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# Effects of various durations of enzyme hydrolysis on properties of starch-based wood adhesive

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#### ABSTRACT

Improving the performance of wood adhesive is important for the development of the veneer industry. This work investigated the effects of various durations of enzymatic hydrolysis to improve and enhance the properties of starch-based wood adhesive (SWA). The results showed that moderate enzymatic hydrolysis for 2 h of starch molecule could improve the properties of SWA. The bonding strength of SWA was increased from 2.72 MPa (0 h) to 6.87 MPa (2 h) in the dry state and from 0.87 MPa (0 h) to 2.69 MPa (2 h) in the wet state. A significant decrease in the viscosity of SWA was also observed after 2 h hydrolysis of starch molecules, which allowed smooth spreading and penetration of adhesive through the wood surface. Meanwhile, the dynamic mechanical analysis and scanning electron microscopy showed that SWA with 2 h enzymatic hydrolysis starch. This study provides important information regarding the possible applications of SWA in the wood industry and presents a potential alternative to less environmentally friendly formaldehyde-based wood adhesives.

#### 1. Introduction

Wood adhesives play a central role in wood processing such as in the furniture, construction, and building industries [1]. However, most wood adhesives for industrial use are based on non-renewable petrochemical resources such as urea–formaldehyde, phenol–formaldehyde, and melamine–formaldehyde because they are inexpensive, easy to use, and have fast curing times [2]. Despite these advantages, the harmful emissions of formaldehyde from finished products initiated the development of wood adhesives that are free of formaldehyde. These concurrent initiatives have prompted the formulation of novel formaldehyde-free adhesives from biomass resources, such as tannin, lignin, soy protein, carbohydrates, and unsaturated plant oils [3–6].

Starch, an inexpensive and renewable natural polymer, is considered one of the most promising candidate materials to replace synthetic polymers. Although starches can be used as adhesives, their bonding capacities are insufficient to glue constructional wood material. Many strategies have been applied to improve the properties of starch-based wood adhesives by strengthening the molecular structure [7-10]. Extensive literature describes the preparation of starch-based wood adhesives by grafting vinyl acetate onto starch, using ammonium persulfate as an initiator [11-14]. Furthermore, the wood adhesive with high amylose content is characterized by high water resistance and superior mechanical properties [15-17], which are essential for practical applications of wood adhesive. Both acid hydrolysis and pullulanase hydrolysis can increase amylose content, but the effect of enzyme hydrolysis is stronger, as compared to acid hydrolysis [18]. The extraction rate of amylose by debranching enzyme is better than that by chemical method, and this process route closely resembles the features of sustainable chemistry.

Pullulanase is a debranching enzyme that can hydrolyze  $\alpha$ -1,6 linkages of polymer and is commonly employed in the saccharification process [19]. This enzyme is useful in producing amylose, glucose, and maltose by debranching the starch molecule with and without

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Received 7 October 2021; Received in revised form 19 December 2021; Accepted 8 February 2022 Available online 15 February 2022 0141-8130/© 2022 Elsevier B.V. All rights reserved.  $\beta$ -amylase, glucoamylase, or  $\alpha$ -amylase, respectively. Notably, this debranching enzyme can assist in regulating the existing approach for starch processing [20,21]. Owing to its industrial relevance, the demand for this enzyme is growing around the world.

In this study, pullulanase was used to investigate the effects of enzyme hydrolysis on cassava starch-based wood adhesives. Apparent viscosity, bonding strength, water resistance, and the starch molecular weight before and after enzyme hydrolysis were tested to confirm the positive effects of enzyme hydrolysis on the starch-based wood adhesive. In addition, the surface properties were investigated through zeta potential and surface tension. Furthermore, the micro-structure and thermo-mechanical characteristics of the adhesive film and film-forming processes were measured. Finally, we selected the suitable enzyme hydrolysis time for the synthesis of starch-based wood adhesives with a novel synthetic strategy. To the best of our knowledge, effects of enzyme hydrolysis on wood adhesive derived from cassava-starch have not been reported yet. Therefore, evaluating the role of starch hydrolysis in enhancing the properties of SWA is an important, strategy to prepare a novel green adhesive for wood application.

#### 2. Materials and methods

#### 2.1. Materials

Cassava starch (moisture content, 14.3%; amylose content, 27.4% (w/w, dry basis); protein content, 0.20% (w/w, dry basis); purity > 99%) was obtained from the Guangxi Academy of Agricultural Sciences (China). Poly (vinyl alcohol) (PVA, polymerization degree: 1700, alcoholysis degree: 87–89%) was purchased from Sinopec Shanghai Petrochemical (Shanghai, China). Vinyl acetate (VAc), ammonium persulfate (APS), sodium bicarbonate (NaHCO<sub>3</sub>), sodium dodecyl sulfate (SDS), sodium hydroxide (NaOH), citric acid monohydrate, tri-sodium citrate dehydrate, ethanol, acetone, urea, glycerin, and hydrochloric acid were procured from Sinopharm Chemical Reagent Co. (Shanghai, China). Enzyme pullulanase (activity: 2000 U/mL) was obtained from Yuan Ye Biological Co. (Shanghai, China). High amylose starch was obtained through enzymolysis (pullulanase) with amylose content of 27.4–39.46% using absorbance at 620 nm. Distilled water was used throughout the preparation.

#### 2.2. Synthesis of starch-based wood adhesive (SWA)

The starch-based wood adhesive was prepared according to the method described by Zia et al. with slight modification [15]. Approximately 25 g of cassava starch was dissolved in 100 mL of buffer solution (32 mL citric acid monohydrate and 68 mL tri-sodium citrate dehydrate) in three necked round-bottomed flasks. Then, the solution was stirred in boiling water for 5 min to gelatinize the starch slurry. After gelatinization, the reaction mixture was placed in a water bath, and enzymolysis was conducted by adding 45 µL of pullulanase enzyme (2000 U/mL) while the reaction mixture was constantly stirred at 50 °C from 0 h (unhydrolyzed starch, control group) to 3 h. After enzymolysis, high amylose starch was obtained. The pH of the reaction mixture was adjusted to 4.5 with 0.5 mol/L hydrochloric acid. Then, PVA solution (1.2% of SWA), SDS (4% of dry starch), and APS (0.3 g) were added under nitrogen protection. After 30 min, the reaction temperature was increased to 70 °C. Subsequently, 50 mL of a mixture of VAc monomer was dropped into the mixture for duration of 2 h. After complete polymerization, the temperature of the reaction mixture was increased to 85  $^{\circ}$ C, which was maintained for 30 min. The mixture was cooled to room temperature. Thereafter, 10 g urea and 3 mL glycerol was added into the flask and pH was adjusted to 6.0 by adding NaHCO<sub>3</sub>.

#### 2.3. Synthesis of copolymer

The copolymer was obtained by preparing the grafted starch samples

in accordance with the previously described procedure without adding PVA [11]. The grafted samples were precipitated with ethanol and washed with distilled water. These samples were then freeze-dried after centrifugation to obtain solid grafted starch samples. These solids were extracted with acetone in a Soxhlet extraction device at 70 °C for 48 h to remove the homopolymers of VAc. Finally, the products were freeze-dried until a constant weight was achieved [16,22]. The percentage of grafting (G%) was calculated using the following equation:

 $%G = \frac{Grafted vinyl acetate polymer weight}{Grafted carbohydrate weight} \times 100$ 

#### 2.4. Adhesive bond performance test

The SWA samples were stored at 23  $\pm$  2 °C for 10 days and then used to glue the wood samples. The shear strengths of the glued samples were evaluated according to the Chinese industry standard HG/T 2727-2010 (China, 2010) [23]. Freshly cut wood pieces (Manchurian ash, approximately 0.81  $\times$  10  $^3$  kg/m  $^3$  ) with dimensions of 25 mm  $\times$  25 mm  $\times$  10 mm were glued using the adhesives at a static pressure of 0.5-1.0 MPa at 25 °C for 24 h (Scheme 1). Prior to the shear strength tests, the glued specimens were stored in a laboratory at 23  $\pm$  2  $^{\circ}C$  and 50%  $\pm$  5% humidity for 48 h. The shear strength of the glued samples in the dry or wet (after immersion in water at 23 °C for 3 h) state was studied using a SUNS shear strength analyzer (ShenZhen Suns Technology Stock, China). The shear strength was calculated by the following equation:  $\sigma_M$ =  $F_{\text{max}}$  / A, where  $\sigma_M$  (MPa) is the shear strength,  $F_{\text{max}}$  (N) is the observed maximum failing load, and A ( $mm^2 = 625 mm^2$ ) is the bonding surface of the sample. The testing speed was 2 mm/min. All tests were replicated six times, and the results are presented as averages.

#### 2.5. Viscosity test

The rotational viscosity was measured using an NDJ-8S rotational viscometer (Shanghai LiChen-BX Instrument Technology, China) with No. 4 rotor at 30 r/min and 30 °C. An extended period was required to obtain a stable reading. All the tests were replicated three times, and the results were presented as averages.

#### 2.6. Molecular weight test

In this study, the molecular weight distribution of enzyme-treated starch samples was detected by GPC-RI-MALLS (gel chromatography differential-angle laser light scattering) series analysis method [24]. The high-performance size-exclusion chromatography separation was conducted in a system with a programmable HPLC IsoPump (G1310A), an autosampler (ALS G1313A), and degasser (G1322A) (Agilent Technology, Santa Barbara, CA, USA). The size-exclusion system comprised three columns in series: OHpak SB-805 HQ, OHpak SB-804 HQ, and OHpak SB-803 HQ (Shodex, Showa Denko, Tokyo, Japan). The separation system was coupled to a multi-angle light scattering Wyatt Dawn Heleos II and Optilab T-rEx (Wyatt Technology, Santa Barbara, CA, USA). The data were processed with ASTRA software (v.5.3.1.5). Mobile phase was 5 mmol LiBr-DMSO solution, degassed and filtered in 0.22 µm Millipore (Pall Corporation, Ann Arbor, MI, USA). All separations were conducted under the same experimental conditions with a volume injection of 100  $\mu$ L at 0.4 mL min<sup>-1</sup>. 5 mg for the starch sample. Then, 1 mL mobile phase was added, and the starch solution was dissolved under 60 °C heat. The solution was centrifuged at 14000 rpm for 10 min, and 100 µL supernatant was obtained for loading.

#### 2.7. Surface tension measurement

The surface tension between SWA and air was measured by a surface tension meter (K100, Krüss, Germany). A platinum plate was used as an electrode while the other was the material to be measured. The



### Unit (mm)

Scheme 1. Shape and dimension of shear test specimen (HG 2727-2010).

parameters of detection speed, immersion depth, and time were set as 5 mm/min, 2 mm, and 1200 s, respectively. Each sample, including water (reference group), was measured three times.

#### 2.8. Zeta potential analysis

The adhesive sample was diluted (1:500) by Zetasizer equipped with red (633 nm) laser and avalanche photo-diode detector [16]. The experiment was conducted using DTS1070 folded capillary cells. Measurements were taken three times and the average values were obtained.

#### 2.9. Dynamic mechanical analysis (DMA)

DMA was conducted by TA Instruments Q800 dynamic mechanical analyzer (DMA) with in temperature program (scanning range: -50-100 °C, rate: 3 °C/min). A compress assembly was used for testing. The adhesive films were cut into a nominal dimension (length: 0.813 mm, width: 1.000 mm, and thickness: 1.000 mm).

#### 2.10. Diffusing wave spectroscopy (DWS) analysis

DWS measurements were conducted using a Horus film formation analyzer (Formulaction, France). The principle of measurement involves sending a laser light (655 nm) into the film. Structural changes in the drying coating sample were revealed by monitoring the speckle rate during the adhesive film formation. The samples were drawn on an aluminum substrate with wet thickness of 50  $\mu$ m.

#### 2.11. Scanning electron microscopy (SEM) analysis

The films of SWA with various durations were made by pouring the adhesive on a Teflon board, gradually evaporating the water on a hot plate at 40 °C, and vacuum drying until a constant weight was achieved. Then, the morphology of the adhesive samples was studied using JSM-7800 Prime field emission SEM operating at 2 kV.

#### 2.12. Statistical analysis

Data was statistically analyzed using DPS 7.05 (Zhejiang University, Hangzhou, China). Significant differences (p < 0.05) between treatment means were evaluated through Duncan's multiple range tests.

#### 3. Results and discussion

## 3.1. Effects of various enzyme-hydrolysis durations on molecular weight of starch

The molecular weight of starch-based wood adhesives with different enzyme hydrolysis durations are shown in Fig. 1. The Mw value of normal cassava starch was  $11.29 \times 10^7$  g/mol. With increase in enzymatic hydrolysis reaction time, the average molecular weight (Mw) of cassava starch was gradually decreased, while the polydispersity coefficient (M<sub>W</sub>/Mn) gradually increased (Fig. 1a and b). This phenomenon might be attributed to the degradation of amylopectin by the debranching enzyme (pullulanase). Under the action of enzyme, branched chained amylopectin was hydrolyzed into short linear chained amylose, so the molecular weight distribution of the system was widen, and thus, an increase in the Mw/Mn value was observed [25]. In addition, Fig. 1c shows that the gyration radius R<sub>Z</sub> of enzyme-hydrolyzed starch was smaller than that of natural cassava starch (217.7 nm). The radius of gyration R<sub>Z</sub> represents the volume of starch molecules in the solution, which is closely related to the branching mode and branching chain length of branched chain molecules [26]. R<sub>Z</sub> was decreased due to the decrease in long-chain amylopectin and the increase in short-chain amylose. Therefore, we further determined the content of amylose in this system (Fig. 1d). The increase in amylose content from 27.4 (0 h) to 39.46 (3 h) also confirmed the results presented above.

### 3.2. Effects of various enzyme hydrolysis durations on apparent viscosity and shear strength of SWA

The effects of enzyme hydrolysis on the adhesive system were further confirmed by the water resistance and bonding strength. As shown in Fig. 2, the shear strength and water resistance showed an increasing



**Fig. 1.** Macromolecular characteristics and amylose content of cassava starch with various enzyme hydrolysis durations. (a) Mw value of enzyme-hydrolyzed starch (0-3 h); (b) polydispersity coefficient (MW/Mn); (c) gyration radius (Rz); (d) amylose content. Bars with different letters denote significant differences (p < 0.05).



Fig. 2. Effects of different enzymatic hydrolysis times on shear strength of starch-based wood adhesive. Bars with different letters indicate significant differences (p < 0.05) among different time points within dry or wet state group.

trend, followed by a decreasing trend. Compared with the control sample, the adhesive that was acid-hydrolyzed for 2 h showed an increase in shear strength from 2.72 MPa (control group) to 6.87 MPa (2 h) in the dry state and from 0.87 MPa (0 h) to 2.69 MPa (2 h) in the wet state. After a certain degree of exposure to enzyme hydrolysis, starch developed many active sites for grafting [15]. Thus, the bonding strength was significantly improved by the grafting reaction. However, the shear strength of the adhesive was decreased when the duration of enzyme hydrolysis exceeded 2 h. The excessively hydrolyzed starch developed many amylose fragments, which led to easy retrogradation and reduced the bond properties of SWA.

The bonding characteristics of the adhesive are used to assess the shear strength in the dry state [27], whereas the water resistance of the adhesive is used to assess the shear strength in the wet state. Compared with the control, the adhesive that was hydrolyzed for 2 h showed increased bonding strength of 152.57% in the dry state and 209.19% in the wet state. Thus, 2 h duration is the best enzyme hydrolysis time to improve the bonding strength and water resistance of SWA. The bonding strength of starch-based wood adhesives inevitably decreases in a humid environment because the water molecules penetrate the wood and act as a plasticizer for hydrophilic polymers [28]. Thus, the shear strength in wet condition was approximately 30%–50% of the shear strength under dry conditions. Wang et al. reported similar results for the bonding characteristics of adhesive derived from starch [29].

The apparent viscosities of starch-based wood adhesives with various enzymatic hydrolysis durations are presented in Fig. 3. The control sample without enzymatic hydrolysis is not shown in Fig. 3 because its



Fig. 3. Variation of viscosity of starch adhesive with various enzymatic hydrolysis durations. Bars with different letters denote significant differences (p < 0.05).

resulting viscosity was extremely high. Viscosity is one of the main indicators of the adhesive coating evaluation. Adhesives with high viscosity do not spread easily, whereas those with low viscosity possess high flowability and can easily lead to losses [30]. As shown in Fig. 3, the viscosity of SWA decreased significantly with the increasing duration of enzymatic hydrolysis. The changes in viscosities suggested that the enzymatic hydrolysis disrupted the structure of the starch molecules. The size of the starch molecules became smaller with increasing duration of enzymatic hydrolysis. Thus, the viscosity of the sample with long hydrolysis time was lower than that of the control sample.

## 3.3. Effects of various enzyme hydrolysis durations on percentage of grafting

The percentage of grafting curve is shown in Fig. 4. Initially, the grafting parameter increased from 41.79% (0 h) to 73.87% (3 h) with increasing duration of enzymatic hydrolysis. The increased grafting



Fig. 4. Effects of various enzyme hydrolysis durations on graft parameter of grafted copolymer. Bars with different letters denote significant differences (p < 0.05).

parameter illustrates the graft copolymerization in the system. The enzyme hydrolysis of the starch promoted grafting along the starch chains because of the poor mobility of the starch macroradicals [31,32]. The enzyme hydrolysis of the starch reduced the molecular weight of the starch, produced more short-chain amylose molecules, and exposed more active sites. This phenomenon increased the probability that the starch macroradicals would collide with the VAc molecules [22]. The amount of copolymer also increased with increasing grafting parameter, thereby enhancing the compatibility between the starch and polymer [11]. This phenomenon also confirms the aforementioned results of increased shear strength of SWA with increasing hydrolysis time (Fig. 3).

## 3.4. Effects of various enzyme hydrolysis durations on zeta potential and surface tension

Surface property is a significant indicator of the quality of liquid adhesives because of its strong effect on the rheological properties and microstructures of adhesive film. In the emulsion system, surface charge and surface are two main factors that affect the dispersion of latex particles. In addition, surface tension is also a significant indicator of wettability in the adhesive substance and directly affects the bonding ability of the adhesive. Figs. 5 and 6 show the surface properties of adhesives with various enzyme-hydrolysis durations measured by zeta potential (Fig. 5) and surface tension (Fig. 6). Fig. 5 shows that the zeta potential of the adhesives decreased from -3.62 (0.5 h) to -7.45 (2 h) eV. The result of the zeta potential showed that the SWA particles had negative charge that could be attributed to negatively charged SDS. As shown in Fig. 6, the surface tension of SWA showed a decreasing trend, followed by an increasing trend. Compared with the control sample, the surface tension of the adhesive decreased from 52.28 mN/m (0.5 h) to 39.33 mN/m (2 h), and then increased to 47.99 mN/m (3 h). These variations in zeta potential and surface tension illustrated that introducing SDS into the adhesive system enhanced the electrostatic repulsion between latex particles and improved the wettability of the adhesive. Furthermore, these results indicated that moderate enzymatic hydrolysis could improve the surface properties and promote the emulsion stability of the adhesive. Generally, the wood adhesive with better wettability is more conducive to spreading and penetration through the wood surface, which helps to improve the adhesion properties of the starch adhesive. These observations confirm the results of dry and wet strength determined in the preceding section. Moreover, we measured the surface tension (71.68 mN/m) between water and air at



Fig. 5. Variation in zeta potential of SWA with various enzymehydrolysis durations.





room temperature, further confirming the reliability of the aforementioned results.

#### 3.5. Dynamic mechanical analysis (DMA)

As a non-reactive adhesive, the SWA adhesive film is the final product of adhesive smeared on the surface of wood. Thus, investigating the thermomechanical performance of adhesive films is important. As one of the most sensitive thermal analytical techniques for detecting the heating transition of polymer matrix [33,34], the dynamic mechanical analysis was measured to study the dynamic change in film and the results are shown in Fig. 7.

As the temperature increased, the E' of all samples decreased gradually, which could be due to the increase in the motion of potential energy of adhesive molecular chains with the increase in temperature. The gradual decline of the deformation resistance of the material, as well as the destruction of the original cross-linking structure between molecules, resulted in a decrease in the E' values of the adhesive. In addition, the samples hydrolyzed for 2 h showed the highest initial storage modulus E', indicating that the adhesive film had better elastic deformation properties, which was consistent with the results of bonding strength.

Furthermore, the loss factor (tan D) curves of adhesive films were used to illustrate that the cured adhesive with enzymatic-hydrolysis duration of 2 h exhibited high elasticity. Generally, tan D is regarded as a credible indicator of glass transition temperature (Tg) reflecting the thermal stability of polymer composites [35]. For adhesive films, the peaks around 50 °C could be regarded as Tg peaks [34]. The Tg temperature of SWA films were 52.27 °C (0.5 h), 56.91 °C (2 h), and 54.30 °C (3 h). The higher thermal stability of the adhesive film showed the strengthened molecular structure and supported the improved bonding strength and water resistance of the adhesive from another aspect [11]. Therefore, the cured adhesive with enzymatic hydrolysis of 2 h exhibited higher mechanical property, which further confirmed that moderate enzymatic hydrolysis of 2 h improved the mechanical performance of starch-based adhesive film to a substantial level.

#### 3.6. Analysis of film-forming process

DWS is an original and non-intrusive tool to investigate the film formation in representative conditions. DWS is conducted to monitor and display the real-time motion of scattering of particles, droplets, and interfaces inside the sample as a function of time [36,37]. In general, the film formation of SWA from dispersion coatings consists of three stages: concentration, packing, and consolidation [23,38]. In the first stage (time 1), water freely evaporated, and the SWA particles inside the film mainly exhibited Brownian motion. The internal particles of the SWA film were ordered gradually in a sequence. The SWA membrane structure was formed at the sorting time called second stage (time 2). The rate of water evaporation increased for the strong capillary siphon. The SWA particles were tightly arranged, and the deformation of particles and interdiffusion/coalescence processes occurred between the latex particles at this time (time 3) [39]. The DWS data of SWA (Fig. 8 and Table 1) showed that the drying process was composed of the aforementioned three stages. The various changes in slope and shape observed on the kinetics could be related to the changes in the film structure. In the first stage (time 1), all SWA samples showed no obvious difference. However, the second and third stages of SWA showed obvious differences. The SWA with enzymatic hydrolysis time of 2 h exhibited the lowest time 2 (587.728 s) and time 3 stage (1411.582 s), which indicated that the SWA



Fig. 7. Dynamic mechanical analysis of adhesive film with various enzyme-hydrolysis durations.



Fig. 8. DWS of SWA with various enzymatic hydrolysis durations.

 Table 1

 Characteristic drying times of SWA with various enzymatic hydrolysis durations.

Sample	Time 1 (s)	Time 2 (s)	Time 3 (s)
0.5 h 2 h 3 h	$\begin{array}{l} 4.856 \pm 1.498^{b} \\ 11.663 \pm 1.839^{a} \\ 9.119 \pm 1.771^{a} \end{array}$	$\begin{array}{c} 1615.427 \pm 81.829^a \\ 587.728 \pm 16.651^b \\ 627.525 \pm 18.325^b \end{array}$	$\begin{array}{c} 2069.710 \pm 136.306^a \\ 1411.582 \pm 85.218^c \\ 1789.829 \pm 67.012^b \end{array}$

Mean  $\pm$  SD values followed by the same column followed by different superscripts are significantly different ( $p \le 0.05$ ).

with enzymatic hydrolysis time of 2 h could easily fabricate continuous flexible films. The fast formation of the adhesive film occurred due to the improved compatibility of the components [38]. Thus, the film formation characteristics of the SWAs indicated that the SWA subjected to enzymatic hydrolysis for 2 h formed a more compact adhesive film. The high-quality adhesive film can help enhance the bonding ability and water resistance of the wood adhesive.

#### 3.7. Morphological characteristics of adhesive films

The effects of various enzymatic hydrolysis durations on the adhesive structure are directly shown by scanning electron micrographs (Fig. 9). This figure shows that compared with the adhesive subjected to enzymatic hydrolysis for 0.5 h (Fig. 9a), those subjected to 2 h (Fig. 9b) and 3 h (Fig. 9c) exhibited smooth and compact films with no evident microphase separations. Combined with the data on bond characteristics, DMA, DWS, and morphological characteristics of SWA, the results showed that moderate enzymatic hydrolysis improved the formation of a continuous adhesive film by SWA. Furthermore, moderate enzymatic hydrolysis greatly improved the component compatibility of SWA and limited the phase separation between the starch and polymer [14]. For non-reactive water-based adhesive, high-quality adhesive films possibly helped enhance their bonding characteristics. In summary, moderate enzymatic hydrolysis could enhance the rate of film-forming behavior of SWA while reinforcing their bonding characteristics.

#### 4. Conclusions

This study evaluated the effects of enzymatic hydrolysis on the characteristics of starch-based wood adhesive. The adhesive sample subjected to the most appropriate enzymatic-hydrolysis duration (2 h) exhibited optimal shear strengths of 6.87 MPa in the dry state and 2.69 MPa in the wet state. The results also revealed that the appropriate duration (2 h) of enzymatic hydrolysis improved the grafting reaction and surface properties of starch-based adhesive. Further analysis showed that these improvements were due to the enhanced thermomechanical performance of adhesive films, improved film-forming rate, and microstructure of adhesive films. In summary, this study provides a novel strategy for designing high-performance bio-based wood adhesive



Fig. 9. SEM of adhesive films with various enzymatic hydrolysis durations: (a) 0.5 h, (b) 2 h, and (c) 3 h.

as an alternative to less environmentally friendly formaldehyde-based wood adhesive.

#### CRediT authorship contribution statement

**Zhenjiong Wang:** Investigation, Conceptualization, Methodology, Writing – original draft. **Zheng Xing:** Investigation, Visualization, Formal analysis, Writing – review & editing. **Qin Zhang:** Investigation, Visualization, Formal analysis, Writing – review & editing. **Dongxia Hu:** Investigation, Visualization, Formal analysis, Writing – review & editing. **Jiasheng Lv:** Investigation, Visualization, Formal analysis, Writing – review & editing. **Chaoyi Wu:** Investigation, Visualization, Formal analysis, Writing – review & editing. **Wenzhi Zhou:** Methodology, Funding acquisition, Supervision. **Zia-ud-Din:** Writing – review & editing, Supervision, Project administration, Conceptualization.

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#### References

- [1] N.S. Sulaiman, R. Hashim, O. Sulaiman, M. Nasir, M.H.M. Amini, S. Hiziroglu, Partial replacement of urea-formaldehyde with modified oil palm starch based adhesive to fabricate particleboard, Int. J. Adhes. Adhes. 84 (2018) 1–8.
- [2] A. Pizzi, Wood products and green chemistry, Ann. For. Sci. 73 (1) (2016) 185–203.
- [3] Y. Zeng, W. Yang, P. Xu, X. Cai, W. Dong, M. Chen, M. Du, T. Liu, P. Jan Lemstra, P. Ma, The bonding strength, water resistance and flame retardancy of soy proteinbased adhesive by incorporating tailor-made core-shell nanohybrid compounds, Chem. Eng. J. 428 (2022), 132390.
- [4] H. Ye, D. Pan, Z. Tian, Y. Zhang, Z. Yu, J. Mu, Preparation and properties of geopolymer/soy protein isolate composites by in situ organic-inorganic hybridization: a potential green binder for the wood industry, J. Clean. Prod. 276 (2020), 123363.
- [5] D. Vnučec, A. Kutnar, A. Goršek, Soy-based adhesives for wood-bonding a review, J. Adhes. Sci. Technol. 31 (8) (2017) 910–931.
- [6] A. Moubarik, B. Charrier, A. Allal, F. Charrier, A. Pizzi, Development and optimization of a new formaldehyde-free cornstarch and tannin wood adhesive, Eur. J. Wood Wood Prod. 68 (2) (2010) 167–177.
- [7] Y. Monroy, S. Rivero, M.A. García, Sustainable panels design based on modified cassava starch bioadhesives and wood processing byproducts, Ind. Crop. Prod. 137 (2019) 171–179.
- [8] Y. Zhang, M.F. Cunningham, N.M.B. Smeets, M.A. Dubé, Starch nanoparticle incorporation in latex-based adhesives, Eur. Polym. J. 106 (2018) 128–138.
- [9] Y. Zhang, Z. Guo, X. Chen, Y. Ma, H. Tan, Synthesis of grafting itaconic acid to starch-based wood adhesive for curing at room temperature, J. Polym. Environ. 29 (3) (2021) 685–693.
- [10] M. Frey, L. Schneider, H. Razi, E. Trachsel, E. Faude, S.M. Koch, K. Masania, P. Fratzl, T. Keplinger, I. Burgert, High-performance all-bio-based laminates derived from delignified wood, ACS Sustain. Chem. Eng. 9 (29) (2021) 9638–9646.
- [11] Z. Wang, Z. Li, Z. Gu, Y. Hong, L. Cheng, Preparation, characterization and properties of starch-based wood adhesive, Carbohydr. Polym. 88 (2) (2012) 699–706.
- [12] X. Zheng, L. Cheng, Z. Gu, Y. Hong, Z. Li, C. Li, Effects of heat pretreatment of starch on graft copolymerization reaction and performance of resulting starchbased wood adhesive, Int. J. Biol. Macromol. 96 (2017) 11–18.
- [13] L. Chen, Y. Wang, P. Fei, W. Jin, H. Xiong, Z. Wang, D. Zia ud, Enhancing the performance of starch-based wood adhesive by silane coupling agent(KH570), Int. J. Biol. Macromol. 104 (2017) 137–144.

- [14] L. Chen, J. Li, Z.-U. Din, C. Hu, H. Xiong, Sustainable bio-based wood adhesive incorporated different functionalized nanoparticles: a performance comparison study, Starch - Stärke 73 (7–8) (2021), 2100042.
- [15] D. Zia ud, H. Xiong, Z. Wang, P. Fei, I. Ullah, A.B. Javaid, Y. Wang, W. Jin, L. Chen, Effects of sucrose fatty acid esters on the stability and bonding performance of high amylose starch-based wood adhesive, Int. J. Biol. Macromol. 104 (2017) 846–853.
- [16] Z.-U. Din, H. Xiong, Z. Wang, L. Chen, I. Ullah, P. Fei, N. Ahmad, Effects of different emulsifiers on the bonding performance, freeze-thaw stability and retrogradation behavior of the resulting high amylose starch-based wood adhesive, Colloids Surf. A Physicochem. Eng. Asp. 538 (2018) 192–201.
- [17] N. Tratnik, P.-Y. Kuo, N.R. Tanguy, P. Gnanasekar, N. Yan, Biobased epoxidized starch wood adhesives: effect of amylopectin and amylose content on adhesion properties, ACS Sustain. Chem. Eng. 8 (49) (2020) 17997–18005.
- [18] H. Zhang, Z. Jin, Preparation of resistant starch by hydrolysis of maize starch with pullulanase, Carbohydr. Polym. 83 (2) (2011) 865–867.
- [19] S.L. Hii, J.S. Tan, T.C. Ling, A.B. Ariff, Pullulanase: role in starch hydrolysis and potential industrial applications, Enzyme Res. 2012 (2012), 921362.
- [20] Y. Zheng, Y. Ou, Y. Zhang, B. Zheng, S. Zeng, H. Zeng, Effects of pullulanase pretreatment on the structural properties and digestibility of lotus seed starchglycerin monostearin complexes, Carbohydr. Polym. 240 (2020), 116324.
- [21] R. Wang, P. Liu, B. Cui, X. Kang, B. Yu, L. Qiu, C. Sun, Effects of pullulanase debranching on the properties of potato starch-lauric acid complex and potato starch-based film, Int. J. Biol. Macromol. 156 (2020) 1330–1336.
- [22] Z. Wang, Z. Gu, Z. Li, Y. Hong, L. Cheng, Effects of emulsifier on the bonding performance and freeze-thaw stability of starch-based wood adhesive, Cellulose 20 (5) (2013) 2583–2590.
- [23] Z. Wang, H. Zhu, J. Huang, Z. Ge, J. Guo, X. Feng, Q. Xu, Improvement of the bonding properties of cassava starch-based wood adhesives by using different types of acrylic ester, Int. J. Biol. Macromol. 126 (2019) 603–611.
- [24] L.A. Bello-Perez, E. Agama-Acevedo, M. Lopez-Silva, J. Alvarez-Ramirez, Molecular characterization of corn starches by HPSEC-MALS-RI: a comparison with AF4-MALS-RI system, Food Hydrocoll. 96 (2019) 373–376.
- [25] N.W.H. Cheetham, L. Tao, The effects of amylose content on the molecular size of amylose, and on the distribution of amylopectin chain length in maize starches, Carbohydr. Polym. 33 (4) (1997) 251–261.
- [26] L.A. Bello-Pérez, P. Roger, B. Baud, P. Colonna, Macromolecular features of starches determined by aqueous high-performance size exclusion chromatography, J. Cereal Sci. 27 (3) (1998) 267–278.
- [27] Z. Wang, Z. Gu, Y. Hong, L. Cheng, Z. Li, Bonding strength and water resistance of starch-based wood adhesive improved by silica nanoparticles, Carbohydr. Polym. 86 (1) (2011) 72–76.
- [28] L. Qiao, A.J. Easteal, Aspects of the performance of PVAc adhesives in wood joints, Pigm. Resin Technol. 30 (2) (2001) 79–87.
- [29] Y. Wang, H. Xiong, Z. Wang, D. Zia ud, L. Chen, Effects of different durations of acid hydrolysis on the properties of starch-based wood adhesive, Int. J. Biol. Macromol. 103 (2017) 819–828.
- [30] Z. Wang, Z. Gu, Z. Li, Y. Hong, L. Cheng, Effects of urea on freeze-thaw stability of starch-based wood adhesive, Carbohydr. Polym. 95 (1) (2013) 397–403.
- [31] L. Chen, Z. Wang, J. Cai, H. Xiong, Z. Xiong, Z.-U. Din, C. Hu, A. Nawaz, A combination of coarse-grain molecular dynamics to investigate the effects of sodium dodecyl sulfate on grafted reaction of starch-based adhesive, Carbohydr. Polym. 218 (2019) 20–29.
- [32] S.H. Samaha, H.E. Nasr, A. Hebeish, Synthesis and characterization of starch-poly (vinyl acetate) graft copolymers and their saponified form, J. Polym. Res. 12 (5) (2005) 343–353.
- [33] N. Pokeržnik, M. Krajnc, Synthesis of a glucose-based surfmer and its copolymerization with n-butyl acrylate for emulsion pressure sensitive adhesives, Eur. Polym. J. 68 (2015) 558–572.
- [34] L. Chen, Z. Xiong, H. Xiong, Z. Wang, Z.-U. Din, A. Nawaz, P. Wang, C. Hu, Effects of nano-TiO2 on bonding performance, structure stability and film-forming properties of starch-g-VAc based wood adhesive, Carbohydr. Polym. 200 (2018) 477–486.
- [35] R.A. El-Ghazawy, A.M. El-Saeed, H.I. Al-Shafey, A.-R.M. Abdul-Raheim, M.A. El-Sockary, Rosin based epoxy coating: synthesis, identification and characterization, Eur. Polym. J. 69 (2015) 403–415.
- [36] H.B. Yamak, H. Yıldırım, Improvement of film properties of vinyl acetate based emulsion polymers by using different types of maleic acid diesters, Prog. Org. Coat. 76 (12) (2013) 1874–1878.
- [37] A. Brun, H. Dihang, L. Brunel, Film formation of coatings studied by diffusing-wave spectroscopy, Prog. Org. Coat. 61 (2) (2008) 181–191.
- [38] L. Chen, Z.-U. Din, D. Yang, C. Hu, J. Cai, H. Xiong, Functional nanoparticle reinforced starch-based adhesive emulsion: toward robust stability and high bonding performance, Carbohydr. Polym. 269 (2021), 118270.
- [39] J.Y. Lee, J.W. Hwang, H.W. Jung, S.H. Kim, S.J. Lee, K. Yoon, D.A. Weitz, Fast dynamics and relaxation of colloidal drops during the drying process using multispeckle diffusing wave spectroscopy, Langmuir 29 (3) (2013) 861–866.