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A novel water-based process produces eco-friendly bio-adhesive made from green cross-linked soybean soluble polysaccharide and soy protein

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Highlights

2. Polysaccharide is crosslinked by a green process to enhance hydrophobicity.
3. Combination of CL-SPSS and SPI formed a stable interpenetrating network structure.
4. Using CL-SSPS creates a smooth and compact cross section of the cured adhesive.
5. Using CL-SSPS improves the water resistance of adhesive by 67.8%.
ABSTRACT

In this study, an eco-friendly soy protein adhesive was developed that utilized two components from soybean meal without addition of any toxic material. A plant-based, water-soluble and inexpensive soybean soluble polysaccharide was used as the novel renewable material to combine with soy protein to produce a soy protein adhesive. Three-plywood was fabricated with the resulting adhesive, and its wet shear strength was measured. The results showed the wet shear strength of plywood bonded by the adhesive reached 0.99 MPa, meeting the water resistance requirement for interior use panels. This improvement was attributed to the following reasons: 1) Combination of cross-linked soybean soluble polysaccharide and soy protein formed an interpenetrating network structure, improving the thermal stability and water resistance of the cured adhesive. 2) Adding CL-SSPS decreased the adhesive viscosity to 15.14 Pa·s, which increased the amount of the adhesive that penetrate the wood’s surface and formed more interlocks.

*Keywords:* green process; cross-linked soybean soluble polysaccharide; bio-adhesive; sodium hexametaphosphate; soy protein water resistance
1. Introduction

Soybean meals have been used as wood adhesives for many years. Early in the 20th century, soybean meals began to be used in the wood composite industry and interior use plywood. Soybeans are the first de-hulled and extracted with hexane to remove the valuable oil, leaving soybean meal that is ground to a fine flour and then dispersed in water at pH greater than 11 (Loman and Ju, 2016). The resulting soybean meal-based adhesive could be cold or hot pressed to bond the plywood. As a natural biomass, soybean is considered a promising material in the wood industry at present because it is abundant, inexpensive, renewable, and naturally fire resistant (Sun, 2005; Mo and Sun, 2013). However, the use of conventional soybean meal-based adhesive has been limited due to its low water resistance and high viscosity.

There have been many attempts to improve the water resistance properties by the modification of soybean meal adhesive, including denaturation, cross-linking agents, synthetic resin modification, nano-material modification and biomimetic modification (Gui et al., 2013; Iman et al., 2013; Liu et al., 2010; Qi et al., 2011; Yue-Hong et al., 2015). Many of these methods have effectively improved the properties of soybean meal adhesives. The use of polyamidoamine-epichlorohydin (PAE) resin as a co-reactant has also been effective in increasing the wet bond strength of soy protein-based adhesives and led to an increase in the use of soybean meal-based adhesives (Jang and Li, 2015). The significant commercial success of soybean/epoxide adhesive has stimulated further development and commercial application of soybean meal-based adhesives (Luo et al., 2015). However, these methods require cross-linkers or
additives derived from non-renewable petrochemicals. Additionally, most of these methods are based on strategies for modifying soy protein to yield improved bonding strength and water resistance. Soybean meals are the least expensive of the refined soy materials used as adhesives, but are also the most complex material, composed of approximately half protein and one-third carbohydrate, as shown in Table S1 (Frihart and Birkeland, 2014). The carbohydrates are likely a main contributor to the poor water resistance of bonded wood products. However, there has been little study of how to modify the carbohydrate to enhance the water resistance and bonding strength of soybean meal-based adhesives (Chen et al., 2012). The carbohydrate material includes a variety of soluble polysaccharides, such as sucrose, raffinose and stachyose, and insoluble components, such as rhamnose, arabinose, galactose, galacturonic acid, glucose, and xylose (Chivero et al., 2015; Pierce et al., 2016). Soybean soluble polysaccharide, a by-product obtained during tofu and soy protein production, is a major component of the carbohydrates material and has a pectin-like structure (Hosny et al., 2015). Structure analysis using the ratio of static to dynamic light scattering parameters revealed that soybean soluble polysaccharide has a highly branched globular shape (Chivero et al., 2016). It has been suggested that soybean soluble polysaccharide could be used in flavor emulsions because of its high water solubility, low bulk viscosity, high temperature stability, emulsifying properties, and its ability to form strong interfacial films (Chivero et al., 2014). Additionally, the high molecular weight of the soluble polysaccharide of soybean imparts the cohesive strength required for a useful adhesive (Maeda et al., 2000). However, there may be drawbacks to the use of this material. One concern is whether the presence of soybean soluble
polysaccharide may interfere with protein-protein interactions to consequently lower bond strength, especially under wet test conditions (Frihart and Birkeland, 2014). Additionally, it is important to test altered forms of the soybean soluble polysaccharide to determine if modification methods that do not rely on organic solvents could similarly improve the material properties. Cross-linked soybean soluble polysaccharide by a green water-based process as a bio-based resource was extended to enhance the water resistance of soy protein adhesive. As we move towards ‘sustainable’ products, clean syntheses, use of biomass reagents and water-based processing become highly desirable.

In this study, the soybean meal-based adhesive was prepared using a novel, green and water-based 2-step process without any toxic additives as shown in Scheme S1. In the first step the soybean soluble polysaccharide was cross-linked to form a network structure using green cross-linker sodium hexametaphosphate (SHMP). The differences between the cross-linked SSPS (CL-SSPS) and the untreated material were characterized using Fourier-transform infrared spectroscopy (FTIR) and thermogravimetric analysis (TGA). The crystallinity was evaluated using X-ray Diffraction (XRD). Next, the cross-linked soybean soluble polysaccharide (CL-SSPS) was used to conjugate soy protein to produce a soybean meal-based adhesive. Three-ply plywood specimen was fabricated to measure the wet shear strength according to Chinese National Standards (GB/T 17657-1999). The mechanisms of the influences of CL-SSPS on the specimens were characterized using Fourier-transform infrared (FTIR) and thermogravimetric analysis (TGA). The present results will guide the further development of soybean meal-based adhesives and can be extended to the preparation of other bio-based polymers using soybean soluble polysaccharide
as the raw material. And the use of protein-polysaccharide bio-based adhesive as drug delivery systems would be highly useful (Cheng et al., 2016; Ding and Yao, 2013).

2. Materials and methods

2.1 Materials

Soy protein isolate (≥ 90% protein, ≤7% moisture, ≤ 6% ash, ≤ 1% fiber, ≥ 85-90% NSI) was provided by Yuwang Ecological Food Industry Company in the Shangdong Province of China, and then milled to 200-mesh flour (SPI). Sodium hexametaphosphate (SHMP), sodium hydroxide (NaOH) and hydrogen chloride (HCl) were analytical grade and from Tianjin Chemical Reagent Co. Soybean soluble polysaccharide (SSPS) was obtained from the Beijing Buerfuji Co. Ltd. Poplar veneer (40 cm × 40 cm × 1.5 cm, 8% moisture content) was obtained from We Nan, Hebei Province of China. Deionized water used in all experiments.

2.2 Preparation of SSPS and cross-linked SSPS gels

Soybean soluble polysaccharide (5 g) was dissolved in deionized water (45g) and then stirred for 1 h at 25 °C to form a homogeneous system. Due to the low purity of industrial grade soybean polysaccharide in this experiment, the polysaccharide solution need to be further purification. The undissolved component was removed by centrifugation at 8000 rpm for 10 min, and the supernatant (45.39 g) was collected as the soluble polysaccharide fraction. The PH was maintained under neutral conditions. And then the polysaccharide gel was dried in an oven at 80 °C, grounding into a powder to obtained the soybean soluble polysaccharide (SSPS), recording as Gel a. The solid content of
polysaccharide gel was directly calculated according to the powder weight of cured soybean soluble polysaccharide gel. The average value of three parallel samples was \(9.5 \pm 0.1\%\).

To create cross-linked polysaccharide gel, soybean soluble polysaccharide (5g) and different amounts of sodium hexametaphosphate (0.75g, 1.5g, 2.25g) were dissolved in deionized water and mixed for 1 h, followed by PH adjustment to 6.8-7.2. The total amount of the adhesive always remained at 50g. Then the dispersion was centrifuged at 8000 rpm for 10 min, and the supernatants were collected and dried, yielding the cross-linked soybean soluble polysaccharide (CL-SSPS). The weights of the three supernatants were 47.14 g, 46.96 g, 46.72 g, respectively. CL-SSPS materials were sealed and stored at 4 °C until further use.

The resulting gels were recorded as gel b, gel c and gel d, respectively. The solid content of gels were directly calculated according to the powder weight of cured cross-linked soybean soluble polysaccharide gel. The value of solid content of the gels were \(10.56 \pm 0.05\%\), \(13.11 \pm 0.2\%\), \(14.60 \pm 0.1\%\), respective.

### 2.3 Characterization of SSPS conjugates (FTIR, TG, XRD and NMR)

The samples were prepared at 120 ± 2 °C in an oven until they reached a constant weight, and then were ground into 200 mesh powder (0.075 mm). The ATR-FTIR spectra were scanned using a Thermo Nicolet 6700 FT-IR (USA) with an ATR accessory from 400 to 4000 cm\(^{-1}\) at a resolution of 4 cm\(^{-1}\) with 32 scans. The XRD spectra were recorded on a D8 advance diffractometer (Bruker, U.S.A) using a cobalt source and 0.02 theta scan ranging from 5° to 60° at 45 kV and 30 mA. The crystallinity of powder samples were calculated by the included software of BRUKER, DIFFRAC.EVA V 3.1. The area method based on the patterns
was adopted by equation (3) and (4). Each adhesive formulation was tested three times and the average value was reported. TGA spectroscopy experiments were performed using a TA Q50 instrument (WATERS Company, USA). About 5 mg of powdered samples were weighed in a platinum cup and scanned from 30 to 600 °C at a heating rate of 10 °C/min in a nitrogen environment while recording the weight change. The thermogravimetric parameters were analyzed using the Universal Analysis 2000 software. The 31P NMR analysis was demonstrated on a Bruker AVANCE III HD 500 spectrometer at 9.4 T, with frequencies of 202.45 MHz. In the 31P NMR analysis, for samples dissolved in D2O, the test temperature maintained at room temperature. Experiments were performed using a 15.4 µs 90° pulse and 32 k data points were recorded over a spectral width of 82 kHz.

\[
\text{Amorphous (%) } = \frac{\text{Global area} - \text{Reduced area}}{\text{Global area}} \times 100\%
\]

Eq. (1)

\[
\text{Crystallinity (%) } = 100\% - \%\text{Amorphous}
\]

Eq. (2)

### 2.4 Preparation of soybean meal-based adhesive

Soy protein flour was milled to 200-mesh flour (SM) using a laboratory grinder. For the SPI adhesive (adhesive 1), soy protein flour (13.5g) was added into deionized water (86.5g) and stirred for 30 min at 20 °C to form a homogeneous system. For the SPI/SHMP adhesive (adhesive 2), SHMP (3g) was added and stirred for 30 min. For the SPI/SSPS adhesive (adhesive 3), SSPS (9g) was dissolved into deionized water (77.5g) and mixed for 10 min before soy protein
flour (13.5g) were added to the solution. To make the SSPS-fortified adhesive, soybean soluble polysaccharide (9g) and different amounts of sodium hexametaphosphate (1.5g, 3g, 4.5g) were dissolved in deionized water and mixed for 30 min before the addition of soy protein to the solution, followed by PH adjustment to 6.8-7.2. Next, soy protein flour (13.5g) was added and stirred for 30 min at 20 °C to develop the soy protein adhesive. The total amount of the adhesive always remained at 100g (free protein content was 12.15%). The relative humidity of the environment was about 45%. The resulting adhesives were recorded as adhesive 4, adhesive 5 and adhesive 6, respectively.

2.5 Preparation of plywood samples

The developed soy protein adhesives were applied to the glue line of the three-layer plywood in order to evaluate the effectiveness of the adhesives. The adhesive was applied at 180 g/m² for a single veneer surface. The coated veneer was stacked between two uncoated veneers so that the grain direction of the two adjacent veneers was perpendicular to each other. The assembled three-layered plywood were hot-pressed at 120 °C and 1.0 MPa for 6 min. After hot pressing, the plywood samples were stored under ambient conditions for at least 12 h before testing.

2.6 Dynamic viscoelastic measurement

The viscosity of the fresh adhesives was determined using a rheometer with a parallel plate fixture (20 mm diameter). The distance was set to 1mm for all measurements. The experiments were conducted under a steady shear flow at 25 °C with a spinning rate of 2 rpm. All measurements were determined by
averaging 5 measurements over 2 minutes.

2.7 Wet shear strength Measurement

The requirement of the interior use plywood (Type II plywood) was determined using a wet shear strength test in accordance with the description in the China National Standards (GB/T 17657-1999). Twelve plywood specimens (2.5 cm × 10 cm) were cut from two plywood panels and submerged into water at 63 ± 2 °C for 3 h, and then dried at room temperature for 10 min before a tension test. The wet shear strength was calculated by the following equation.

\[
\text{Bond Strength (MPa)} = \frac{\text{Tension Force(N)}}{\text{Gluing Area(m}^2\text{)}} \times 100\%
\]

Eq. (3)

2.8 Characterization of adhesive samples

Fourier Transform Infrared (FTIR) Spectroscopy

Samples of the modified soy protein adhesive were ground into powder and used for FTIR characterization. The powders were mixed with potassium bromide at a ratio of 1:100, and then the mixture was pressed in a special mold at 12 MPa for 1 min to obtain a pressed powder sample. The FTIR spectra of the cured adhesives were recorded on a Nicolet 7600 spectrometer (Nicolet Instrument Corporation, Madison, WI) from 500 to 4000 cm\(^{-1}\) with 4 cm\(^{-1}\) resolution using 32 scans.

Thermogravimetric (TG) measurement

The thermal stability of the cured adhesive samples was tested using a TGA
instrument (TA Q50, WATERS Company, USA). About 5 mg powdered samples were weighed in a platinum cup and scanned from 30 to 600 °C at a heating rate of 10 °C/min in a nitrogen environment while recording the weight change. The thermogravimetric parameters were analyzed using the Universal Analysis 2000 software.

**Scanning Electron Microscopy (SEM)**

Adhesive samples were placed in an oven at 120 ± 2 °C until reaching a constant weight. To begin testing, the samples were coated with 10 nm Au/Pd film using a Q150T S Turbo-Pumped Sputter Coater/Carbon Coater (Quorum Technologies Ltd., UK). A Hitachi S-3400N (Hitachi Science System, Ibaraki, Japan) scanning electron microscope was used to observe the fractured surface of the adhesive.

**Moisture uptake measurement**

The moisture uptake measurement of the cured adhesive was determined by gravimetric analysis. Six pieces of cured adhesive were placed into a constant temperature humidity chamber with 80% humidity and 50 °C temperature. The weight of the adhesive chunks was measured every two hours until a constant value was obtained. The moisture uptake value was calculated by the following equation, where $\alpha$ is the value of the adhesive before treatment and $\beta$ is the value of the adhesive after treatment.

$$\text{Moisture uptake value (\%)} = \frac{\text{Weight}(\beta) - \text{Weight}(\alpha)}{\text{Weight}(\alpha)} \times 100\%$$

Eq. (4)
3. Results and discussion

3.1 FTIR spectroscopic and $31^P$ NMR analysis of SSPS/CL-SSPS

Fig.1. (A) FTIR spectra of the different gel samples: a (SSPS), b (SSPS/SHMP-1.5 wt%), c (SSPS/SHMP-3 wt%), d (SSPS/SHMP-4.5 wt%) e (SHMP). (B) The X-ray diffraction patterns of different gel samples: a (SSPS), b (SSPS/SHMP-1.5 wt%), c (SSPS/SHMP-3 wt%), d (SSPS/SHMP-4.5 wt%), e (SHMP). (C) The theromgravimetric (TG) and derivative thermogravimetic (DTG) curves of the different gel samples: a (SSPS), b (SSPS/SHMP-1.5 wt%), c (SSPS/SHMP-3 wt%), d (SSPS/SHMP-4.5 wt%), e (SHMP). (D) $31^P$ NMR spectra of the different gel samples: b (SSPS/SHMP-1.5 wt%), c (SSPS/SHMP-3 wt%), d (SSPS/SHMP-4.5 wt%), e (SHMP). (E) The cross-linking reactions between SHMP and SSPS.

FTIR spectroscopy allows study of the interactions between the cross-linker and polysaccharide and was used to investigate the characteristics of the cross-linked polysaccharide. The FTIR spectra of the soy soluble polysaccharide conjugates
are shown in Fig.1A. A peak observed at approximately 3303 cm\(^{-1}\) was assigned to the free bound O-H and N-H bending vibration.\(^2\) The strongest absorption at 1027 cm\(^{-1}\) was observed in the SSPS sample, resulting from the rhamnogalacturonan moiety (KačUráková et al., 2000). The band in 1630–1600 cm\(^{-1}\) is derived from C=O stretching vibration (Chatjigakis et al., 1998). The bands corresponding to the stretching vibration of COO\(^-\) were located at 1410 cm\(^{-1}\) (Monsoor, 2005). The peak at 888 cm\(^{-1}\) suggested that soybean soluble polysaccharide gel molecules were linked by β-glycoside bonds (Zong-Cai et al., 2011).

Comparison of spectra of adhesive a and b revealed that the peaks at 1410 cm\(^{-1}\) and 1036 cm\(^{-1}\) were gradually weakened due to the reaction between the SHMP and the –OH groups on the soybean soluble polysaccharide molecules. The peak shifted from 1608 to 1621 cm\(^{-1}\) (blue shift) in the spectrum of the gel with SHMP, indicating that the cross-linked soybean soluble polysaccharide formed more stable chemical linkage, having more dense structure than that in the SSPS gel. When SSPS is crosslinked and form a network, the structure become dense and ordered, so that, the vibration of functions need more energy, which presents a blue shift. A similar blue shift is observed at the FTIR spectrum in the Dobritoiu and Luo’s research (Dobritoiu and Patachia, 2013; Luo et al., 2015). This is in agreement with gel crystallinity decrease. This dense structure was caused by the chemical reaction between SHMP and SSPS and increased the cross-linking density of adhesive. Interestingly, a new peak was formed at 1260 cm\(^{-1}\), attributed to C-O stretching (Yang et al., 2015). This indicated that SHMP might have reacted with soybean polysaccharide to form a network structure to increase the degree of cross-linking. Phosphorus 31 NMR is a good technique to
point out some differences in the structural built-up of the four SSPS samples. The one-dimensional proton NMR revealed a by comparing the $^{31}$P NMR spectra of SHMP, SSPS/SHMP-1.5 wt%, SSPS/SHMP-3 wt%, and SSPS/SHMP-4.5 wt% samples in Fig.1D. The spectra of SHMP showed two strong characteristic peaks at $\delta=-9.71$ and $\delta=-21.52$, two peaks were the overlapping SHMPt peak caused by the terminal P atom and the overlapping SHMPc peak caused by the internal P atom, respectively (Peng et al., 2005). Because of the absence of phosphorous element in soybean soluble polysaccharide, there was no phosphorus peak in the $^{31}$P spectra of soybean soluble polysaccharide. From Fig.1D, two changes in the $^{31}$P NMR spectra have been detected. The peak at $\delta=-9.71$ was gradually weakened, while the peaks at $\delta=-21.52$ and $\delta=-23.57$ was almost disappeared. These phenomena indicated that there was a reaction between polysaccharide and sodium hexametaphosphate. This was consistent with the result of FTIR. The cross-linking network structure of SSPS/SHMP is illustrated in Fig.1E.

### 3.2 X-ray diffraction analysis

XRD was used to study the crystallinity of the SSPS sample and the results are shown in Fig.1B. The SSPS matrix showed a broad diffraction peak at $2\theta=17.7^\circ$, which indicated a high degree of crystallinity of the soybean soluble polysaccharide (Shi and Zhong, 2015). The crystallization peak was gradually weakened in the X-ray diffraction patterns after adding SHMP, which suggested that presence of SHMP affected the crystallinity of the gel. This confirms the results discussed above (FTIR analysis). As shown in Fig.1B, crystallinity decreased from 19.7 to 9.7% after adding 4.5 wt% SHMP to the adhesive sample, indicating that a reaction occurred between SHMP and the soybean soluble
polysaccharide that increased the cross-linking density of the cured gel, thus decreasing crystallinity (as shown in Fig.1B).

3.3 Thermogravimetric (TG) analysis

The thermogravimetric (TG) and derivate thermogravimetric (DTG) curves of the SSPS and CL-SSPS gel specimens are presented in Fig.1C. The gels both displayed a similar behavior in the first stage of weight loss. This stage was observed primarily between 50–200 °C and it is related to the loss of free and absorbed water. SSPS gel maximal thermal degradation peak was close to 248.54 °C. With the addition of SHMP, there was an apparent decrease in the degradation peak of the gels, indicating the generation of different structure in the gels after cross-linking. Additionally, the maximal degradation peak shifted to 254.14 °C in the spectra of gel b, indicating an increase in the thermal stability of the gel (Soares et al., 2005). Also evident in Fig.1C, the SHMP exhibited very little degradation, indicating that the treatment may increase the thermal stability of the gel system due to the stability of SHMP. The data of Fig.1C presents the thermogravimetric parameters for SSPS and CL-SSPS gels. After heating at 600 °C, the residual mass of the SSPS gel was 30.27%. And with the increase of SHMP, the residual mass of the gels was 44.99%, 52.10%, 60.83%. This result confirmed that the CL-SSPS gel had better thermal stability after forming the cross-linked structure (Ghosh Dastidar and Netravali, 2013).

Preparation of Modified Soy Protein Adhesive

Based on previous studies of the soybean meal-based adhesives, we designed a new formula that maintains the same proportions as soybean meal-based
adhesive in the specifications for the modified soy protein adhesive. The adhesive formulations are shown in Section 2.4. To confirm the positive effect of CL-SSPS on the soy protein-based adhesive, three-ply plywood samples were fabricated with the resulting adhesive, and the wet shear strength was measured.

3.4 Wood wettability of the different adhesive samples

Contact angle is a significant parameter of an adhesive’s affinity for an adherent, where smaller contact angle indicates better wettability. The contact angle of the soy protein isolate (SPI) adhesive was 115.8°, indicating that SPI adhesive had a poor wettability and surface tension. For a wood adhesive, the viscosity is also an important physical property governing the behavior of wood adhesives. The soy protein adhesive is a typical non-Newtonian fluid, where shows the images of contact angles of different adhesives on the wood substrate and the initial apparent viscosity of adhesive samples. With the addition of SSPS, the contact angle of adhesive 2 had an obvious reduction, reaching 80.6°. After adding SHMP (from 1.5 to 4.5%), the contact angle of the adhesive was gradually increased from 80.6° to 96.3°, respectively. Meanwhile, it could be founded that the initial apparent of adhesive samples followed a similar trend with the contact angles variation. The initial apparent viscosity of SPI adhesive was 22.07 Pa·s and the adhesive showed difficulties in flowing, making it difficult to fully distribution on the veneer. The use of high viscosity adhesive is unacceptable for wood composites, as it leads to difficulty penetrating the wood surface to allow insufficient mechanical bonding between the veneer pieces, resulting in a low bond strength. The viscosity of the SPI/SHMP adhesive was 24.02 Pa·s. The viscosity was maintained at a same level after addition of SHMP, indicating SHMP
had no effect on soy protein in the adhesive system. The viscosity was 5.59 Pa·s, when using the SPI-SSPS mixtures to prepare the adhesive. When SSPS was mixed with SPI, the acidic backbone of the soybean soluble polysaccharide was combined with the amino groups of the protein molecules. Thus, the whole mixture carried a negative charge and prevented the aggregation of protein particles, causing a significant reduction in viscosity (Xia and Abdalhai, 2015). This low viscosity could be attributed to phase separation. After adding SHMP, the viscosity of the adhesive 4 increased by 62.3% to 9.07 Pa·s. This is likely due to two reasons: Firstly, with the introduction of SHMP, the relative molecular size of the soybean soluble polysaccharide increased and the chain length increased, which result in an increase of viscosity. Second, when the SHMP was cross-linked with the polysaccharide, strong attraction between the protein aggregates and the larger volume of protein microgel particles led to phase separation and thus increased the viscosity of the adhesive (Shirashoji et al., 2010). When the content of SHMP reached 4.5 wt%, the viscosity of adhesive 6 further increased to 15.14 Pa·s, which is suitable for adherence to the industry standards in wood composites.

Table 1. The droplet picture on the wood substrate in 5 s after the deposition and apparent viscosity of the adhesive samples: 1(SPI adhesive), 2 (SPI/SHMP adhesive) 3 (SPI/SSPS adhesive), 4 (SPI/SSPS/SHMP-1.5 wt% adhesive), 5 (SPI/SSPS/SHMP-3 wt% adhesive), 6 (SPI/SSPS/SHMP- 4.5 wt% adhesive).
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3.5 Wet shear strength measurement

![Graph showing wet shear strength measurement](image)
Fig. 2. The wet shear strength of plywood panels with the different adhesives: 1 (SPI adhesive), 2 (SPI/SHMP adhesive), 3 (SPI/SSPS adhesive), 4 (SPI/SSPS/SHMP-1.5 wt% adhesive), 5 (SPI/SSPS/SHMP-3 wt% adhesive), 6 (SPI/SSPS/SHMP-4.5 wt% adhesive).

The wet shear strength measurements of the plywood bonded by the different adhesives are shown in Fig. 2. The wet shear strength of the plywood bonded by the SPI adhesive was 0.59 MPa, lower than the interior use panel bond strength requirement (≥0.7 MPa). The isolated protein can easily aggregate through polar and hydrophobic interactions that provide strength under both dry and wet conditions. However, this kind of bond is primarily based on intermolecular hydrogen bonding, which is easily broken by moisture, resulting in a low water resistance. When SHMP was added into the adhesive, the wet shear strength increased slightly to 0.62 MPa. Addition of SHMP could improve the uniformity of the soy protein in the adhesive system, thus enhancing the water resistance of the adhesive. With the addition of SSPS, the wet shear strength of the plywood bond by SPI/SSPS adhesives was 0.45 MPa, likely due to the weakening of hydrogen bonds under wet conditions, decreasing the bridging effect between protein molecules and soybean soluble polysaccharide. After the addition of SHMP, the wet shear strength of plywood bonded by the different adhesive formulations increased gradually. When 1.5 g SHMP was added to the adhesive, the wet shear strength increased by 18.7% to 0.70 MPa, sufficient to meet the interior use panel bond strength requirement. However, based on the standard deviation of the measurements of adhesive 4 in Fig. 2, the wet shear strength of the plywood specimens cannot be guaranteed over 0.70 MPa. After further adding SHMP to 3 g, the wet shear strength was improved to 0.99 MPa, an
increase of 67.8% compared to adhesive 1.

This significant increase in the wet shear strength can be attributed to the cross-linking reaction between SSPS and SHMP. Cross-linked soybean soluble polysaccharide and soy protein form a stable interpenetrating network structure to improve the water resistance. The introduction of SHMP increased adhesive flowability, promoting interlocking to improve the water resistance of the adhesive. However, by increasing the SHMP content to 4.5 g, the bond strength decreased to 0.85 MPa. This suggests that excess SHMP remains in the adhesive, affecting the formation of interpenetrating network structure and resulting in a reduction in strength.

3.6 FTIR spectroscopic analysis of the adhesive samples

![FTIR spectra of different adhesive samples](image)

Fig.3. FTIR spectra of different adhesive samples: 1 (SPI adhesive), 2 (SPI/SHMP...
adhesive), 3 (SPI/SSPS adhesive), 4 (SPI/SSPS/SHMP-1.5 wt% adhesive), 5 (SPI/SSPS/SHMP-3 wt% adhesive), 6 (SPI/SSPS/SHMP-4.5 wt% adhesive).

Fig. 3 shows the FTIR spectra of SPI and SPI + cross-linked polysaccharide conjugates. A typical soy protein spectrum shows three major peaks at 1663, 1518, and 1231 cm⁻¹, which are assigned to C=O stretching (amide I), N-H deformation (amide II), and C-N stretching and N-H vibration (amide III), respectively. There were no new peaks in the spectrum of adhesive 2, indicating that there are no reactions between SPI and SHMP. A simple mixture does not allow soy protein and polysaccharide to react, as shown in Fig. 3. As SHMP addition increased from 0 to 4.5 g in the adhesive formulation, the peak of amides III and I gradually decreased, indicating that the modified adhesives had a more dense structure than the SPI adhesive. This dense structure was caused by the conjugate of SPI and SSPS to form a solid structure, which increased the cross-linking degree of the adhesives (Tajik et al., 2013). No ester peak was detected at 1720-1730 cm⁻¹ for SPI combined with CL-SSPS, indicating that the conjugation took place without the formation of an ester linkage (Tran and Rousseau, 2013). As shown in Fig. 3, no new absorption bands were observed in the FTIR spectra, suggesting that no chemical interaction occurred between SPI and SSPS, only the formation of the interpenetrating network for increased stabilization as in Scheme 1.
Scheme 1 The interpenetrating network of the SPI/SSPS/SHMP adhesive

3.7 SEM analysis of the adhesive samples

Fig.4. The fracture surface micrograph of different cured adhesive samples: 1 (SPI adhesive), 2 (SPI/SHMP adhesive), 3 (SPI/SSPS adhesive), 4 (SPI/SSPS/SHMP-1.5 adhesive).
wt% adhesive), 5 (SPI/SSPS/SHMP-3 wt% adhesive), 6 (SPI/SSPS/SHMP-4.5 wt% adhesive). (The data* represent the moisture uptake of different cured adhesive samples)

Scanning electron micrographs of the fracture surface of the different cured adhesives are shown in Fig. 4. Irregularity of a large area with some cracks was observed on the fracture surface of the SPI adhesive. The entire fracture surface appeared loose and disordered due to water evaporation in the adhesive, which was easily intruded by moisture, resulting in low water resistance of the SPI adhesive. After SSPS addition, the fracture surface of the blended adhesive was rougher and the pore sizes were bigger. The bigger pore size of the adhesives with SSPS addition suggested that more protein dissolved in the water bound in the holes and less protein took part in gelation for a decrease in adhesive strength (Wang et al., 2013). After SHMP was added, fewer holes and cracks were observed, and the fracture surface of the cured adhesive became smoother and more compact. This result was attributed to the conjugate between SPI and SSPS forming an interpenetrating network that limited the evaporation of moisture, which resulted in the improvement of the water resistance of the adhesive (Salarbashi et al., 2015).

The moisture uptake of the different cured adhesive samples is also shown in Fig. 4. Adding SHMP to the adhesive sample, the moisture uptake of the cured adhesive decreased, suggesting the adhesive with SHMP efficiently prevented moisture intrusion and improved moisture resistance compared to adhesives lacking SMHP. This was in agreement with the results of Flauzino's research (Flauzino Neto et al., 2013). When incorporating 4.5 wt% of SHMP, the moisture uptake decreased from 6.9% to 3.2%. From Fig. 4, it could be concluded that
reduced surface roughness corresponded to improved moisture resistance of the cured adhesive. The smooth surface of the adhesive with SMHP was attributed to increased adhesive flowability after SMHP incorporation and the fact that SMHP conjugated with soy protein molecules to form a stable system.

3.8 Thermal stability of the adhesive samples

Fig.5 Thermogravimetric curves (TG) and derivate thermogravimetric (DTG) curves of different adhesive samples: 1 (SPI adhesive), 2 (SPI/SHMP adhesive), 3 (SPI/SSPS adhesive), 4 (SPI/SSPS/SHMP-1.5 wt% adhesive), 5 (SPI/SSPS/SHMP-3 wt% adhesive), 6 (SPI/SSPS/SHMP-4.5 wt% adhesive).

The thermal stabilities of the soy protein adhesive and modified soy protein adhesive were characterized using TGA analysis as shown in Fig.5 presents the TG and DTG curves of different adhesive samples. Soy protein thermal degradation started at 287.43 °C and the maximal degradation peak was around
288.54 °C. With the addition of SHMP, the mixture adhesive peaked at 321.40 °C, suggesting the presence of SHMP influenced thermal degradation. When SSPS was added, the initial and maximal degradation temperature decreased. A reduction in thermal stability can be promoted by changes in the protein structure, due to the rupture of low energy intermolecular bonds that maintain the protein conformation (Kamińska and Sionkowska, 1999). With SHMP in the soy protein adhesive formulation, the thermal degradation of the adhesive occurred in three stages. The first stage (I), over a temperature range of 70 °C to 200 °C, was related to the evaporation of residual moisture. The second stage (II) was the initial degradation stage, from 200 °C to 300 °C, and was weight loss due to small molecular degradation and the breakage of some unstable chemical bonds (Kumar et al., 2004). Comparing adhesive 3 and 4, adhesive 4 showed a new peak at 308.98 °C, which was attributed to the degradation of the skeleton structure. This result was possibly due to the breakage of C-C and C-O bonds. Upon increasing the SHMP further, the peak at stage (III) shifted to a high temperature, with a peak at 316.84 °C for adhesive 6, indicating that the thermal stability of the adhesive increased with SHMP addition. The increase in thermal stability was related to the interpenetrating network structure formation, resulting in an improvement of the adhesive water resistance. The residual mass (%) after heating at 600 °C, values were between 23 and 54% for all studied systems. These residual masses represent inorganic compounds derived from thermal degradation. The adhesive 6 exhibited the largest residual mass, indicating the excellent thermal stability of this adhesive system.

Table 2. Thermogravimetric parameters for soy protein adhesive samples
### 4. Conclusions

The present research proposes and tests a novel scheme for developing an eco-friendly soy protein adhesive without the use of a toxic cross-linker. The 2-step process consisted of cross-linking soybean soluble polysaccharide in soybean meal and then conjugating soy protein. The resulting modified SPI adhesive showed enhanced thermal and mechanical properties and reduced moisture absorption. The wet shear strength of plywood bonded by the adhesive reached 0.99 MPa, meeting the interior use plywood water resistance requirement. This improvement was attributed to the following reasons: (1) the formation of a stable interpenetrating network structure from the conjugation between the SPI and CL-SSPS; (2) creation of a smooth and compact fracture surface that prevented moisture intrusion; (3) the adhesive viscosity decreased by 31.4% to 15.14 Pa·s compared to SM adhesive. These results made the bonded plywood suitable for industrial application.

In the current research, this easy, environmentally, friendly and low-cost method to conjugate protein can be useful in making inexpensive bio-based adhesive
with enhanced water resistance. This technology also can be extended to the use of soybean meal carbohydrates for other green composites for many applications.

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**References**


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