# Bonding Performance of Plywood with Pig Blood Adhesives Prepared by a Novel Method

Keke Du,[a,b,c,§] Cheng Yong,[b,c,§] Mingjie Guan,[a,*] and Fan Zhang [a]

Renewable and eco-friendly pig blood adhesives (PBAs) were obtained by modifying PBAs with a mild alkaline solution and glutaraldehyde and exposing them to an ultrasound treatment. The PBAs were subjected to thermogravimetric (TG) analysis and Fourier transform infrared (FTIR) spectroscopy, and the bonding strength and bonding interface of plywood were analyzed to evaluate bonding performance. The results showed that the alkaline solution unfolded the structure of blood proteins, and the glutaraldehyde crosslinked the blood protein. In addition, the ultrasound greatly increased protein expansion under weak alkaline conditions. The thermal stability of the PBAs exposed to ultrasound treatment under weak alkaline conditions was improved, but the crosslinking agent was not used, and the permeability, which was analyzed by a fluorescence microscope, improved. Further, the average and effective penetration depths on the bonding interface increased 53% and 55%, respectively. The bonding strength of plywood prepared with modified PBAs was greatly improved and compliant with relevant requirements.

*Keywords: Pig blood adhesive; Ultrasound; Crosslinked structure; Bonding interface; Fluorescence*

*Contact information: a: College of Materials Science and Engineering, Nanjing Forestry University, Nanjing, Jiangsu Province 210037, China; b: Recycling Agriculture Research Center, Jiangsu Academy of Agricultural Sciences, Nanjing, Jiangsu Province 210014, China; c: Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization, Nanjing, Jiangsu Province 210014, China; §: Keke Du and Cheng Yong contributed to this work equally; *Corresponding author: mingjieguan@126.com*

## INTRODUCTION

At present, most adhesives used for the bonding of wood are formaldehyde-based, such as phenolic resin and melamine resin. These adhesives are nonrenewable, and formaldehyde represents a significant threat to human health (Irle and Bolton 1991; Gryta *et al.* 1996; Schmidt *et al.* 2006; Mo *et al.* 2015; Li *et al.* 2018). Due to recent increases in both environmental consciousness and waste production, biological adhesives based on renewable resources have become a popular research topic (Ferdosian *et al.* 2017).

Plant protein-based adhesives are some of the most representative of bio-based adhesives. Many studies have been carried out on various plant protein-based adhesives, such as corn protein (Santoni and Pizzo 2013), wheat protein (D'Amico *et al.* 2010; Nordqvist *et al.* 2012), and soy protein (Wang *et al.* 2008; Lin *et al.* 2012). Soy protein is the most widely used protein adhesive (Pizzi 2006). However, soy protein has a high viscosity, which limits the application of soy protein-based adhesives. In addition, the use of food crops for adhesive production may raise food shortage concerns, especially in countries with large populations, such as China.

Blood is a natural, biodegradable resource with low viscosity that is mainly derived from animal husbandry residues and byproducts of the meat processing industry. Blood has a high protein content and is primarily used as animal feed with low value added after

processing. In addition, blood has been used in eco-friendly composite materials, such as bio-plastics (Bier *et al.* 2012a,b; Low *et al.* 2012). Up to now there have been few studies on the preparation of adhesives from pig blood. Yang *et al.* (2006) compared the properties of protein-based phenolic resin adhesives modified by soy protein, peanut protein, and blood protein and found that the properties of blood protein-based phenolic resin adhesive were similar to those of pure phenolic resin adhesives. Li *et al.* (2018) prepared a blood meal-based adhesive modified by polyvinyl alcohol, sodium dodecyl sulfate, and three glycidyl diamine. The relevant properties of the modified blood meal-based adhesives were found to be superior to those of unmodified adhesives. However, the adhesives prepared in these studies were made with dried blood meal. Drying is an energy-intensive process, and the blood drying process is not necessary for the preparation of pig blood adhesive (PBA), which should be prepared with an economical and simple method. Lin and Gunasekaran (2010) used fresh cow blood as a raw material to prepare adhesives *via* alkali modification, and the adhesive bonding shear strength was found to be independent of the pH, and it was comparable to that of phenol formaldehyde in the dry condition. Unfortunately, Lin and Gunasekaran (2010) did not discuss the phenomenon in terms of microscopic mechanisms.

Recently, much attention has been paid to the chemical modification of blood proteins due to the maturity of soy protein adhesive technology. However, the excessive use of chemical reagents can cause great damage to the environment. In contrast, ultrasound treatment is a mild and eco-friendly modification method. Many studies have shown that ultrasound can destroy the quaternary and/or tertiary structure of proteins, produce small molecular subunits, and improve the solubility and emulsifying capability of proteins by cavitation (Suslick and Price 1999; He *et al.* 2005; Chen *et al.* 2011). Jambrak *et al.* (2014) reported that ultrasound treatment can decrease particle sizes and increase the specific free surface of proteins. Cheng *et al.* (2019) reported that the molecular interactions of proteins are enhanced by ultrasound pretreatment. These findings indicate that a small amount of alkali can unfold the secondary structure of proteins with ultrasonic waves. More importantly, the use of fewer chemicals can reduce environmental damage.

In this study, the fresh pig blood was modified using a facile method of adding a small amount of chemical reagents and using ultrasound to prepare protein adhesives. This approach not only avoids the energy consumption in the blood drying process, but it also reduces the damage of excess chemical reagent to the environment. Ultrasound treatment destroys the quaternary and tertiary structure of proteins, alkaline solution can promote the hydrolysis of proteins, and glutaraldehyde can improve the waterproof property of adhesives as a crosslinking agent. This study aimed to develop an eco-friendly adhesive for biomaterials using pig blood. The basic physical properties, thermal stability, functional groups, wet shear strength (WSS), and bonding interface of plywood prepared with PBAs were measured to investigate the performance and mechanism of the modified PBAs.

## EXPERIMENTAL

### Materials

Fresh pig blood with a pH of 7.36 was obtained from Xiaolingwei slaughterhouse in Nanjing, China. The blood was added with an appropriate amount of anticoagulant and stored at 4 °C for no more than a week. The NaOH and glutaraldehyde used were purchased from Beijing SLB Technology Co., Ltd. (Beijing, China). Poplar (*Populus tomentosa*

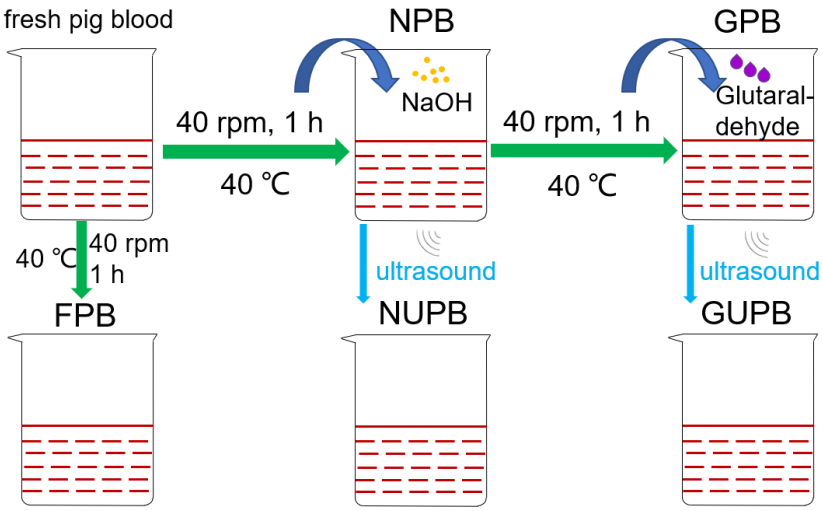
Carr.) veneers (50 cm × 50 cm × 0.2 cm) with a 9% moisture content were purchased from Guannan, Jiangsu Province, China.

## Preparation of Adhesives

Fresh pig blood (100 g) was stirred for 1 h at 40 °C and coded as FPB. NaOH (1 g) was added to the fresh pig blood (100 g), stirred for 1 h at 40 °C, and coded as NPB. Glutaraldehyde (1 g) was then added into the NPB, stirred for 1 h at 40 °C, and coded as GPB.

NaOH (1 g) was added to fresh pig blood (100 g), stirred for 1 h at 40 °C, exposed to ultrasound treatment (40 kHz, 500 W), and coded as NUPB. Glutaraldehyde (1 g) was then added into the NPB, stirred for 1 h at 40 °C, exposed to ultrasound treatment (40 kHz, 500 W), and coded as GUPB.

The production process of adhesive is shown in Scheme 1.




**Scheme 1.** Flow chart of the preparation of adhesives

## Determination of Basic Properties

The FPB, NPB, NUPB, GPB, and GUPB adhesives (20 mL of each sample) were separately placed in a test tube for 30 min to observe whether stratification or bubbles would be produced.

The solid content of each adhesive was measured according to GB/T 14074 (2006). Approximately 3 g (M) of adhesive was placed in an oven until a constant weight (m) was reached at a temperature of 100 ± 2 °C. The solid content was calculated with Eq. 1,

$$Solid\ content = m/M \times 100\% \quad (1)$$

where $m$ is constant weight (g) and $M$ is the mass (g) of the adhesive. All measurements were performed in triplicate.

The FPB, NPB, NUPB, GPB, and GUPB adhesives (20 mL of each sample) were placed in a test tube for 1 h, and then the pH was measured. All measurements were performed in triplicate.

## Methods

*Thermogravimetric analysis (TGA)*

The thermal degradation behavior of the pre-freeze dried adhesives was measured on a S II 7200 TGA instrument (Hitachi Ltd., Tokyo, Japan) with the following conditions: 5 mg of adhesive powder, a scanning temperature from 25 °C to 600 °C, and a heating rate of 10 °C/min under an $N_2$ atmosphere.

*Fourier transform infrared (FTIR) spectroscopy*

The adhesive samples were pre-freeze dried completely and ground into powder. Fourier-transform infrared spectroscopy was used to obtain spectra of the adhesives with a Tensor 27 infrared spectrometric analyzer (Bruker, Karlsruhe, Germany) using KBr pellets in the spectral range of 400 $cm^{-1}$ to 4000 $cm^{-1}$ with a 4 $cm^{-1}$ resolution using 32 scans.

*Wet shear strength (WSS) measurement*

The adhesives were applied to 3-ply poplar veneers (40 cm × 40 cm × 0.2 cm). The glue-spread of single glueline was 360 $g/m^2$ and hot-pressed at 120 °C for 5 min at 1.20 MPa in a QLB-D hydraulic press (Shanghai First Rubber Machinery Factory, Shanghai, China). The plywood samples were stored at a temperature of 21 to 23 ℃ and humidity of 60 to 63% for at least 24 h after hot pressing. The bonding shear strength of the plywood was measured in wet conditions according to GB/T 17657 (2013). The specimens (2.5 cm × 10 cm) were submerged in water at 63 ± 2 °C for 3 h and then dried at room temperature for 10 min, and their wet shear strengths were tested using a tensile machine (CMT5504, Shenzhen XinSanSi Co. Ltd., Shenzhen, China) at an operating speed of 10.0 mm/min.

*Morphology of the bonding interface*

A cross-section of 5 mm × 5 mm × 20 μm in size was created from each sample with a TU-213 ultramicrotome (Yamato Kohki Industrial Co., Ltd., Yamato, Japan) at room temperature after the specimens had been softened by soaking in water for 2 w. The cross-sections were then dehydrated with graded ethanol (30%, 50%, 75%, 95%, and 100%). Then, 0.5% of the fluorescent dye toluidine blue-O was added dropwise over 30 min, and each sample was cleaned twice with deionized water before observation. Finally, the sample was observed under a BX51 fluorescence microscope (Olympus Corporation, Tokyo, Japan). The microscopy images were processed with graphics processing software (ImageJ, National Institutes of Health, v1.8.0, Bethesda, America) to calculate the average penetration depth (AP), effective penetration depth (EP), and permeation area (*A*) of the adhesive layer. The AP was obtained by taking the average of the five maximum penetration depth values of the adhesive layer, *A* is the area of the adhesive on the entire fluorescent image, and *A* divided by the length of the adhesive line is the EP (Gavrilovic-Grmusa *et al.* 2016; Qin *et al.* 2016).

# RESULTS AND DISCUSSION

## Basic Performance of the Adhesives

Solid content is an important indicator for evaluating the quality of adhesives. Table 1 shows that FPB had the lowest solid content of 18.2%, and the solid content of NPB and GPB increased to 19.0% and 22.1%, respectively. The reasons for the increase of solid content may be the addition of solid NaOH and the evaporation of water during the

preparation of adhesives. The spherical structure of the protein molecule was destroyed, which exposed more active groups that were beneficial for further crosslinking modification of glutaraldehyde. The effect of ultrasound treatment on the solid content of PBA differed, as the solid content of NUPB was higher than that of NPB, and the solid content of GUPB was lower than that of GPB. This may have been due to an increase in soluble protein under cavitation and the thermal and mechanical action of ultrasound treatment, which could have caused enhanced protein solubility (Liu *et al.* 2011; Zhu 2015; Wu 2016). However, the mechanical action of ultrasound may destroy the crosslinked structure formed by protein and glutaraldehyde (Li *et al.* 2012). In spite of this, the solid contents of NUPB and GUBP were still higher than that of FPB, so ultrasound treatment was shown to be a feasible modification method. In addition, the pH of the PBAs decreased after modification with glutaraldehyde, probably because the amino groups covalently react with glutaraldehyde (Silva *et al.* 2004; Betancor *et al.* 2006).

**Table 1.** Basic Performance of the Adhesives

| Adhesive | Solid Content (%) | pH | Appearance |
|---|---|---|---|
| FPB | 18.2 | 7.4 | Fizzy and stratified |
| NPB | 19.0 | 7.7 | Not stratified |
| NUPB | 19.6 | 8.0 | Not stratified |
| GPB | 22.1 | 7.0 | Not stratified |
| GUPB | 21.3 | 7.1 | Not stratified |

## TG and DTG Analysis

As shown in the TG curve in Fig. 1(a), the thermal degradation of PBAs was divided into four stages: 0 °C to 120 °C, 120 to 230 °C, 230 °C to 400 °C, and above 400°C. The weight change in the first stage was related to the loss of free water and bound water, and the second stage was due to the separation of the protein quaternary structure and the decomposition of small molecules. The third stage was mainly due to the degradation of porcine blood protein skeleton structure and the non-covalent bond breaks at this stage. The fourth stage was due to the thermal decomposition of blood (Barreto *et al.* 2003; Soares *et al.* 2005; Schmidt *et al.* 2005; Schmidt and Soldi 2006). The TG curve shows that the residue weights of GUPB and NUPB were relatively large, which suggests that NUPB and GUPB had better heat resistance. This result may have been due to NUPB and GUPB generating more small molecules with good thermal stability under the action of ultrasound treatment.

The DTG curve shows that the highest degradation temperatures of FPB, NPB, and GPB gradually increased. This contrast indicated that some structural changes occurred in the composites. This may have been due to the degradation of peptide and amide bonds by NaOH, which exposes many reactive groups that can crosslink under the catalysis of NaOH. Then, the larger-formula-weight molecules were generated, and the stability of NPB was improved. The reaction continued to produce crosslinked structures after glutaraldehyde was added, and the thermal stability of GPB was further improved (Tian *et al.* 2016). The highest degradation temperature changes of NPB and GPB were not obvious after ultrasound modification. However, for NPB and GPB, the peak was shaper than NUPB and GUPB, respectively. It may be that ultrasound can be used to enhance protein-protein interactions *via* alterations to the protein structure; thus, there are more macromolecules inside NUPB and GUPB and their thermal stability improved (Li *et al*. 2012; Esteghlal *et al*. 2019; Pathak *et al.* 2020).

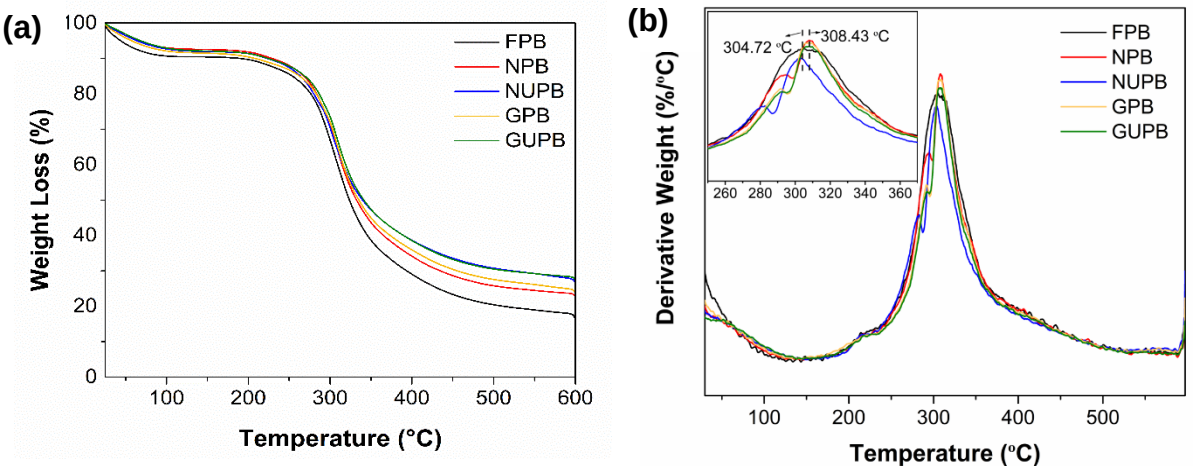



**Fig. 1.** TG (a) and DTG (b) curves of FPB, NPB, NUPB, GPB, and GUPB adhesives

## FTIR Spectrophotometric Analysis

Figure 2 shows the FTIR curves of the PBAs. Fourier-transform infrared analysis can reveal possible chemical and physical interactions in the adhesives. The wide peak at 3000 to 3500 $cm^{-1}$ in Fig. 2a was due to a large number of free N-H and O-H groups (Yuan *et al.* 2017). The half-peak width of GPB was much narrower than that of FPB, which indicates that the free amino acid residues in the protein were reduced and glutaraldehyde crosslinked with protein. NaOH destroyed the structure of the protein molecules and increased the number of free amino acid residues, which resulted in a wider half-peak width of NPB than that of FPB. The half-peak width of NUPB was narrower than that of NPB, and the half-peak width of GUPB was wider than that of GPB. This may have occurred because ultrasound can cause protein aggregation and reduce free amino acid residues but destroys crosslinked structures formed by glutaraldehyde and protein.

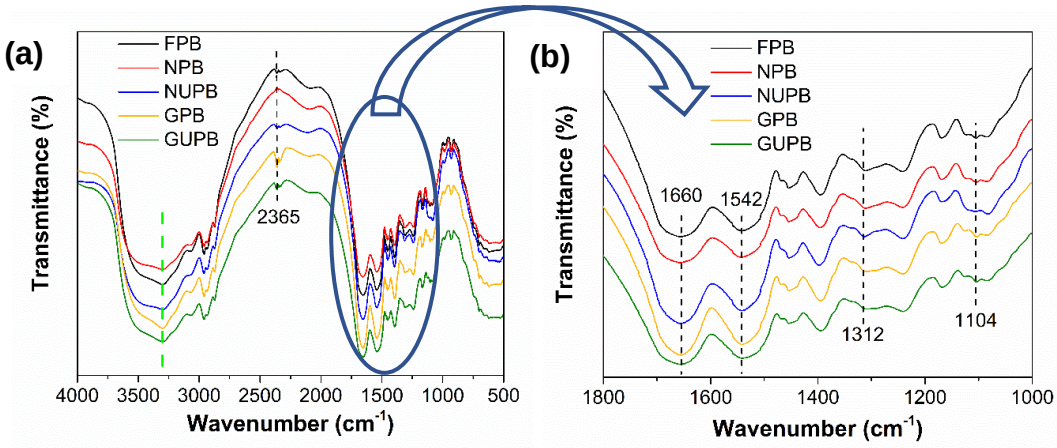



**Fig. 2.** The FTIR curves of FPB, NPB, NUPB, GPB, and GUPB adhesives

The characteristic absorption peaks of proteins are 1660, 1542, and 1312 $cm^{-1}$, which are the C=O stretching of amide I, the N-H bending of amide II, and the C-N stretching and N-H bending of amide III, respectively. The absorption peaks of GPB and GUPB were much weaker than those of FPB at 1312 $cm^{-1}$ (Fig. 2b), indicating that glutaraldehyde was crosslinked with protein. Compared with the spectrum of the FPB adhesive, the absorption peak of NUPB disappeared at 1104 $cm^{-1}$ (attributed to C-O

stretching), which may have occurred because the protein subunits were degraded by the ultrasound treatment. The reaction mechanism of NaOH, glutaraldehyde, and ultrasound treatment with pig blood protein molecules is shown in Fig. 3.

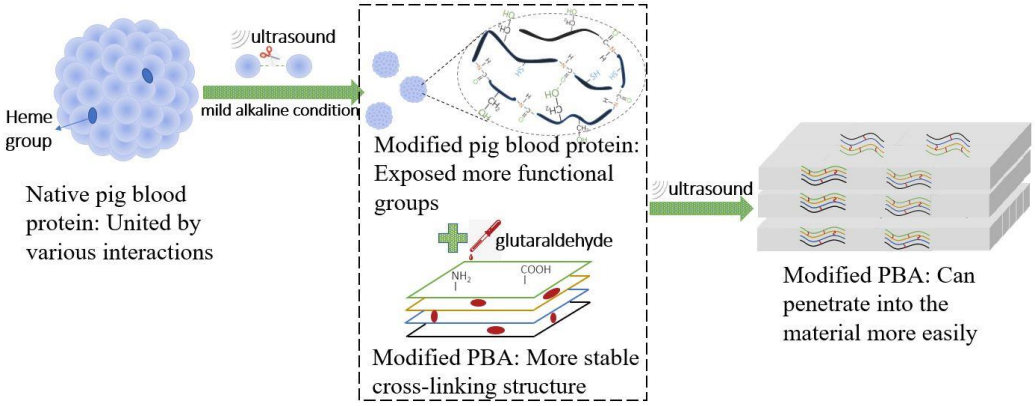



**Fig. 3.** A schematic illustration of the reaction mechanism of NaOH, glutaraldehyde, and ultrasound treatment with pig blood protein molecules

## Bonding Interface Analysis

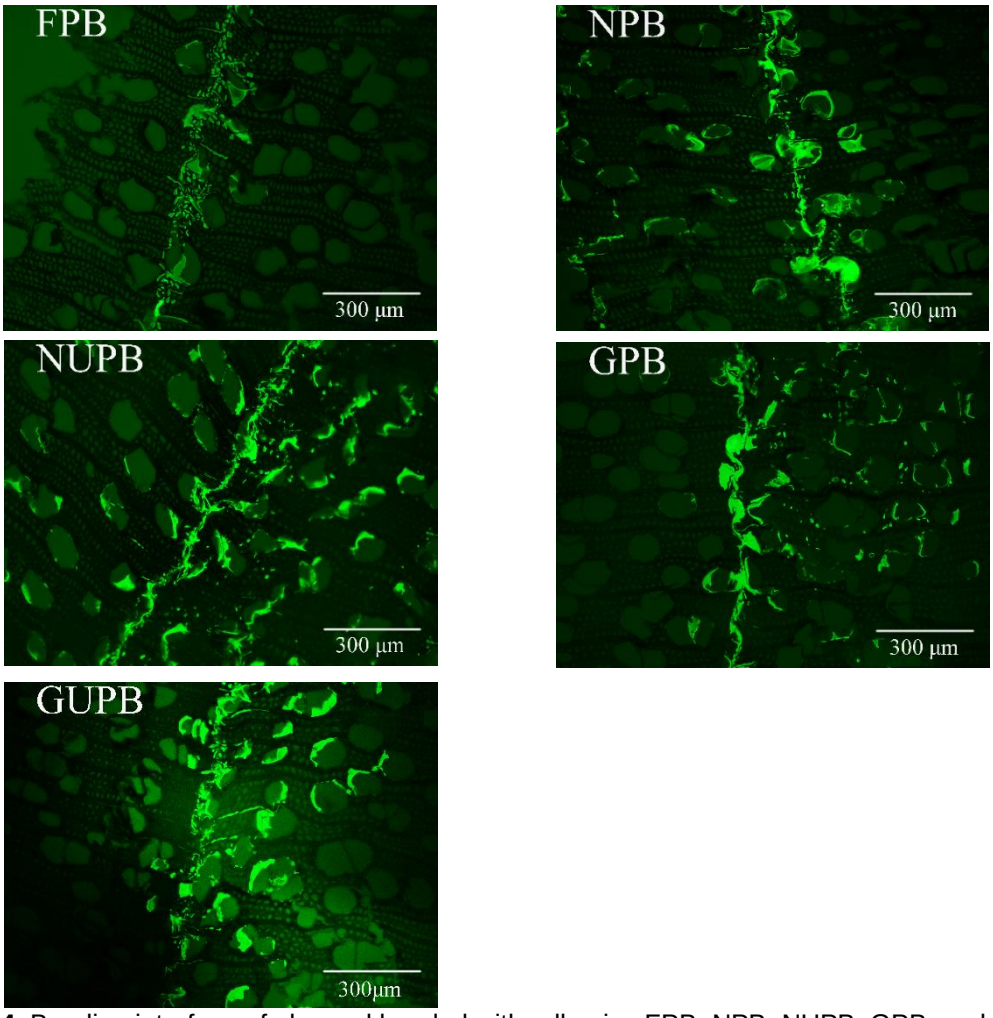



**Fig. 4.** Bonding interface of plywood bonded with adhesive FPB, NPB, NUPB, GPB, and GUPB

The distribution of adhesives in the plywood bonding interface is shown in Fig. 4. Figure 4 shows that the penetration depth of the adhesives in the two adjacent veneers was not symmetrical or consistent.

The adhesives were mainly distributed in the cell cavity and cell wall of the parenchyma cells, ray cells, and ducts of poplar, and some adhesives flowed into the interior of the veneer through a crack in the veneer. The amount of FPB infiltration into the veneer and poplar cells was low and mostly was present in the bonding interface. More NPB, NUPB, GPB, and GUPB penetrated the inside of the veneer and poplar cells and formed a "glue ring" for better permeability.

The results of quantitative analysis are shown in Table 2. The results indicate that the A, AP, and EP values of FPB were the lowest and that the permeability of FPB was the worst, and the A, AP, and EP values of the NPB, NUPB, GPB, and GUPB were improved after modification. The A and AP values of NPB were the largest, and the EP value of NUPB was the largest. The EP values of NUPB and GUPB increased 10% and 12%, respectively, relative to the EP values of NPB and GPB, which indicates that the fluidity of PBAs improved under ultrasound treatment.

**Table 2.** Analysis Results of Plywood Bonding Interface

| Adhesives | *A* (μm$^2$) | AP (μm) | EP (μm) |
|---|---|---|---|
| FPB | 36280 ± 1814 | 35.41 ± 0.18 | 237.23 ± 1.19 |
| NPB | 68115 ± 3405 | 65.79 ± 0.33 | 384.30 ± 1.92 |
| NUPB | 62489 ± 3124 | 54.21 ± 0.27 | 423.45 ± 2.13 |
| GPB | 55204 ± 2706 | 55.48 ± 0.28 | 367.83 ± 1.84 |
| GUPB | 60205 ± 3010 | 60.59 ± 0.30 | 412.25 ± 2.06 |

## WSS of Plywood

The WSS of plywood made using PBA is shown in Fig. 5. The WSS of FPB was only 0.16 MPa, which is far less than the strength requirement of adhesives for plywood (≥ 0.7 MPa) (GB/T 17657 2013).

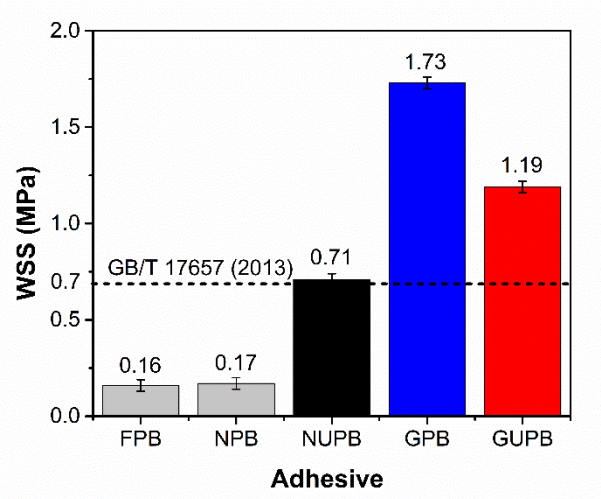



**Fig. 5.** The WSS of plywood bonded with FPB, NPB, NUPB, GPB, and GUPB

The WSS of plywood made with NPB was slightly increased. This may have been due to the ability of NaOH to open the structure of protein molecules and expose active groups for crosslinking, but the structure of protein molecules could not be fully opened

with mild alkaline conditions. As the protein molecules disperse and unfold in solution under the cavitation of ultrasound treatment, the interaction between the wood and the protein increases during the curing process (Nordqvist *et al.* 2010; Sui *et al.* 2017; Zhu *et al*. 2017), and a better bond strength is achieved. Therefore, the WSS of the plywood made with NUPB increased 318% relative to that of NPB, and it reached 0.71 MPa. The WSS of the plywood made with GPB was the highest, which was attributed to the fact that the addition of glutaraldehyde led to the formation of complex crosslinked structures, which improved the performance of the resultant adhesive (Wang *et al.* 2007). The WSS of the plywood made with GUPB decreased 31% compared to that of GPB. This might be because the cross-linking structure of GUPB was less than that of GPB after ultrasound treatment (Li *et al.* 2016). However, the plywood made with GUPB still met the strength requirement.

## CONCLUSIONS

1. After modification with a small amount of NaOH and glutaraldehyde and exposure to ultrasound treatment, the thermal stability and bonding performance of the modified pig blood adhesives (PBAs) were improved.
2. The glutaraldehyde-treated pig blood (GPB) had a more stable crosslinked structure, better thermal stability, and excellent bonding performance. In addition, the bonding strength of the ultrasonicated version (GUPB) was lower than that of GPB because ultrasound treatment slightly destroyed the formed crosslinked structure.
3. In weak alkalinity conditions, the use of ultrasound helps pig blood protein molecules unfold sufficiently and improve the permeability of PBA on the bonding interface. Therefore, the wet shear strength (WSS) of GPB increased 318% and reached the bonding strength requirements of plywood as per GB/T 17657 (2013).
4. As it allows a reduction of chemical reagents and cost, the preparation of PBAs with ultrasound treatment under mild alkaline conditions is a green preparation method.

## ACKNOWLEDGMENTS

This work was financially supported by the National State Science Foundation of China (Grant No. 21808093) and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

## REFERENCES CITED

Barreto, P. L. M., Pires, A. T. N., and Soldi, V. (2003). "Thermal degradation of edible films based on milk proteins and gelatin in inert atmosphere," *Polymer Degradation and Stability* 79(1), 147-152. DOI: 10.1016/s0141-3910(02)00267-7

Betancor, L., López-Gallego, F., Hidalgo, A., Alonso-Morales, N., Mateo, G. D.-O. C., Fernández-Lafuente, R., and Guisán, J. M. (2006). "Different mechanisms of protein immobilization on glutaraldehyde activated supports: Effect of support activation and immobilization conditions," *Enzyme and Microbial Technology* 39(4), 877-882. DOI: 10.1016/j.enzmictec.2006.01.014

Bier, J. M., Verbeek, C. J. R., and Lay, M. C. (2012a). "An eco-profile of thermoplastic protein derived from blood meal. Part 1: Allocation issues," *The International Journal of Life Cycle Assessment* 17(2), 208-219. DOI: 10.1007/s11367-011-0349-8

Bier, J. M., Verbeek, C. J. R., and Lay, M. C. (2012b). "An ecoprofile of thermoplastic protein derived from blood meal. Part 2: Thermoplastic processing," *The International Journal of Life Cycle Assessment* 17(3), 314-324. DOI: 10.1007/s11367-011-0355-x

Chen, L., Chen, J., Ren, J., and Zhao, M. (2011). "Effects of ultrasound pretreatment on the enzymatic hydrolysis of soy protein isolates and on the emulsifying properties of hydrolysates," *Journal of Agricultural and Food Chemistry* 59(6), 2600-2609. DOI: 10.1021/jf103771x

Cheng, Y., Donkor, P. O., Ren, X., Wu, J., Agyemang, K., Ayim, I., and Ma, H. (2019). "Effect of ultrasound pretreatment with mono-frequency and simultaneous dual frequency on the mechanical properties and microstructure of whey protein emulsion gels," *Food Hydrocolloids* 89, 434-442. DOI: 10.1016/j.foodhyd.2018.11.007

D'Amico, S., Hrabalova, M., Müller, U., and Berghofer, E. (2010). "Bonding of spruce wood with wheat flour glue—Effect of press temperature on the adhesive bond strength," *Industrial Crops and Products* 31(2), 255-260. DOI: 10.1016/j.indcrop.2009.11.001

Esteghlal, S., Gahruie, H. H., Niakousari, M., Barba, F. J., Bekhit, A. E., Mallikarjunan, K., and Roohinejad, S. (2019). "Bridging the knowledge gap for the impact of non-thermal processing on proteins and amino acids," *Foods* 8(7), 262. DOI: 10.3390/foods8070262

Ferdosian, F., Pan, Z., Gao, G., and Zhao, B. (2017). "Bio-based adhesives and evaluation for wood composites application," *Polymers* 9(2), Article number 70. DOI: 10.3390/polym9020070

Gavrilovic-Grmusa, I., Dunky, M., Djiporovic-Momcilovic, M., Popovic, M., and Popovic, J. (2016). "Influence of pressure on the radial and tangential penetration of adhesive resin into poplar wood and on the shear strength of adhesive joints," *BioResources* 11(1), 2238-2255. DOI: 10.15376/biores.11.1.2238-2255

GB/T 14074 (2006). "Testing methods for wood adhesives and their resins," Standardization Administration of China, Beijing, China.

GB/T 17657 (2013). "Test methods of evaluating the properties of wood-based panels and surface decorated wood-based panels," Standardization Administration of China, Beijing, China.

Gryta, M., Majchrzak, J., Kaledkowski, B., and Szemien, M. (1996). "Phenolic-formaldehyde resin modified with melamine and urea," *Przemysl Chemiczny* 75(1), 12-14.

He, Y., Cao, Y., and Liu, Y. (2005). "Initiation mechanism of ultrasonically irradiated emulsion polymerization," *Journal of Polymer Science Part B: Polymer Physics* 43(18), 2617-2624. DOI: 10.1002/polb.20544

Irle, M. A., and Bolton, A. J. (1991). "Physical aspects of wood adhesive bond formation with formaldehyde-based adhesives. Part III. The creep behavior of formaldehyde based resins at different relative humidities," *Holzforschung* 45(1), 69-74. DOI: 10.1515/hfsg.1991.45.1.69

Jambrak, A. R., Mason, T. J., Lelas, V., Paniwnyk, L., and Herceg, Z. (2014). "Effect of ultrasound treatment on particle size and molecular weight of whey proteins," *Journal of Food Engineering* 121, 15-23. DOI: 10.1016/j.jfoodeng.2013.08.012

Li, Q., Lin, T., and Wang, X. (2012). "Effects of ultrasonic treatment on wool fibre and fabric properties," *The Journal of the Textile Institute* 103(6), 662-668. DOI: 10.1080/00405000.2011.597569

Li, S., Yang, X., Zhang, Y., Ma, H., Liang, Q., Qu, W., He, R., Zhou, C., and Mahunu, G. K. (2016). "Effects of ultrasound and ultrasound assisted alkaline pretreatments on the enzymolysis and structural characteristics of rice protein," *Ultrasonics Sonochemistry* 31, 20-28. DOI: 10.1016/j.ultsonch.2015.11.019

Li, X., Li, J., Luo, J., Li, K., Gao, Q., and Li, J. (2018). "A novel eco-friendly blood meal-based bio-adhesive: Preparation and performance," *Journal of Polymers and the Environment* 26(2), 607-615. DOI: 10.1007/s10924-017-0976-7

Lin, H., and Gunasekaran, S. (2010). "Cow blood adhesive: Characterization of physicochemical and adhesion properties," *International Journal of Adhesion and Adhesives* 30(3), 139-144. DOI: 10.1016/j.ijadhadh.2009.10.003

Lin, Q., Chen, N., Bian, L., and Fan, M. (2012). "Development and mechanism characterization of high performance soy-based bio-adhesives," *International Journal of Adhesion and Adhesives* 34, 11-16. DOI: 10.1016/j.ijadhadh.2012.01.005

Liu, B., Ma, H.-L., Li, S.-J., Tian, W.-M., and Wu, B.-G. (2011). "Study on the structure of defatted wheat germ protein isolate under ultrasonic treatment," *Spectroscopy and Spectral Analysis* 31(8), 2220-2225. (In Chinese) DOI: 10.3964/j.issn.1000-0593(2011)08-2220-06

Low, A., Lay, M., Verbeek, J., and Swan, J. (2012). "Decoloring hemoglobin as a feedstock for second-generation bioplastics," *Preparative Biochemistry & Biotechnology* 42(1), 29-43. DOI: 10.1080/10826068.2011.563401

Mo, X. F., Fan, D. B., Qin, T. F., and Chu, F. X. (2015). "Curing characteristics and adhesion performance of phenol-fornaldehyde resins with composite additives," *Journal of Tropical Forest Science* 27(2), 248-254.

Nordqvist, P., Khabbaz, F., and Malmström, E. (2010). "Comparing bond strength and water resistance of alkali-modified soy protein isolate and wheat gluten adhesives," *International Journal of Adhesion & Adhesives* 30(2), 71-79. DOI: 10.1016/j.ijadhadh.2009.09.002

Nordqvist, P., Thedjil, D., Khosravi, S., Lawther, M., Malmström, E., and Khabbaz, F. (2012). "Wheat gluten fractions as wood adhesives—Glutenins *versus* gliadins," *Journal of Applied Polymer Science* 123(3), 1530-1538. DOI: 10.1002/app.34312

Pathak, R., Leong, T. S. H., Martin, G. J. O., and Ashokkumar, M. (2020). "Amino acid and secondary structure integrity of sonicated milk proteins," *Australian Journal of Chemistry* 73(2-3), 170-179. DOI: 10.1071/ch19372

Pizzi, A. (2006). "Recent developments in eco-efficient bio-based adhesives for wood bonding: Opportunities and issues," *Journal of Adhesion Science and Technology* 20(8), 829-846. DOI: 10.1163/156856106777638635

Qin, L., Lin, L., and Fu, F. (2016). "Microstructural and micromechanical characterization of modified urea-formaldehyde resin penetration into wood," *BioResources* 11(1), 182-194. DOI: 10.15376/biores.11.1.182-194

Santoni, I., and Pizzo, B. (2013). "Evaluation of alternative vegetable proteins as wood adhesives," *Industrial Crops and Products* 45, 148-154. DOI: 10.1016/j.indcrop.2012.12.016

Schmidt, V., Giacomelli, C., and Soldi, V. (2005). "Thermal stability of films formed by soy protein isolate–sodium dodecyl sulfate," *Polymer Degradation and Stability* 87(1), 25-31. DOI: 10.1016/j.polymdegradstab.2004.07.003

Schmidt, K., Grunwald, D., and Pasch, H. (2006). "Preparation of phenol-urea-formaldehyde copolymer adhesives under heterogeneous catalysis," *Journal of Applied Polymer Science* 102(3), 2946-2952. DOI: 10.1002/app.24441

Schmidt, V., and Soldi, V. (2006). "Influence of polycaprolactone-triol addition on thermal stability of soy protein isolate based films," *Polymer Degradation and Stability* 91(12), 3124-3130. DOI: 10.1016/j.polymdegradstab.2006.07.016

Silva, C., Sousa, F., Gübitz, G., and Cavaco-Paulo, A. (2004). "Chemical modifications on proteins using glutaraldehyde," *Food Technology and Biotechnology* 42(1), 51-56.

Soares, R. M. D., Scremin, F. F., and Soldi, V. (2005). "Thermal stability of biodegradable films based on soy protein and corn starch," *Macromolecular Symposia* 229(1), 258-265. DOI: 10.1002/masy.200551132

Sui, X., Bi, S., Qi, B., Wang, Z., Zhang, M., Li, Y., and Jiang, L. (2017). "Impact of ultrasonic treatment on an emulsion system stabilized with soybean protein isolate and lecithin: Its emulsifying property and emulsion stability," *Food Hydrocolloids* 63, 727-734. DOI: 10.1016/j.foodhyd.2016.10.024

Suslick, K. S., and Price, G. J. (1999). "Applications of ultrasound to materials chemistry," *Annual Review of Materials Science* 29, 295-326. DOI: 10.1146/annurev.matsci.29.1.295

Tian, Z., Liu, W., and Li, G. (2016). "The microstructure and stability of collagen hydrogel cross-linked by glutaraldehyde," *Polymer Degradation and Stability* 130, 264-270. DOI: 10.1016/j.polymdegradstab.2016.06.015

Wang, Y., Mo, X., Sun, X. S., and Wang, D. (2007). "Soy protein adhesion enhanced by glutaraldehyde crosslink," *Journal of Applied Polymer Science* 104(1), 130-136. DOI: 10.1002/app.24675

Wang, W. H., Li, X. P., and Zhang, X. Q. (2008). "A soy-based adhesive from basic modification," *Pigment & Resin Technology* 37(2), 93-97. DOI: 10.1108/03699420810860446

Wu, Z. (2016). *Crosslinking Modification of Protein-based Adhesives and Mechanism*, Ph.D. Dissertation, Beijing Forestry University, Beijing, China.

Yang, I., Kuo, M., Myers, D. J., and Pu, A. (2006). "Comparison of protein-based adhesive resins for wood composites," *Journal of Wood Science* 52(6), 503-508. DOI: 10.1007/s10086-006-0804-5

Yuan, C., Chen, M., Luo, J., Li, X., Gao, Q., and Li, J. (2017). "A novel water-based process produces eco-friendly bio-adhesive made from green cross-linked soybean soluble polysaccharide and soy protein," *Carbohydrate Polymers* 169, 417-425. DOI: 10.1016/j.carbpol.2017.04.058

Zhu, W. (2015). *Chemical Crosslinking of Soybean Protein and Its Effects on the Properties of Soybean Protein-based Wood Adhesives*, Master's Thesis, Northeast Forestry University, Harbin, China.

Zhu, X., Wang, D., Li, N., and Sun, X. S. (2017). "Bio-based wood adhesive from camelina protein (a biodiesel residue) and depolymerized lignin with improved water resistance," *ACS OMGEA* 11(2), 7996-8004. DOI: 10.1021/acsomega.7b01093

Article submitted: March 20, 2020; Peer review completed: June 4, 2020; Revised version received and accepted: June 23, 2020; Published: August 19, 2020.
DOI: 10.15376/biores.15.4.7586-7597