively examined in recent years to replace fossil-based adhesives with bio-based materials and to eliminate formaldehyde-related health concerns. Many bio-based materials have been considered for making wood adhesives (Dunky, 2020; Solt et al., 2019). Among protein-based adhesives, soy is the most studied because it is produced in large volumes, has the highest protein content of the major seed crops, and has successful commercial products. Soy flour-based adhesives are now used in over half of the interior plywood produced in North America, as well as a significant fraction of engineered wood flooring (Mike Birkeland, Solenis LLC, personal communication).

Soy flour is used instead of the higher protein content and stronger soy protein isolate because the flour is far less expensive. A main difference between soy flours is the PDI (protein dispersibility index). PDI is the measure of protein dispersed in solution such that it resists centrifugation under specified conditions (AOCS, 2017). During soy processing, hexane from the oil extraction step can be removed while the soy flakes remain at low temperature to keep the proteins largely in their native configuration (90 PDI flour), or toasted by

greater heat exposure to denature the proteins and improve digestibility for animal feeds (20 PDI flour) (Sun, 2005a). Thus, the effect of dry heating of flour on performance can be estimated by comparing 90 versus 20 PDI flours. However, there is little information on the impact of wet heating on the shear strength of soy adhesives.

Some studies of soy adhesives have used commercial soy protein isolate (CSPI) (Dunky, 2020; Mousavi et al., 2021; Vnučec et al., 2017), while a few use laboratory-produced isolate (Sun, 2005b). Those using CSPI have not indicated an awareness that in making CSPI suitable for food applications, the protein structure is greatly altered. In addition, it should be noted that the CSPIs have considerable variation in properties (Egbert, 2004, Frihart & Birkeland, 2016). Although almost all soy materials produce bonds with high dry strength, soy flours have only a small fraction of the wet strength obtained by most CSPIs (Lorenz et al., 2015). The process for isolating soy protein is to make a dilute aqueous dispersion of the soy flour at pH 8–10 and remove the higher molecular weight insoluble carbohydrates by centrifugation (Petruccelli & Añon, 1994). Proteins and lower molecular weight soluble carbohydrates in the supernatant are then typically separated from each other by acidification to precipitate the proteins near their isoelectric points followed by centrifugation. Since carbohydrate-based adhesives such as starch are extremely water sensitive, carbohydrates in soy flour were often falsely assumed to be the main cause of the water sensitivity of soy flour adhesives relative to commercial isolate (Lorenz et al., 2015). Lorenz et al. compared the wet bond strength of the native configuration, laboratory made soy protein isolate (LSPI), to the native flour from which it was isolated proving that the carbohydrates were not the major factor causing their difference in strength (Lorenz et al., 2015). Also, adding carbohydrates (soluble, insoluble, or in combination) to CSPI caused only a moderate decrease in wet strength, demonstrating that the carbohydrates were only a small part of the difference in wet strength between soy flour and CSPI (Lorenz et al., 2015). Still unexplained was why the LSPI was much lower in wet strength than CSPI even though they had similar protein contents. Lorenz et al. (Lorenz et al., 2015) proposed that the difference between CSPI and LSPI might be that CSPI is jet cooked, that is, exposed to high temperature and shear forces followed by extremely fast cooling and drying. Jet cooking is used widely in industry for making dispersions of proteins and starches. Most of the literature on jet-cooking is in patents (Duren, 1972; Hawley et al., 1972; Johnson, 1999; Monagle et al., 2006), while only limited journal literature is available on jet cooking of soy materials (Wang & Johnson, 2001; Yang et al., 2014).

As various protein denaturation methods have been shown to impact the performance of the resulting adhesives (Dunky, 2020), we suspected that the jet cooking denaturation process might have a significant impact on CSPI adhesive performance. Therefore, this paper investigates how different hydrothermal treatments, wet autoclaving and jet cooking, impact the adhesive properties of soy aqueous dispersions. Here we show that jet cooking can account for the large wet strength difference between commercial and laboratory SPIs and discuss how strength differences likely result from differences in protein configuration. The adhesive properties of jet cooked flour are also investigated.

EXPERIMENTAL PROCEDURES

Commercial and lab-prepared soy materials

Soy flours of 200 mesh with 90 and 20 protein dispersibility index (PDI) (AOCS, 2017) were provided as Prolia™ 200/90 and Prolia™ 200/20 by Cargill, Inc. (Cedar Rapids, IA). Flours contained 53% protein, 6% moisture, ~18% insoluble carbohydrate (hemicellulose, pectin, cellulose, starch), ~14% soluble carbohydrate (sucrose, stachyose, raffinose), and ~5% ash (Liu, 1997). Commercial soy protein isolates (CSPIs) PRO-FAM^{R} 646, 891, and 974, all with protein contents above 90%, with a maximum of 4% fat and 5% ash, were provided by ADM (Decatur, IL) and chosen for differences in dry and wet shear strength and viscosity before autoclaving. All CSPI results are from PRO-FAM^{R} 974 unless otherwise indicated. All experiments used single lots of soy flours. Other chemicals were sourced from Sigma-Aldrich (St. Louis, MO).

The procedure used to make LSPI from the previous study (Lorenz et al., 2015) was impractical for the volumes needed, leading to scaling up the process using equipment at the Forest Products Laboratory pilot plant to make Pilot Plant SPI (PPSPI). This process involved adding 9 kg of 90 PDI soy flour to 91 L of reverse-osmosis water and adjusting to pH 8.2 ± 0.2 with ~1.6 L of 1.5 M NaOH. After vigorous agitation using a 25 cm dia. Mixer blade 10 cm from the bottom of the tank rotating at 240 rpm for at least 1.5 h., the mixture was centrifuged at 9600 G using a Sharples model AS16 continuous flow separator (Separation Equipment Sales, Inc., New York) fed at 2 L min^{-1}. The insoluble carbohydrate fraction was set aside. The supernatant fraction was then slowly acidified to pH 4.5 using ~2 L of 1.5 M H_2SO_4 and centrifuged as before to separate the precipitated protein isolate from soluble carbohydrate supernatant. The precipitated isolate was dispersed as a 15% slurry in reverse-osmosis water, then neutralized to pH 7 using 0.69 L of 1.5 M NaOH per kg of protein and stirred for at least 30 min. After pH stabilization. This minimally processed PPSPI, as well as some of the soluble and insoluble carbohydrates,

were frozen separately in pans and lyophilized, then ground in a mortar/pestle to pass a 100-mesh screen. The PPSPI was 98% protein using the bicinchoninic acid (BCA) procedure (Smith et al., 1985) and, when run on SDS-PAGE gels, had a protein molecular weight distribution typical of soy proteins in the literature (Qi et al., 2013), though some PPSPI remained at the loading well.

Adhesive preparation

To make soy dispersions, dry material was added to deionized water and stirred by hand with a spatula for 15 min. The solids content was 20% unless otherwise specified, and all measurements were made on the same day of jet cooking or adhesive preparation.

Autoclaving

Autoclaving studies were done with 15% solids adhesives due to the large increase in viscosity with autoclaving. The soy adhesive preparation was placed in a tall beaker and filled to 10% of its volume to minimize sample loss during depressurization. A small autoclave (model 750, Fisher Scientific, Pittsburgh, PA) was used to heat the samples to 120°C, well above denaturation temperature for the dominant soy proteins (Sun, 2005c). After 10 min. The autoclave was cooled down to about 60°C (approximately 30 min.) before placing the beaker with the sample in a water bath to cool to room temperature. One batch of each adhesive was autoclaved and properties of the autoclaved and control measured within 4 h.

Jet cooking

A laboratory jet cooker was constructed (Figure 1) with the goal of processing the smallest possible volumes, as necessitated by the considerable labor involved in producing PPSPI. The method followed the patent of Hawley et al., claim #9, though their apparatus was not well described (Hawley et al., 1972). Briefly, soy dispersions at 20% solids were driven by a gear pump (model H1F, Liquiflo, Garwood, NJ) into a 9.5 mm ID stainless steel tube surrounded by an 18 mm ID tube where pressurized steam was added. The pressure then carried the combined product through a 9.5 mm ID tube with lengths of 0.4–18 m. Tubes longer than 1 m were insulated. Residence times were approximately 2 s for the 0.4 m tube and 60 s for the 18 m tube, but because of the unknown flow field of the soy material through the residence tube, residence time is likely non-uniform. Upon exiting the tube through an orifice 1 mm^2, the soy dispersion was quickly cooled by depositing on the side of a rotating stainless-steel drum immersed in an ice water bath. The target flow rate for the dispersion was 300 ml min^{-1}. Temperature was set by steam pressure and monitored with thermocouples. Temperatures at the exit were within 2° of the injection point. At least 100 ml of material was run through the tube and discarded before samples of 100 ml were collected. The solids content dropped from 20% to ~16% during jet cooking due to the added steam. The pH before cooking was 6.3–6.8 and increased on average 0.25 pH units with jet cooking for both flour and PPSPI. Within one workday soy flour or PPSPI was mixed in water, jet cooked under different conditions, viscosity and solids content measured, and D 7998 shear samples made with jet cooked products and the non-jet cooked starting material as a control. There were seven different batches of each of the flours yielding 42 jet cooked adhesives prepared from the 20 PDI flour and 32 prepared from the 90 PDI flour. PPSPI had to be produced twice to supply enough material for jet cooking. A paired *T*-test found no significant difference in the ABES shear strength of the jet cooked material from the two PPSPI batches (avg difference = 0.31 MPa, SD = 0.60, $p = 0.44$).

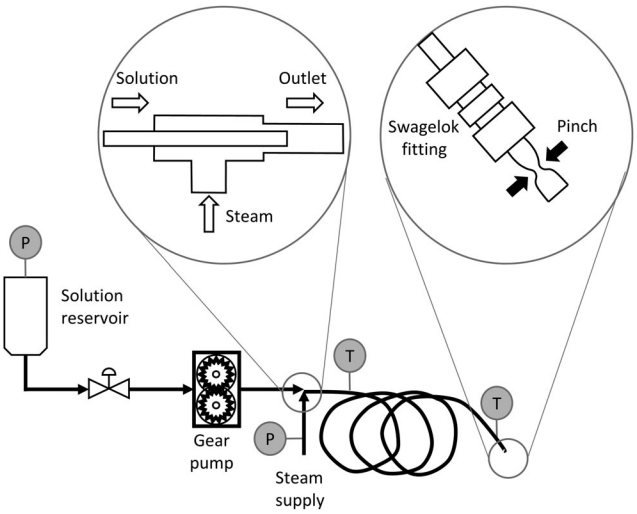

FIGURE 1 Jet cooking apparatus. *T* = thermocouple, *P* = pressure gauge

Jet cooking conditions were chosen to determine the impact of temperature and length of the residence tube on bond strength. A full list of jet cooking runs and resulting properties are shown in Supporting Information. With PPSPI, 23 jet cooking runs were roughly evenly distributed between 166 and 185°C, and 0.4, 6, 12, or 18 m tube length, with two more runs at 140°C/6 m tube. The 20 PDI flour had 34 runs at 120, 140, 166, or 185°C and 0.4 or 1.9 m tube, with 8 more across the temperature range with 6 or 12 m tube. The 90 PDI flour, the first to be cooked, had 29 runs at 150–155°, 166–170° or 185°C, labeled

150, 166, and 185°C; 6, 12, or 18–21 m (labeled 18 m) tube, and three additional runs with a 0.4 m tube at 140, 166, and 185°C. For every soy material, there were at least six replicates of one jet cooking condition and at least seven conditions with two or more replicates.

VISCOSITY MEASUREMENT

At least 50 ml of adhesive was stirred by hand for 30 s with a spatula while trying to minimize air entrapment. The spindle of a Brookfield viscometer model DV-E (Brookfield Engineering Laboratories, Middleboro, MA) was then immediately lowered into the sample and the viscosity reading taken 10 s after spindle immersion. Only one measurement was acquired for each material, as the coefficient of variation (COV, or standard deviation/mean) on a single sample and the same operator is typically below 10%, and we were concerned only with large changes.

Because of the very strong shear-thinning nature of soy protein dispersions, observed viscosity values are highly dependent on sample history, shear experienced by the sample in the seconds before measurement, the size of the spindles, and speeds. Entrained air also strongly effects viscosity readings. We, therefore, encourage soy dispersion viscosity values to be interpreted cautiously.

ABES (AUTOMATIC BOND EVALUATION SYSTEM) SHEAR STRENGTH

Bond strength of the adhesive was evaluated using the ABES method as described in ASTM D7998 (ASTM, 2019) using smooth sugar maple (*Acer saccharum*) veneers (Columbia Forest Products, Greensboro, NC) bonded in a parallel orientation. This method measures the changes in cohesive shear strength while minimizing the influence of variables such as adhesive viscosity, solids content, open or closed assembly time, or spread rate (Frihart & Lorenz, 2013). Approximately 10 mg of adhesive was applied with a spatula to 5 mm on the end of one piece of the veneer (117 × 20 × 0.6 mm), which was immediately overlapped by 5 mm with another (uncoated) piece of veneer and hot pressed in the ABES (Automated Bonding Evaluation System) (AES, Corvallis, OR) at 1.3 MPa for 120 s. More than 10 mg (wet weight) of soy adhesive per sample (100 g m^{-2}) squeezes out of the bond and is unnecessary. Bonding temperature was 120°C unless otherwise specified. The bonded wood samples were equilibrated by storing at 21°C and 50% relative humidity at least overnight before testing. Wet samples were soaked in water for 4 h. at room temperature. Then, 3–6 (autoclave) or 6–10 (jet cooking) specimens each, dry or wet, were tested in tensile shear with

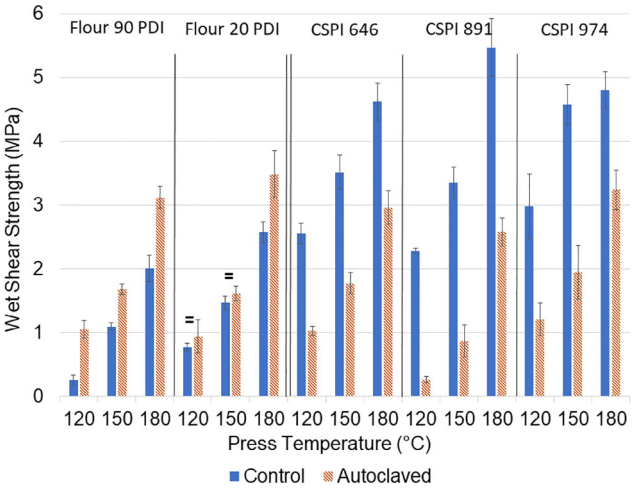

FIGURE 2 ABES wet shear strength of soy flour and three commercial isolates before and after autoclaving. Three pairs of bars represent bonding temperatures of 120, 150, and 180°C. Values different by 0.2 MPa or more are statistically significant. A "=" indicates that the control (nonautoclaved) and autoclaved values are not statistically different at $p = 0.05$. Values for 120°C flour controls were measured on the same day, with the same material, as the autoclaved sample, and vary slightly from Table 2.

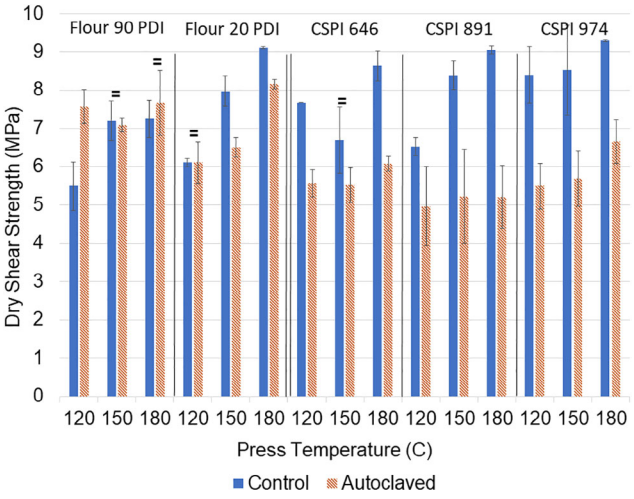

FIGURE 3 ABES dry shear strength of soy flour and isolates before and after autoclaving, bonded at 120, 150, and 180°C. values different by 0.6 MPa or more are statistically significant. A "=" indicates that the control and autoclaved values are not statistically different at $p = 0.05$. Values for 120°C flour controls were measured on the same day, with the same material, as the autoclaved sample, and vary slightly from Table 3.

the ABES. The pooled COV between replicates of single sample was 8% across all the strength data over 1 MPa. In Figures 2 and 3, a = symbol was placed above samples where the control and autoclaved sample were not significantly different at a 95% confidence level by a Student's *T*-test. An ABES wet strength value of at least 3 MPa is considered necessary for commercial viability because adhesives with lower strength values typically

do not pass the 3-cycle soak test (ANSI/HPVA, 2016) in plywood (Frihart et al., 2009).

Statistical analysis

Initial effect screening and data exploration was conducted with JMP (SAS, Cary, NC). Linear effects models of the impact of soy material, temperature, and tube length on strength and viscosity were constructed in Excel (Microsoft, Redmond, WA). Student's *T*-tests and *F*-tests followed the methods of Box et al. (1978). Correlation coefficients were calculated using Microsoft Excel in office 365. For the *T*-tests, the variance of the two groups under consideration were first *F*-tested for differences at the $p = 0.05$ level. In all cases, there was no evidence to reject the assumption of homoscedasticity. All *p*-values assumed two-sided distributions.

RESULTS AND DISCUSSION

Wet autoclave treatment

Initial experiments heated aqueous soy dispersions in an autoclave, and by necessity these were allowed to cool slowly. Autoclaving investigates the impact of wet heating, as opposed to the dry heating used in making 20 PDI soy flour. Water is potentially important because it strongly plasticizes soy proteins (Sun, 2005c). Autoclaving provides no shear and slow cooling, very different from jet cooking used to make commercial soy protein isolates, which involves high-steam pressures, high turbulence, short residence time, and is followed by quench cooling (Wang & Johnson, 2001).

In Figure 2, the impact of dry heating (toasting) during production of the 20 PDI flour is seen by comparing it with untoasted 90 PDI flour; the 20 PDI provides significantly higher wet strength at every press temperature. The figure also shows that autoclaving (wet heating) significantly improved the wet strength of 90 PDI flour at all press temperatures but autoclaving significantly improved 20 PDI only at the 180°C press temperature. Figure 2 also supports the prior observation that pressing at higher temperature improves wet bond strength no matter what soy material is used (Frihart et al., 2016). The similarity of 20 and 90 PDI strength after autoclaving (no statistical difference) suggests that the hydrothermal treatment might bring both flours to a similar configurational state, potentially rearranging the protein aggregation state induced by their different processing histories. Autoclaving also greatly increased the viscosities of the soy flour and insoluble carbohydrate dispersions (Table 1) which may include pasting (dissolution) of the starch present in flour at ~0.6% (Karr-Lilienthal et al., 2005), and possibly changes in protein aggregation state when exposed to heat and moisture.

TABLE 1 Viscosity (cP @ 10 RPM) of soy flours, isolates, and carbohydrates removed during isolation of the PPSPI at 15% solids, to indicate order-of-magnitude viscosities before and after autoclaving

	Control (cP)	Autoclaved (cP)
Flour 90 PDI	550	30,000
Flour 20 PDI	<10	18,000
PPSPI	900	4000
Insoluble carbohydrates	<10	130,000
Soluble carbohydrates	<10	<10
CSPI 646	36,000	37,000
CSPI 891	31,000	450
CSPI 974	57,000	6000

Note: While these were not replicated, typical coefficients of variance are 10%. Dramatic changes are observed in all the materials except soluble carbohydrates and CSPI 646.

Having shown that autoclaving improves the wet bond strength of the 90 and 20 PDI flour, the question was whether the change was due to alteration of the proteins, carbohydrates, or both. Since it was previously shown that autoclaving greatly improved the wet bond strength of largely carbohydrate-free native-configuration LSPI to 1.50 MPa (Lorenz et al., 2015), the change to the proteins is likely to be the source of the strength improvement, even though the strength was still far below that of the commercial SPI. Figure 2 shows the wet strength of the three commercial SPIs (low carbohydrate and jet cooked by the manufacturer) decreased significantly with the autoclave treatment, further supporting the idea that wet bond strength is highly sensitive to protein aggregation state. As with the flours, autoclaving could produce large viscosity changes in the PPSPI dispersion, (Table 1) further supporting the conclusion that protein aggregation state could be dramatically changed by the autoclave treatment.

Figure 3 shows dry strength results with and without autoclaving. In contrast to the wet strength, the unaltered flours show good dry strength. Carbohydrates may contribute to the dry strength of the flour, obscuring any protein-related strength changes. The commercial isolates, however, were almost pure protein. Their clear and consistent loss of dry strength with autoclaving suggests that autoclaving of CSPIs was detrimental to cohesive strength development of proteins during the bonding process.

Jet cooking and bond strength

Since the autoclave treatment did not convert the native PPSPI into a CSPI equivalent, we jet cooked the PPSPI, and flour as well. The result of jet cooking 90 PDI flour, 20 PDI flour, and PPSPI are shown in Table 2 (ABES wet strength), Table 3 (ABES dry strength), Table 4 (viscosity) and in Appendices S1, S2, and S3, respectively.

TABLE 2 ABES wet shear strength after jet cooking under various temperatures (120–185°C) and tube lengths (0.4–18 m)

Soy	Tube length, m	*N*	Avg, MPa	95% CI, ±	*p* value[a]
90 PDI[b]	0.4–18	28	1.52	0.17	0.0000
90 PDI control[c]		6	0.00	0.00	
20 PDI[b]	0.4–18	42	1.31	0.12	0.0235
20 PDI control[c]		7	0.94	0.39	
PPSPI[d]	0.4–12	21	3.10	0.20	0.0000
PPSPI[e]	18	4	2.41	0.33	0.0000
PPSPI control		6	0.59	0.65	

Note: No statistically significant effect of temperature was detected. Samples pressed at 120°C.
[a]*p* Value of students *T*-test versus the respective control, that is, noncooked material.
[b]*T*-test of difference between 20 versus 90 PDI flour after jet cooking gave a $p = 0.039$.
[c]Strength values of control measured on same day, with same batch of soy as the treated material. Values are slightly different from control specimens reported in Figure 2.
[d]*T*-testing for tube lengths detected no difference among 0.4, 6, and 12 m tubes.
[e]*T*-test $p = 0.006$ for 18 m tube versus 0.4–12 m tubes.

TABLE 3 ABES dry shear strength after jet cooking at various temperatures (120–185°C) and tube lengths (0.4–18 m). Samples pressed at 120°C

Soy	Temperature, °C	Tube length, m	*N*	Avg, MPa	95% CI, ±	*p* value[a]
90 PDI[b]	150	0.4–18	15	6.84	0.34	0.0000
90 PDI[b,c]	166–185	0.4–18	13	6.26	0.22	0.0000
90 PDI control[d]			6	5.23	0.25	
20 PDI[c]	120–185	0.4–12	38	6.40	0.16	0.8880
20 PDI control[d]			6	6.43	0.42	
PPSPI[e]	140–185	0.4–12	16	7.81	0.23	0.0000
PPSPI[f]	140–185	18	4	7.31	0.49	0.0000
PPSPI control			5	5.14	0.56	

[a]*p* value of students *T*-test versus the respective control, non-cooked material.
[b]*T*-test $p = 0.014$ for 90 PDI cooked at lower versus higher temperature.
[c]*T*-testing did not show a difference among 200/90 (166–185°C cook), 200/20 jet cooked, and 200/20 control.
[d]Strength values of control measured on same day, with same batch of soy as the treated material. Values are slightly different from control specimens reported in Figure 3.
[e]T-testing for tube lengths of 0.4, 6, 12 m did not show a difference among them.
[f]T-test $p = 0.044$ for 18 m tube versus 0.4–12 m tubes.

Table 2 shows the wet strength of 90 PDI (considered native configuration) flour, 20 PDI (thermally denatured) flour, and PPSPI (native SPI) before and after jet cooking, all tested at ~16% solids. Just like in Figure 2, the non-jet cooked (control) denatured 20 PDI flour had more wet strength than the native configuration 90 PDI flour, which had virtually none. After jet cooking, the average wet strength of both flours significantly increased to 1.52 MPa for 90 PDI and 1.31 MPa for 20 PDI. The difference between jet cooked flour strength (20 vs. 90 PDI) was small but statistically significant ($p = 0.039$). *T*-testing did not show any significant impact of temperature or tube length. The fact that the 20 PDI and 90 PDI flours had very similar average values after jet cooking further suggests that their differing states of aggregation/denaturation before jet cooking were largely reconfigured by the more energetic and wet jet cooking environment.

Table 2 also shows that jet cooking dramatically improved the wet strength of PPSPI from 0.59 MPa (uncooked control) to an average of 3.10 MPa when jet cooked for the shorter tube length/ residence times. Further analysis showed that with the longest tube length (18 m), and presumably longest cooking time, the wet strength of PPSPI only increased to 2.41 MPa. This might be because prolonged heating above 100°C has been associated with protein degradation (Kinsella, 1979), though we saw no significant effect of temperature on strength in this case. The average wet strength value (3.10 MPa) for PPSPI treated with the shorter residence times (0.4–12 m tubes) is comparable to the wet ABES strength observed with CSPIs in Figure 2 and in the literature (Frihart & Birkeland, 2016). Since native state PPSPI (this study) and laboratory SPI (Lorenz et al., 2015) had such poor wet strength and jet cooking resulted in large strength gains, jet cooking of commercial SPIs likely contributes significantly to their high wet

TABLE 4 Viscosity (cP @ 20 RPM) of soy materials after jet cooking under various conditions

Soy	Temperature, °C	*N*	Avg, cP	95% CI	*p* Value[a]
90 PDI	150–185	20	61,300	14,600	0.0014
90 PDI control[b]		4	3400	1900	
20 PDI[c,d]	120–140	18	30,300	3800	0.0000
20 PDI[e]	166–185	19	72,700	15,900	0.0000
20 PDI control		7	4100	700	
PPSPI[f]	140–185	25	*		
PPSPI control		2	11,500		

Note: Control at 20% solids, jet cooked at 16%.
[a]*p*-value of students *T*-test versus the respective control.
[b]Controls are higher viscosity than Table 1 because of higher solids content.
[c]Different spindles are required at different viscosities. For example, spindle #3 was used before jet cooking and spindle #6 or #7 after.
[d]*T*-testing $p = 6 \times 10^{-6}$ for 20 PDI flour cooked at lower and higher temperature.
[e]*T*-test did not show a difference between 200/90 and high temperature 200/20, $p = 0.274$.
[f]After jet-cooking the PPSPI was gelled and so viscosity was not measured.

strength. The jet cooked flour wet strength values are below the 3 MPa minimum value for a commercially viable product. Obtaining higher strength values with flour from jet cooking, if possible, will require different conditions than those explored here. The highest wet strength from 90 PDI flour was 2.45 MPa at 150°C (using an 18 m tube) and for 20 PDI flour, 2.10 MPa at 185°C (using a 0.4 m tube). Interestingly, these maximal values are comparable to that found when carbohydrates were added to CSPI 974 (Lorenz et al., 2015), suggesting that the presence of carbohydrates may not interfere with soy protein cohesive strength development during jet cooking. Though probably not sufficient on its own, the improved wet strength from jet cooking flour might increase competitiveness of commercial formulations by requiring less co-reactant to achieve wet strength targets.

Dry strength values are also impacted by jet cooking, as shown in Table 3. The native-state 90 PDI flour went from 5.23 MPa (uncooked control) to above 6 MPa with jet cooking. The 90 PDI jet cooked at 150°C had higher ($p = 0.014$) dry strength (6.84 MPa) than those cooked at higher temperatures (6.26 MPa). In contrast, jet cooking the previously denatured 20 PDI did not result in any change, staying at ~6.4 MPa. Tube length was not significant for either flour.

PPSPI dry strength improved significantly, from 5.14 MPa (uncooked control) to 7.81 MPa after most jet cooking treatments. As with the wet strength values, the dry strength for the longest tube was significantly lower at 7.31 MPa, but there was no dependence on temperature.

Jet cooking and viscosity

Table 4 shows the viscosity values, which reflect the changes in protein (and carbohydrate, if present) structure by jet cooking. Jet cooked PPSPI gelled on cooling so viscosity could not be measured. The viscosity of flour always increased at least 6-fold with jet cooking. Using T-testing to identify significant differences revealed that 20 PDI flour has higher viscosity when jet cooked above 140°C, resulting in two groups: one at 30,000 cP (120–140°C), and the other at 73,000 cP (166–185°C). The value for the jet cooked 90 PDI flour (61,000 cP) was not significantly different than the higher temperature 20 PDI flour results. Insoluble carbohydrates likely contributed substantially to flour viscosity with jet cooking, since isolated insoluble carbohydrates exhibited extremely high viscosities when autoclaved (Table 1). There was some correlation between wet strength and viscosity of jet cooked 90 PDI flour ($R^2 = 0.69$, $N = 21$), but not with jet cooked 20 PDI flour ($R^2 = 0.14$, $N = 42$).

While both hydrothermal treatments dramatically increased viscosity, jet cooking had a larger effect than autoclaving. The 20 and 90 PDI flour viscosities were ~2–4× higher with jet cooking (Table 4) than autoclaving (Table 1), even though jet cooked values were recorded at higher shear rates and soy dispersions are shear thinning (see initial viscosity with spindle 3, and 10 vs. 20 rpm in appendices). Also, PPSPI was gelled with jet cooking, but the viscosity was only 4000 cP after autoclaving (Table 1). Gels are commonly formed when soy proteins are heated in water (Renkema & van Vliet, 2002), but carbohydrates are known to interfere with this process. It is difficult to analyze the effect of heating on flour protein viscosity because insoluble carbohydrates in flour (~20% of flour mass, [Karr-Lilienthal et al., 2005]), including starch, are likely contributing significantly to the viscosity after hydrothermal treatments.

Summary of factors influencing wet strength

Figure 4 graphically summarizes the effects of various treatments on the wet strength of soy adhesives, when

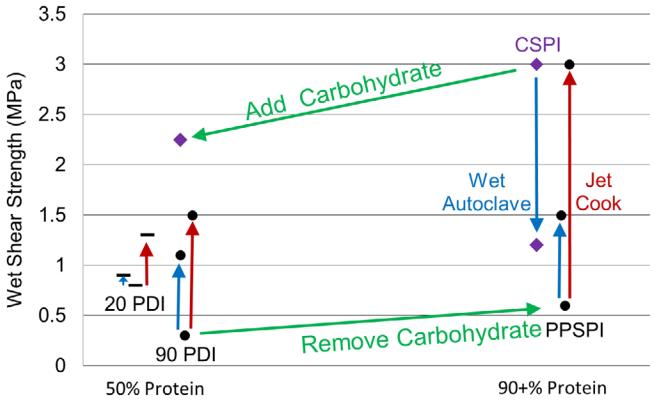

FIGURE 4 The wet strengths of bonds made with different soy formulations with emphasis on protein content from 50% for flour to 95% for SPI, and exposure to different heating conditions. Starting materials: Black circle, 90 PDI flour; black bar, 20 PDI flour; purple diamond, CSPI PROFAM® 974. *Source*: "Add carbohydrate" data are from Lorenz et al. (2015)

pressed at 120°C. **Black circles** (•) indicate 90 PDI flour as the starting material, in the bottom left corner, with ~50% protein content and low wet strength. **Black dashes** (–) indicate 20 PDI starting material. **Purple diamonds** (♦) indicate CSPI starting material, in the top right, with high protein content and high wet strength. Lorenz (2015) showed that adding carbohydrate to CSPI (see "Add Carbohydrate" label) did not produce a wet strength comparable to (uncooked control) 90 PDI flour. Likewise, uncooked PPSPI was marginally better than the 90 PDI flour it was made from (Remove carbohydrate), confirming the observation of Lorenz that higher protein content is clearly not the major factor responsible for the enhanced wet strength of commercial SPIs relative to flour. Dry thermal denaturation of soy during processing (20 PDI flour) improved wet strength relative to 90 PDI flour. Wet autoclaving (blue arrows) improved 90 PDI flour strength but had little impact on the previously denatured 20 PDI flour. Lab jet cooking (red arrows) of either 20 or 90 PDI flour gave the highest wet strength flour-based adhesives. For PPSPI, wet autoclave hydrothermal denaturation provided some strength improvement, but the hydrothermal treatment by jet cooking was much more effective, bringing PPSPI up to the level of the CSPI. Wet autoclaving significantly reduced the strength of commercially jet cooked CSPI, indicating that the advantage attained by jet cooking can be lost by subsequent hydrothermal treatment. This leads to two conclusions: (1) jet cooking is likely a significant contributor to the strength of commercial SPIs, and (2) proteins in commercial jet cooked isolates have quite different adhesive performance than protein in soy flour and, therefore, may not be appropriate models for many studies of soy adhesive properties.

The results of this study clearly show both that soy proteins are affected by hydrothermal treatment, and the treatment history of the proteins has a strong influence on the water resistance of the resulting adhesive. We propose that differences in the protein conformation and/or aggregation are the main causes of the differing bond performances in these experiments.

We further hypothesize that jet cooking induces a metastable state in the protein—a transient high strength-producing configuration that was lost with the autoclave treatment, a slow hydrothermal process. Two of the three commercial isolates, which had been previously jet cooked, also saw dramatic viscosity reductions after autoclaving (Table 1). The jet cooking conditions by the manufacturer are proprietary, so it could not be determined why one isolate did not reduce in viscosity after autoclaving.

Bond formation and strength development

We propose that increased wet strength of bonds formed at high temperature, as well as with jet cooked materials, could be a result of improved film coalescence. Film coalescence is the process where individual particles form a cohesive film through water loss and bond formation between particles. Here "particle" means any object in the soy adhesive dispersion, from a soy cell wall fragment to a single protein subunit to a large protein aggregate. Soy flour adhesives, when pressed at room temperature until dry, do not coalesce; they form a powder with no strength (data not shown). The dry strength data shows that under the bonding conditions in this study, all the soy materials coalesced or flocculated. Wet cohesive strength requires not only flocculation, but coalescence where intermolecular interactions bind particles, and these bonds persist when wet. The strongest of these would likely be polymer chain entanglement, followed by hydrophobic associations. Covalent bonds could also contribute but we do not see evidence for significant differences in the formation of covalent bonds in our samples and so did not address that question.

Protein interactions in water are primarily the result of hydrophobic associations (Petsko & Ringe, 2004), so it seems reasonable that hydrophobic interactions will play a large role in the wet strength of protein adhesives. Figure 2 and the literature show that the available heat-treated (lower PDI) soy flours have marginally better wet strength than native soy flour (Frihart & Satori, 2013). Autoclaving and jet cooking flour in this work improved wet strengths, supporting the hypothesis that protein aggregation state is important, and further, that water can influence aggregate formation, because autoclaving flour with no water does not improve the wet strength (data not shown). Ball-like clumps of aggregated protein as well as strands have

been observed after hot, wet treatments (Amagliani & Schmitt, 2017). We suspect that balls or clumps of multiple protein chains are the most common forms of protein aggregate when heated under dry conditions such as in the manufacture of toasted 20 PDI flour, where proteins would be expected to minimize their surface area. Hydrophobic interactions and possibly chain entanglement hold the ball together when placed in water. These interactions likely form during heat treatment and could contribute to wet strength if they persist after bond formation. This is consistent with the common belief that denaturation improves protein adhesive performance and explains the inverse relationship between flour PDI and wet strength (Figure 2, and other work Frihart & Satori, 2013; Zhang et al., 2018). These changes are supported by the viscosity data in that the ball like structures have lower viscosity than extended polymer chains. The contribution of preformed aggregates to cohesive strength has been discussed (Vnučec et al., 2017).

Chain entanglement occurs when polymers from adjacent particles inter-diffuse. The fact that increased heat, whether during bonding (Figures 2 and 3), or post-treatment (Frihart et al., 2016), invariably improves both wet and dry cohesive strength clearly shows that heat improves inter-particle bonding. While this could be solely from a better network of hydrophobic interactions, we believe the extra motion afforded by higher temperature bonding likely increases inter-particle chain entanglement.

Rapid quenching hypothesis

One hypothesis that could explain the jet cooking effect could be the rapid cooling during completion of the jet cook. We propose that the rapid cooling step of jet cooking could kinetically trap proteins in conformations favored by high temperature, and these conformations coalesce better than proteins that were given time to adjust their conformation to room temperature conditions, such as during autoclaving. In the jet cooker, above the denaturation temperature (Sun, 2005c) and plasticized by water, proteins will be somewhat extended, as favored by entropy. The high shear in the tube and at the outlet of the jet cooker might also contribute to protein extension. The high shear environment in the jet cooker will tend to align protein chains, favoring inter-chain associations over the intra-chain associations that typically dominate protein structure.

Starting in this extended and possibly aligned state, they are quickly cooled in an environment with strong intermolecular associations. Like quench-cooling, the polymers have such restricted mobility and limited time that they do not reconfigure to lower energy states, resulting in energetically unfavorable molecular interactions. Later, during bond formation, the groups with unfavorable interactions will be much more likely to reconfigure and form new, favorable interactions with adjacent proteins, because the existing interactions are relatively weak. Critically, the new interactions could form bonds between particles. For example, polymer segments could be trapped in strained configurations during quench-cooling, primed to move during bond formation. This added molecular mobility during bond formation could result in chain entanglements between particles or increase the likelihood of finding favorable interactions between adjacent particles. In addition, proteins in a more extended conformation will have a larger surface area, leading to a larger fraction of inter- rather than intramolecular contacts, further contributing to bonding.

The quench-cooling hypothesis is primarily supported by the large increase in wet and dry strength of PPSPI with jet cooking (Tables 3 and 4). The autoclaving data (Figures 2 and 3) is consistent with the idea that autoclaving and slow cooling of commercial SPIs allowed the proteins to adopt more compact conformations, resulting in fewer inter-chain and inter-particle interactions leading to reduced wet and dry strength compared to jet cooking. Also, the lower strength of autoclaved flour relative to jet cooked flour (Figure 4) is consistent with this hypothesis. The quench cooling hypothesis is also consistent with the commercial application of jet cooking. Different jet cooking process conditions result in a wide variety of products optimized for diverse functions such as foam stabilization, gelation, or solubility (Egbert, 2004). The variety of physical characteristics of the same chemical structures under different jet cooking conditions is another indication that jet cooked proteins are trapped in metastable states.

We have presented some hypotheses that attempt to explain the large strength improvement associated with jet cooking of soy materials. Other mechanisms are certainly possible, including higher hydrophobicity at the protein or aggregate surface, larger aggregate sizes, chemical changes suggested by the rise in pH or others. While this work shows the clear impact of jet cooking on bond performance, further studies will be needed to test these proposed mechanistic hypotheses. Our hope is that through understanding the weaknesses of soy adhesives, and mechanisms for overcoming them, we can illuminate mechanisms for improving the industrial use of these materials.

CONCLUSIONS

The role of jet cooking in the development of soy adhesive wet strength was investigated because protein concentration could not account for the strength differences observed between soy flour and commercial soy protein isolate (Figure 4). Jet cooking of pilot plant soy protein isolate brought its wet strength up to the level of commercial, jet cooked isolates. The large effect of jet

cooking suggests that the proteins in commercial soy protein isolates, which are almost always jet cooked, have very different states of aggregation than the proteins in soy flour typically used in industrial adhesives. This brings into question the validity of soy adhesive research that uses the commercial isolate as a model for the proteins in flour. It also reinforces the point that denaturation is a very vague term, and one needs to define specifically the process for denaturation before the impacts on performance can be estimated. Despite the positive attributes of jet cooked soy, processing costs and a significant increase in viscosity make commercial application of jet cooking in adhesives unlikely.

We propose several factors that, if induced by jet cooking, could contribute to the wet cohesive strength of jet-cooked soy protein materials, including: (1) The fast cooling of jet-cooked material could trap the proteins in conformations with some nonequilibrium associations, similar to quench-cooling. During bond formation, proteins in these states are more likely to form new associations, which can be inter-particle, thus increasing cohesive strength. (2) Quench-cooled proteins are likely to be more extended with more exposed surface, thus increasing the number of inter-particle interactions and improving the possibility of chain entanglement between proteins of different particles. (3) The high shear inside a jet cooker will increase protein chain extension and alignment, resulting in more inter-chain interactions, improving film cohesion. (4) Large protein aggregates formed by many denaturation processes contain internal water-resistant interactions which could contribute to wet cohesive strength.

AUTHOR CONTRIBUTIONS

Conceptualization: Christopher G. Hunt, Linda F. Lorenz, Charles R. Frihart; *Methodology*: Christopher G. Hunt, Linda F. Lorenz, Carl J. Houtman, Charles R. Frihart; *Investigation*: *all Formal Analysis*: Christopher G. Hunt, Carl J. Houtman; *Writing original*: Christopher G. Hunt, Carl J. Houtman, Charles R. Frihart; *Editing: all*; *Supervision/administration*: Christopher G. Hunt; *Funding*: Christopher G. Hunt, Charles R. Frihart.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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